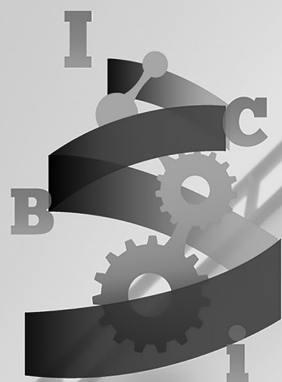

I Congreso Internacional de Biotecnología e innovación (ICBi)

09 al 12 de julio
Lima - Perú
2018

Resúmenes

Material suplementario S1

Citación:
Villena G, Hernández- Macedo M, Samolski I. 2020. Memorias Del Primer Congreso Internacional de Biotecnología e Innovación - ICBi 2018. Material suplementario S1. Revista Peruana De Biología 27 (1), 5-14. https://doi.org/10.15381/rpb.v27i1.17624

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Gretty Katherina Villena Chávez

Keynote conference

Section I: Agricultural and Animal Biotechnology

Genomics tools in animal breeding

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Keywords: SNPs; candidate genes; GWAS; disease; livestock

Integration of developing countries into international agricultural markets requires animal production systems to be competitive and this will demand to increase efficiency of animal production. Conventional selection schemes have been useful to increase productivity in livestock (milk, beef, wool, eggs) by using quantitative genetics and many changes in the productive systems. The animals that have the best combination of genes for a given trait has been accomplished by using pedigree information (parents, grandparents, cousins, and/or offspring). Nevertheless, the use of animal breeding schemes is difficult in many production systems because no pedigree information exists and the improvement under the quantitative model in many important traits (i.e. resistance to disease, traits with low heritability, traits limited to one sex; longevity, etc.) was not stimulant. The molecular and technological development in the last three decades became most of us astonished. Thomas H. Roderick in 1986 coined genomics as the discipline that studies the genome. The development of genomics was prompted by the availability of the human genome sequence and since then, the genome sequence of most livestock species is available. The new paradigm quantitative-molecular in animal breeding was growing and expanding the opportunities of using molecular techniques. As an example, the bovine genome has a minimum of 22000 genes, of which 14345 are orthologues shared among different mammalian species. The estimated size of the bovine genome is 2.87 Gigabases. The availability of high throughput sequencing techniques not only enabled the development of the genome sequencing projects for livestock, but also allowed for the development of microarrays with thousands of single nucleotide polymorphisms (SNP). Today there are commercially available several types/sizes of microarrays for cattle, sheep and goats. They allow the genotyping of individuals and the consequent inclusion and use them in paternity test, detect carriers of genetic diseases, genetic diversity, genome-wide association studies and in many others fields. In Argentina there are some large database in dairy cattle but limited to productive traits and pedigree. There was not database available that include for the same cow, productive, health, pedigree and genotype information. We studied somatic cell score (SCS) and others traits associated with mastitis in a Holstein and Holstein x Jersey population with a candidate gene and a genome-wide association study (GWAS) approaches. In the first study we identified two SNP associated with SCS and in the GWAS at least five chromosomal regions associated with different mastitis-related traits were detected. The other important disease in dairy cattle is the leukemia. Very high rate of positive cows are reported in many countries and there is only a few plan to stop transmission and a safe vaccine preventing BLV infection is not available yet. Even when most of infected animals courses BLV infection with no evident symptoms, dairy farms are adversely impacted by unregistered lost profits derived from deaths because of lymphosarcoma occurring in 5-10% of lactating cows. Using a candidate genes approach and a GWAS we found strong association between certain alleles of the bovine leukocyte antigen (BoLA) DRB3.2* gene and some SNPs affecting bovine leukemia virus infection level in dairy cattle. On the other side, small ruminant farming is a good means of livelihood improvement in most Latin American and the Caribbean countries but infection with gastro-intestinal parasite (GIP) incurs serious loss to farmers. Among the GIPs, Haemonchus contortus, is the most important ones in the northeast in Argentina. The situation is further compounded by the emergence of anthelminthic resistant parasites, which means the control of parasite by using chemicals will be more and more difficult. In this context, new options for breeding sheep are necessary to explore and can contribute to alleviate losses and chemical contamination. Breeding sheep for resistance to parasites would be a natural and sustainable method of controlling GIP; however, a good dataset on genotypes and phenotypes is a prerequisite for designing any good breeding program. We are using an artificial challenge larvae 3 protocol in lambs to measure several GIP phenotypic traits and productive (wool and growth) traits, too. In addition, we are genotyping animals with a set of candidate to immune innate response SNPs genes and the ovine 15K bead SNPs chip in order to use of molecular approaches to discover genetic variation underlying parasite resistance in sheep. At the same time as these valuable new genomic resources for animals are being used, livestock breeders in developing countries are facing real challenges in delivering more balanced breeding objectives that seek to broaden selection goals beyond traditional productivity traits to include sustainability and welfare traits such as disease resistance, robustness, reproductive efficiency and longevity. For the reasons already indicated, molecular genetic diagnostics used in selective breeding are most valuable for these traits but need to be country developed and validated. These can be considered cases which genomic research in developing countries should pursue the identification of causative mutations underlying larger genetic effects on sustainability traits, or the identification of very closely linked genetic markers that overcome the limitations of conventional quantitative and QTL- quantitative trait loci - for use in selection. This quantitative-molecular model works towards integrative and predictive biology approaches that improve the ability to move from genomic sequence to trait consequence.

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Keynote conference

Section I: Agricultural and Animal Biotechnology

Evaluation and regulation of the use of genetically modified plants in Latin America. Considerations

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Keywords:

Continuous advances in the understanding of molecular and cellular biology (in vitro culture) have allowed biotechnology to genetically modify viruses, bacteria and higher organisms. Undoubtedly, this has had an impact beyond the scientific sphere on the social order, which is the objective. It is to be expected that transgenesis arises a high level of caution in its application because of its novelty and the magnitude of the impact that it can have, which generates questions that must be scientifically argued to understand its safety for society and the environment. Therefore, it is essential to state and justify in each case the need for applying a genetic transformation to a crop, the safety of the crop, its genetic stability, and even its labeling as GMO to enable consumers freedom of choice. Since the 1990s, the use of genetically modified organisms has been studied, evaluated and regulated, mainly in relation to food and environment safety. However, at present the main economic blocs (United States, Europe, Latin America and Asia) have not reached an agreement, not even domestically, which could allow them to advance beyond unenthusiastic regional initiatives regarding regulations that may or may not exist and may or may not be applied. While it is true that there has been a consistency in agricultural and technological development in the countries of Latin America, which is leading the cultivation of GMOs due to relations with multinationals in the sector, it is also true that between the state and private sectors of these countries, there is a critical mass of qualified scientific personnel and laboratory structure that allow to reach a consensus to assess and regulate, even begin to address nationally research related with GMOs. The commercial approval of a GMO necessarily has to pass a period of evaluation in each country. Hence, a structure allowing the successful management of biosecurity must be created in each country. In the first stage of approval, all the information required must be provided to evaluate independently each event that presents the possible risks (General Information, Event Information, Agronomic and Biosecurity Information, Information on Establishments and Sowing Sites) by the different institutions, and once approved favorably, the exchange of information (Sowing, Progress and Closure Reports) and inspections to verify what has been approved are maintained, always having a contingency plan and additional commitments. Nowadays it is a necessity and a governmental obligation to promote social knowledge, and generate debate on this issue with scientific rigor and a global vision that allows arriving at favorable positions for all the parties.

Keynote conference

Section I: Agricultural and Animal Biotechnology

Advances in understanding the alpaca genome

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Keywords:

There are a few and isolated research efforts to study the alpaca genome. Avila et al (2015) reported an integrated cytogenetic map that includes 230 markers distributed along the 37 alpaca chromosome pairs. Two alpaca reference genomes are available at NCBI (Vicugna_pacos-2.0.2 and Vi_pacos_V1.0). However, the information is reported at scaffold level and not by chromosomes. Currently, microsatellite markers are used for genetic diversity analysis and paternity test in alpacas, and mitochondrial DNA for phylogenetic studies. However, modern markers like single nucleotide polymorphisms (SNPs) are scarcely known in alpacas. SNPs are now widely used in animal genetic improvement for cattle, poultry, pig, etc., where the identification of parents for the next generation is done by genomic selection. In the past two years, our research group has been characterizing alpaca SNPs based on the use of a high density BovineHD BeadChip–Illumina and an alpaca/hamster radiation hybrid cell panel. Our work has identified bout 50,686 bovine SNPs that are also present in the alpaca genome. Some of these SNPs have been ordered into 33 linkage groups and 12 genes have been assigned to chromosomes in alpacas using fluorescent in situ hybridization (FISH). Likewise, 50 candidate SNPs in the region of the KRTA and KAP genes were discovered with bioinformatics tools. A second research project seeks to find more SNPs by sequencing six alpaca genomes and 150 reduced libraries originating from 150 animals. It is expected that enough alpaca specific SNPs will be discovered to build an alpaca beadchip with at least 50,000 SNPs to enhance our understanding of the alpaca genome and of their association to phenotypic production traits.

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Keynote conference

Section I: Agricultural and Animal Biotechnology

Uso potencial de las biotecnologías reproductivas con células madre testiculares (SSC) en el rescate de la variabilidad genética de la alpaca peruana

Martha Valdivia Cuya

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Keywords:

Actualmente el desarrollo de biotecnologías reproductivas usando aislamiento, cultivo, criogenia y transplante de células madre espermatogoniales (SSC: Spermatogonia stem cell) abre nuevas expectativas para la reproducción animal en animales seleccionados. Las SSC denominadas también células madre adultas permiten la conservación de la fertilidad en organismos pre púberes (sin producción de espermatozoides) así como en organismos adultos. El balance armonioso de los procesos de autorrenovación y de diferenciación de las células madre espermatogoniales permiten el mantenimiento de la fertilidad en un animal adulto; estos procesos de autorrenovación y de diferenciación pueden llevarse a cabo in vivo e in vitro. En alpacas, se ha observado la dificultad en la colecta de semen de forma rutinaria, lo que aunado a la alta viscosidad del semen afectan el uso rutinario de programas de inseminación artificial en alpacas usando semen congelado. Ante esto, la criogenia de SSC pone en evidencia su utilidad y uso potencial en la conservación y rescate de la variabilidad de la alpaca usando bancos de SSC. Nuestras investigaciones en los últimos seis años han estado dedicadas a evaluar el uso de células madre testiculares de alpaca proveniente de animales adultos; en primer lugar el trabajo consistió en aislar células espermatogoniales, de testículos adultos post mortem, mediante dos digestiones enzimáticas con las cuales se ha logrado obtener suspensiones celulares y con estas células evaluar la presencia de SSC logrando identificarlas mediante genes marcadores como Zbtb16 e integrina ?1 mediante PCR y mediante citometría de flujo usando una sonda fluorescente específica para células madre (DBA). Estas herramientas nos han facilitado realizar cultivo y criopreservación de las SSC de alpacas; así como evaluar la calidad usando marcadores de actividad mitocondrial y apoptosis celular. Hasta el momento ya hemos generado un sistema de cultivo de las SSC de alpacas; dos protocolos para congelamiento de SSC uno para biopsias testiculares y otro para suspensiones celulares, los cuales han sido evaluados mediante citometría de flujo usando un marcador específico para SSC denominado FITC conjugated Dolichos biflorus Agglutinin (DBA). Asimismo, nuestro control de calidad de las SSC posdescongelamiento ha sido analizada mediante citometría de flujo, usando un sistema de Flow Cellect Mitopotential Red Kit identificando el potencial mitocondrial de las SSC mediante la sonda mitosense y la apoptosis celular con la sonda 7AAD.

Keynote conference

Section I: Agricultural and Animal Biotechnology

Línea de base molecular de la estructura poblacional de 9 tipos raciales locales de maíces amiláceos y posible flujo génico en zonas de coexistencia con cultivares híbridos de maíz amarillo duro

César López Bonilla[1], Jorge Luis Quispe Velasquez[1], Rosa Espejo Joya[1], Roberto Mansilla Samaniego[1], Enrique Fernandez Northcote[1], Gloria Gonzales Zeña[2]

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Keywords: SSR, AFLP, MAD, flujo génico, diversidad génica, polimorfismo.

El Perú, importante Centro de Diversidad Racial de Maíz; sin embargo, los estudios del estado de la diversidad sólo se desarrollaron a nivel morfológico, hace 60 años (Grobman, 1961). Hoy existe la ley de moratoria para OVMs que expira el 2021 y poco se ha avanzado es el desarrollo de capacidades y las líneas de base de muchos cultivos. Sin embargo, el consumo de maíz amarillo duro (MAD) ha aumentado, mientras que los rendimientos no, requiriéndose introducir semilla híbrida mejorada que podría ser GM o no. Para aportar en el desarrollo de las líneas de base y capacidades en bioseguridad, este estudio plantea el objetivo de estudiar molecularmente, el estado de las estructuras poblacionales de 9 tipos raciales del banco de germoplasma y como modelo la zona de coexistencia en Lambayeque. Del banco de germoplasma de la UNALM, se tomaron 200 granos de 9 tipos raciales amiláceos: 5 de costa (2 coexisten con MAD) y 4 de sierra, pero se vienen sembrando en costa para consumo como choclo. Además, se realizaron colectas de muestras en campos y lugares de venta de semillas de maíces Alazán y Mochero se están cultivando en Lambayeque. Además, se estudiaron 5 híbridos de MAD producidos por el INIA y 3 de Pionner. Se han constituido un total de 23 poblaciones, en las que hemos extraído el ADN con el método Doyle and Doyle (1990) con CTAB 2%, de 24 individuos por población. Se estudiaron 22 pares de cebadores SSR del Maize GDB y se eligieron 3 que produjeron mayor polimorfismo; además, se estudiaron 6 combinaciones de AFLPs, eligiéndose las 2 mejores. La visualización se realizó en geles de poliacrilamida al 6%; y, los datos se analizaron con los programas Pop Gene 32 y Arlequín 3.5. Tres provincias y 8 distritos de Lambayeque cultivan maíz; de los cuales Illimo, Túcume, Mochumí y Jayanca evidencian coexistencia con maíces Mochero, Alazán y Chancayano con MAD. Hemos encontrado que los maíces nativos de costa y sierra son muy polimórficos, con una diversidad génica de Nei superior a 0.50. con los SSR y hasta 0.25 con AFLPs. Además, comparten entre el 50% a 67% de alelos comunes con los tipos raciales del Banco de Germoplasma UNALM, pero en frecuencias génicas diferentes. Además, se evidencia que los maíces han experimentado eventos recientes de cuellos de botella, tal como lo indica el índice pequeño de Garza – Williamson. La alta variación alélica y la falta de coincidencia entre los maíces del mismo tipo racial, podría deberse a la manera como se mueve la semilla. Los agricultores venden a los mercados locales y estos a su vez abastecen de semilla para las campañas. En relación al estado de diferenciación alélica entre maíces nativos y MAD, no observamos los mismos alelos, indicando que no han ocurrido eventos de flujo génico a pesar de mantenerse en coexistencia por más de 70

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años. Según los resultados logrados, los maíces nativos a pesar de cultivarse en parcelas pequeñas y no habiendo muchos agricultores, se mantiene con un alto polimorfismo alélico, una alta diversidad génica y una diferenciación genética muy marcada de MAD, indicando que no ha ocurrido flujo génico en muchos años de coexistencia.

Keynote conference

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Contribution

Section I: Agricultural and Animal Biotechnology

Potato genes candidates for resistance to Globodera pallida, identified with ARNseq in the radicular transcriptome

De la Cruz, GF.1*; Carreño, H.2; Ponce O.2; Lozano, R3 ; Neyra, E2.

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Keywords: Nematodes, bioinformatics, differential expression, reads.

Globodera pallida is the plant-parasitic nematode with the major distribution in the Peruvian Andes, that affect potato and causes significant economic losses. We aim to identify potato´s resistance genes against this pathogen. In a first approach, 49 native Andean potato varieties were evaluated under the simple lattice design 7x7, were inoculated with 23 cysts of G. pallida (viability 378 juvenile-eggs/cyst)/experimental unit in field, identifying 46 resistant and 3 tolerant cultivars. In a second approach, two native Andean potato clones Maria Huanca (Solanum andigena) resistant clone (CIP 279142.12) and Chimbina Colorada (Solanum chaucha) (CIP 701013) susceptible clone to G. pallida were inoculated with juveniles-2 (J2) in roots .The experimental design for each potato variety was consisted of a control treatment without inoculation (Tc) and a treatment with G. pallida inoculation (Ti), each with 3 repetitions. Five inoculation points were determined at the level of the root elongation zone in each experimental plant. Total RNA was extracted from each of the 12 samples taken in the Tc and Ti after 72 hours post inoculation (hpi); these were sequenced using massive transcriptome sequencing techniques (ARNseq). Sequence screening using bioinformatic pipeline reported good quality reads, the mapping to the reference genome (clone DM1-3516 R44 genome version 3.0.34) reported 27,717 and 27,750 genes expressed in the resistant and susceptible variety respectively at 72 hpi (pvalue <0.05). When they were compared the differential expression of genes between the two varieties 91 candidate genes of resistance to G.pallida were obtained (considering: fold change ? 2 in up-regulated transcript, and fold change ? -2 in down-regulated transcript, pValue <0.05, opposite behavior of expression of genes) plus 9 considered R genes (fold change ? 1). It is concluded that there were differences in the expression of resistance genes NBS-LRR domain, genes related to metabolic pathways of plant hormones, signal transmission and cell wall formation.

Contribution

Section I: Agricultural and Animal Biotechnology

Viruses detection in arracacha (Arracacia xanthorrhiza) using Next-generation sequencing

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Keywords: crinivirus, vitivirus, sRSA

Arracacha (Arracacia xanthorrhiza Bancroft) is a root crop cultivated in several countries of South America, which commercial product (storage root) has many nutritive properties (such as high calcium content, vitamin A); moreover, its fine starch makes it easily digestible. Because there are very few studies in Andean roots and tubers, this work is focused on the characterization of sequences associated with viruses in arracacha samples by means of sRSA (small RNA sequencing and assembly), as well as to detect those virus-associated sequences by PCR in both arracacha and potato. Evaluations were performed on host plants and the viruses identified using the deep sequencing technology were molecularly characterized. For the analysis, the program VELVET was used to produce contigs using the small RNA reads obtained from next generation sequencing. These contigs were used to search for similarity in the GenBank database using BLASTx. Novel sequences related to crinivirus and vitivirus were identified in arracacha. Based on the genomic sequences obtained, primers were designed to amplify corresponding sequences from further arracacha accessions. The genomic organization of these sequences would confirm their viral activity. On the other hand, these sequences have been found in arracacha and potato plants collected in farmers' fields in Peru and Colombia; but attempts of mechanical transmission and by a number of insect vectors were negative. However, the genomic organization found in the sequences strongly indicates they are new viruses capable of infecting arracacha and potato. This work is of particular importance for the diagnosis when exchange of germplasm is made and for obtaining virus-free material, both for arracacha and potato, because they are viruses that may be present in both crops.

Trabajos presentados al ***I Congreso Internacional de Biotecnología e innovación (ICBi)***, 9- 12 de julio de 2018, Universidad Nacional Agraria La Molina, Lima, Perú.

Editoras:
Ilanit Samolski Klein
Maria Lucila Hernández-Macedo
Gretty Katherina Villena Chávez

Contribution

Section I: Agricultural and Animal Biotechnology

Time of conservation under refrigeration of gelified semen of alpaca (Vicugna pacos)

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Keywords: camelids, gelatin, gel, sperm, survival

Alpaca semen freezing technology is not yet available for routine use. While these investigations continue, it is necessary to explore other alternatives. The conservation of semen in solid medium or gel tested in other species, has been able to maintain viability for a few days. The objective was to evaluate the maintenance time of motility and membrane functionality of alpaca spermatozoa in medium gel at 5° C. A total of 18 ejaculates from 7 breeding males was randomly assigned to each of the 3 treatments. The first treatment (Gel) consisted in the centrifugation of samples at 4448 g for 7 min in PureSperm80® medium, then recovered, diluted, gelled in gel (280 ° Bloom) and kept in refrigeration at 5 °C. The second (Tris) It was centrifuged in Tris medium, gelled and preserved in a similar way to the previous one. The third (Control) was not centrifuged, but mixed with dilutor-Tris-yolk, and preserved as the first. All treatments were performed with 6 repetitions. Every day for 7 days, were evaluated both the individual motility as well as membrane functionality of alpaca spermatozoa. Using a general linear model of mixed effects with repeated measures were analyzed these sperm parameters. A rapid loss of motility was observed in the "Control" group, slow in "Tris" and slower in "Gel". Motility decreased as time went by in all treatments. A motility close to 50% at 48 hours had the Gel treatment. Regarding the response on membrane functionality, similar behavior has been observed. Although, "Gel" was close to 50% on the third day, "Tris" on the second and "Control" on the first day. Using gelled semen refrigeration it is possible to achieve motility and membrane functionality close to 50% at 48 hours of storage time.

Contribution

Section I: Agricultural and Animal Biotechnology

GC-MS analysis of Sanky oil (Corryocactus brevistylus BRITTON & ROSE) an endemic cactus from Peru.

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Keywords: cactácea, bioactive compounds, in vitro culture, oil extraction, gas chromatography.

Sanky (Corryocactus brevistylus BRITTON & ROSE) is an endemic cactus from Peru. The fruit is consumed fresh and is a source of bioactive compounds with high antioxidant capacity that can improve human health and nutrition. It is currently used in traditional medicine. The objective of this work was to determine by GC-MS the compounds present in the oil obtained from callus induced in vitro and sanky seeds. The protocol for obtaining calluses in vitro was performed using modified Murashige & Skoog (MS) medium and supplemented with auxins and cytokinins at 0.0, 0.6 and 0.9mg/L. the extraction of oil was done using a soxhlet extractor and n-hexane as solvent, the analysis of the present compounds in the oil was determined by GC-MS. A completely random design with three repetitions was used. In vitro callus induction was obtained on modified MS medium with 0.9 mg/L of ANA and 0.9 mg/L of Zeatin. The callus fresh weight was of 0.906 g and 0.36 g of dry weight. The oil concentration was 42.44uL and 10 uL in 0.1 g of dry and pulverized sample of callus and seeds respectively. GC-MS revealed the presence of eight biochemical compounds in callus and twelve in seeds, being Butylated Hydroxytoluene (BHT) the most abundant whit peak area of 94.4% in seeds and 93.5% in callus. BHT of natural origin was also found in leaves of Mesembryanthemum crystallinum, Trichilia emetica and Cytisus triflorus L'Hérit. BHT is a phenolic compound whose importance lies in its antioxidant capacity and Its use extends in cosmetic, food, pharmaceutical and farming industry. These results showed that, the seeds had higher relationship percentage to BHT than callus oil.

Trabajos presentados al ***I Congreso Internacional de Biotecnología e innovación (ICBi)***, 9- 12 de julio de 2018, Universidad Nacional Agraria La Molina, Lima, Perú.

Editoras:
Ilanit Samolski Klein
Maria Lucila Hernández-Macedo
Gretty Katherina Villena Chávez

Contribution

Section I: Agricultural and Animal Biotechnology

Antibiotic resistance of Salmonella Spp, Escherichia Coli in baby alpacas (Vicugna pacus) with and without diarrheal enteropathies

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Keywords: Alpacas, enteropathies, antibiotics, antibiotic resistance

Antibiotic resistance of Salmonella sp and Escherichia coli was evaluated. These bacteria were isolated from 300 samples of baby alpacas rectal swabs with enteropathy characteristics in 6 rural communities of Huancavelica-Peru. The analysis were performed from 10 to 60 days and the prevalence E. coli and Salmonella sp was determined by conventional microbiology (triple sugar-iron- agar [TSI], lysine iron agar [LIA], sulfur-indole-motility [SIM] and catalase). The antibacterial susceptibility test was performed by disk diffusion method. The antibiotics evaluated were: A-Gentamicin, B-Novomycin, C-Tetracycline, D-Enrofloxacin, E-Ampicillin, F-Amikacin, G- Ceftriaxone and H- Penicillin. In baby alpaca with diarrheal enteropathies positive were found the prevalence of 100% E. coli, 40.0% Salmonella sp, 40% E. coli-Salmonella sp and without positive diarrheal enteropathies 48.3% E. coli, 14.0% Salmonella sp and 9.3% E. coli-Salmonella sp. The inhibition halos (CMI) demonstrated that E. coli and Salmonella sp isolated from baby alpacas with diarrheal enteropathies were resistant to Ampicillin (10,4± 0.3), (9,3± 0.2); Novomycin (11,1± 0. 2), (11,2± 0. 1); Tetracycline (8,2± 0.1), (9,2± 0.3); Penicillin (9,1± 0. 4), (11,1± 0. 3); Gentamicin (10,1± 0. 4), (10,2± 0. 3ª). In strains without diarrheal enteropathies the CMI demonstrated resistance to Gentamicin (10,3± 0.1), (8,2± 0.1); Tetracycline (9,2± 0.4), (8,2± 0.4); Ampicillin (11,2± 0.1), (9,3± 0.2); Penicillin (10,2± 0. 4), (10,1± 0. 3). The Salmonella sp and E. coli isolates from baby alpaca with and without diarrheal enteropathies of the Huancavelica -Perú communities exhibited high resistance to multiple antibiotics usually available in the veterinary market.

Contribution

Section I: Agricultural and Animal Biotechnology

Isolation and molecular characterization of sulfur-oxidizing bacteria present in the blood of Anadara tuberculosa (Sowerby, 1833) "Concha negra"

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Keywords: Overexploitation, sulfur, microbial fauna, symbiotic relationship, soxB

The pustulose ark, Anadara tuberculosa, is an emblematic species of the East Pacific mangrove ecosystem. This specie has been ancestrally collected as a staple food and as a basic economic income for coastal communities. Most of natural stocks of A. tuberculosa are over exploited and some populations are close to collapse in several countries. The natural habitat of the A. tuberculosa is characterized by high sulfur concentrations due to the organic matter decomposition and the important variations of abiotic factors which probably stimulate the particular microbiota development that could be specific and would benefit its survival. It is known that some mollusks family (Lucinidae, Solemnydae) can establish a symbiotic relationship with chemo- and phototrophic sulfur-oxidizing prokaryotes (SOP), which allow them to transform the reduced sulfur compounds to sulfate using the acquired energy to fix inorganic carbon and transmit it to its host. In this context, the objective of this study was to investigate and detect SOP from the blood of A. tuberculosa by classical microbiology techniques using specific culture media and by polymerase chain reaction (PCR) targeted to the soxB gene that encodes the SOXB component of the enzymatic oxidase complex of the periplasmic thiosulfate. The presence of SOP had been evidenced in all blood samples by both culture dependent method and PCR. Six bacterial strains have been isolated and identified on the basis of their 16S rRNA sequences. Their DNA sequences analysis determined that they belong to the classes Bacilli (Bacillus megaterium, B. subtilis y Staphylococcus hominis) and Gammaproteobacteria (Acinetobacter lwoffi, Micrococcus flavus y Kocuria rhizophila). Furthermore, detect the soxB gene in B. megaterium and B. subtilis. Interestingly, these bacteria are classified as chemoorganotrophic, however, the physiological versatility of the microorganisms determines that the latterly mentioned classification is not strictly reliable since at the genetic level they are shown to have sulfur-oxidizing genes, which confirms that it is necessary to carry out molecular analysis targeted to specific genes in order to conduct more valid characterizations. Altogether these results suggest a symbiotic relationship between A. tuberculosa and SOP, and open the way for potential utilization of these SOP as probiotic for aquaculture and bioremediation programs.

Trabajos presentados al ***I Congreso Internacional de Biotecnología e innovación (ICBi)***, 9- 12 de julio de 2018, Universidad Nacional Agraria La Molina, Lima, Perú.

Editoras:
Ilanit Samolski Klein
Maria Lucila Hernández-Macedo
Gretty Katherina Villena Chávez

Contribution

Section I: Agricultural and Animal Biotechnology

Quantitative variation of characters associated to ploidy level in androgenic plants of aguaymanto (Physalis peruviana L.)

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Keywords: Pollen size and viability, Stomata size, Physalys peruviana

Aguaymanto fruit (Physalis peruviana L.) is an excellent source of vitamin A and it is a biofortified food to combat vitamin A deficiency (VAD) and reduce the risk of tuberculosis infection associated with VAD (Aibana et al., 2017). In order to generate pure lines with enhanced carotenoid content by double haploid technique. It is necessary to study the characters associated to ploidy level such as pollen viability, pollen diameter, chloroplast number per guardian cell, stomatal length and stomatal width. Our results showed that (a) Pollen viability was 83.83% (donor plant), 11.74% (androgenic caj01) and 89.22% (androgenic caj02). (b) Pollen diameter was 29.1135 ?m ± 0.168 (donor plant), 28.6716 ?m ± 0.10.75 (androgenic caj01) and 28.2304 ?m ± 0.1126. (c) Chloroplast number per guardian cell was 10.68 ± 0.18 (donor plant), 5.6 ± 0.11 (androgenic caj01) and 10.74 ± 0.24 (androgenic caj02). (d) Stomatal length was 37.9732 ?m ± 0.4248 (donor plant), 23.2972 ?m ± 0.2008 (androgenic caj01) 34.8190 ?m ± 0.2703 (androgenic caj02). (e) Stomatal width was 27.4513 ?m ± 0.2898 (donor plant), 18.9309 ?m ± 0.1101 (androgenic caj01) and 24.7987 ?m ± 0.1496 (androgenic caj02). The findings allow us to conclude that only androgenic (androgenic caj02). is a haploid. This work was supported by Innovate Peru under grant No 451-PNICP-BRI-2014.

Contribution

Section I: Agricultural and Animal Biotechnology

Analysis of genetic variability of Suri populations (Pterocnemia pennata) in three rescue centers from Puno and Lambayeque using microsatellite markers

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Keywords: Pterocnemia pennata, microstellites, molecular sexing, genetic diversity.

The Suri (Pteronemia pennata), an emblematic bird from Peruvian south, is categorized in danger of extinction by the Peruvian Supreme Decree and the IUCN red list, a factor that may be influenced by the diminution of its genetic diversity. In addition, the lack of a clear sexual dimorphism makes assisted reproduction difficult in a rescue center. The objective of this study was to analyze the genetic variability and to determine the sex of the population of Suri in Perú. The level of genetic variability of 66 specimens kept at rescue center at Sumac Kantati, PELT (modules Humajalso Tupala and Humajalso Chapuco) in Puno and at the Suri-Sican rescue center in Lambayeque, were analyzed by Short Tandem Repeats-Microsatellite and successfully sexed using the kw1 DNA marker. About Ram30, Ram 14 and Emu33 microsatellites, our results showed two alleles for both Ram30 and Ram14, otherwise only one allele for Emu33. The analysis of the captive populations showed a Ho of 0.223. In the total population, Ram14 and Emu33 showed a high frequency of homozygotes with 79% and 100% respectively, unlike Ram30 that showed a high frequency of heterozygotes with 85%. Additionally, we found that these populations are not in Hardy-Weinberg equilibrium (FIS = -0.575) and have a Nei index with a GST=0.008. This is the first study of genetic variability reported for Suri species in Peru so that we conclude that among the captive populations there is a low genetic differentiation in their allelic frequencies, however despite presenting low levels of heterozygotes, the locus Ram30 and Ram14 allows genetically selection of the best reproducers.

Contribution

Section I: Agricultural and Animal Biotechnology

Identification of reference genes from RNA-seq data in Lupinus mutabilis Sweet

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Keywords: RNA-seq, gene expression analysis, reference genes

RNA-seq is a high throughput transcriptomic technique that allows to study changes in gene expression. Transcript validation is required and it is done by semiquantitative RT- PCR and Real Time qPCR. The accuracy of these techniques

Trabajos presentados al ***I Congreso Internacional de Biotecnología e innovación (ICBi)***, 9- 12 de julio de 2018, Universidad Nacional Agraria La Molina, Lima, Perú.

Editoras:
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relies on the selection of suitable reference genes to avoid quantification errors. Thus, the development of RNA- seq is an opportunity for the identification of new stable transcripts under different conditions. The objective of this work is to identify reference genes from RNA-seq data of 12 libraries of Lupinus mutabilis (Sweet). The first selection criterion was a variation coefficient (<0.10) among all FPKM values per each gene, which allowed selecting 270 candidate genes. The second selection criterion was a p-value (<0.05) for equality of variances between subgroups and groups, where 23 candidate genes were obtained. Kudo et al. (2016) Genes Genet. Syst. 91: 111–125

Contribution

Section I: Agricultural and Animal Biotechnology

Preliminary results on implementation of CRISPR/Cas9 system for White Spot Syndrome Virus (WSSV) receptor-directed mutagenesis in Litopenaeus vannamei shrimp spermatids

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Keywords: Crispr/Cas9, Pacific White Shrimp, LvRab7, B-integrin, mutation, WSSV virus.

The white shrimp Litopenaeus vannamei production is an economic activity very important for many tropical countries around the world. However, White spot syndrome virus (WSSV) is the worst viral pathogen. This infection involves interactions between the virion and host receptors. The prevention of WSSV infection has been explored through various strategies based on vaccines, interfering dsRNA, probiotics, plant extracts, immunostimulants and genetic breeding. This last one showed to be more convenient and efficient in terms of results but very laborious during several generations with the selection of survivors against experimental infections. Based on information related to the molecular interactions between WSSV and its host, the mutation of virus receptors shrimp proteins has emerged as a potential new strategy to select WSSV resistant shrimps, CRISPR/Cas9 system being particularly attractive for his efficiency and simplicity. Two shrimp receptor genes have been selected (LvRab7, ?-integrina) and characterized at the DNA level to identify and characterize exon sites as the best candidates for directed-mutagenesis. Subsequently, optimal RNA guides have been designed and the corresponding plasmids have been acquired and massively produced. A protocol for in vivo transfection of male gametes (spermatids) has been optimized and a protocol for detection and characterization of induced mutations has been established on basis of next generation sequencing of targeted gene PCR amplicones from spermatozoid DNA extracted from control and treated shrimps. The first preliminary analysis of the NGS data are inconclusive; however, some of our samples present certain genetic modification clues. In addition, our analyzes also revealed a high variability in the LvRab7 gene of resistant shrimps resulting from a breeding program compared to non-resistant shrimps, converting it a possible marker of resistance. This work is original for the shrimp L. vannamei, but previous successful results have already been achieved for another species of shrimp and two other crustacean model species.

Contribution

Section I: Agricultural and Animal Biotechnology

Mutagenesis at the N-terminal end of the Cry1Ab toxin of Bacillus thuringiensis var. Kurstaki

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Keywords: bioinsecticides, Bacillus thuringiensis, toxicity.

Pesticidal proteins expressed by strains of B. thuringiensis include antifungal compounds, ? exotoxins, vegetative insecticidal proteins (Vip) and ? endotoxins that include Cry proteins (crystalline) and Cyt (cytolytic) proteins (Hofte and Whiteley 1989, Schnepf et al., 1998, OECD, 2007). Preparations containing microbial pesticides, including Cry proteins, that interact widely with each other to influence the toxicity and specificity of insects, thus generating their biocontrol (Schnepf et al., 1998, OECD, 2007). Site-directed mutagenesis is a molecular biology technique used to create point mutations in a DNA chain (Sandra et al., 1978), which allows to study the molecular processes. To design simple mutants S39C, T239C, R93C and D74C and double mutants S39C-T239C and R93C-D74C, to evaluate the integrity of the mutated Cry1ab protein. The mutated Cry1Ab proteins were purified ion exchange columns, quantified by the Bradford method and activated with trypsin by observing the presence of each mutated protein in acrylamide gels (SDS-PAGE). Single and double mutations could be introduced into the Cry1Ab gene, a 130 kDa band was evidenced in the Cry1Ab protoxins of the S39C and S39C-T239C mutants, the others did not present clear bands, the quantification of the Cry1Ab S39C toxin was 1, 84 ?g/?l in S39C-T239C is 2.26 ?g/?l, the activation of the protoxin was evidenced with a band of 55 kDa. We were able to design the single mutants S39C, T239C, R93C and D74C and double mutants S39C-T239C and R93C-D74C, we could only obtain, purify, quantify and activate the Cry1Ab toxin of the candidate S39C and S39C-T239C mutants for future oligomerization and toxicity assays in Manduca sexta larvae (hornworm).

Trabajos presentados al ***I Congreso Internacional de Biotecnología e innovación (ICBi)***, 9- 12 de julio de 2018, Universidad Nacional Agraria La Molina, Lima, Perú.

Editoras:
Ilanit Samolski Klein
Maria Lucila Hernández-Macedo
Gretty Katherina Villena Chávez

Contribution

Section I: Agricultural and Animal Biotechnology

"In vitro" culture of meristems of four varieties of Dianthus caryophylus L. "standard carnation"

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Keywords: survival, mortality, benzylamino purine, INDOL-3-acetic acid.

The objective of this investigation was the protocol elaboration for the introduction In-Vitro of 4 varieties of standard carnation for its propagation. Therefore, meristem culture of cuttings of 4 varieties of standard carnation (Virginia, Candy, Amapola and Ronja) was used. This material was obtained in the city of Huaraz and was selected from the meristem culture and the clean material of phytopathogenic agents was obtained, suitable for its massive spread. The experiment was carried out at the facilities of the Biotechnology Research Center of the UNPRG - Lambayeque (CIB). The sample was transported with 2 different concentrations of 6-Benzylamino Purine (BAP) and Indol-3-acetic acid (AIA), of which the response of the 4 varieties at these concentrations was observed. The survival and death rate of 2 treatments in the 4 carnation varieties was evaluated; these contained the Murashige & Skoog medium, sucrose (3%), glycine (2mg / L), agar (7g / L); varying only in the use and quantity of plant hormones. The treatment N° 1 (T1) was constituted of 6-Benzylamino Purine (3.0mg / L) and Indole-3-Acetic Acid (1.5 mg / L), Treatment No. 2 (T2) 6-Benzylamino Purine (5.0mg / L) and Indole-Induced Acid 3-acetic acid (2.5 mg / L). The results showed that treatment No.1 (T1) was the most appropriate for the sowing of meristems in the 4 varieties of Standard Carnation, exhibiting a mortality rate of 15% compared to a mortality rate of 57.5% for Treatment No. 2 (T2). The death by vitrification was also evaluated, and a high rate of vitrification in the 4 varieties of Standard Carnation for treatment 2 (T2) was observed with a total of 41 (51.2%) vitrified explants, compared to 7 (8) , 7%) in the treatment 1 (T1).

Contribution

Section I: Agricultural and Animal Biotechnology

Metabolic Profile of avocado (Persea americana) cv. Hass at physiological maturity from two harvest seasons

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Keywords: Primary and secondary metabolites, polar metabolites, fatty acids, tocopherols, phytosterols

Avocado cv. Hass is a fruit of great economic relevance for Peru, as it leads the export of fruits, due to its sensory, nutritional and functional attributes that are closely related to the type and quantity of metabolites present. These metabolites up to date have been little studied. The objective of this research was to characterize some of the primary metabolites (sugars, sugar-alcohols, organic acids and fatty acids) and secondary metabolites (phytosterols and tocopherols) in avocado cv. Hass from the Region of Lima at physiological maturity from two harvest seasons (May and July 2017 at 26.1% and 31.3% dry matter, respectively). A total of 10 avocados for each season were used for metabolomics analysis based on GC-FID, GC-MS / MS, UPLC-DAD and HPLC-Fluorescence platforms. The results showed that perseitol and mannoheptulose (seven carbon sugars) was the most predominant sugars, and ranged from 1.71 to 1.83 and, from 0.54 to 0.88%, dry weight (d.w.), respectively. Manoheptulose, sucrose and myo-inositol were found to be significantly higher (p <0.05) in avocados from the first harvest; while glucose and fructose showed higher contents in the last harvest. The main organic acids found were citric (ranged from 0.84 to 0.99%, d.w.) and malic (ranged from 0.31 to 0.88%, d.w), with the quinic, shikimic and succinic acids being found in smaller amounts; however the malic, quinic and shikimic acids decreased significantly in the last harvest. On the other hand, the predominant fatty acids were oleic and palmitic (ranged from 39.2 to 42.5% and 26.4 to 28.05%, respectively), followed in smaller quantities by linoleic, palmitoleic and ?-linolenic acids. The ?-sitosterol was the phytosterol with greater participation, followed in smaller quantities of campesterol and stigmasterol. Within the tocopherols, ?-tocopherol was the most abundant, being twice the amount of ?-tocopherol. In general, fatty acids, phytosterols and tocopherols did not show significant differences in concentrations between harvest seasons. The results indicate that the avocado cv. Hass is a fruit that has important functional metabolites; in addition, that the harvest seasons evaluated mainly influenced the content of polar metabolites: sugars, alcohol-sugars and organic acids which are involved in the biochemical processes associated to the ripening process of the fruit.

Trabajos presentados al ***I Congreso Internacional de Biotecnología e innovación (ICBi)***, 9- 12 de julio de 2018, Universidad Nacional Agraria La Molina, Lima, Perú.

Editoras:
Ilanit Samolski Klein
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Contribution

Section I: Agricultural and Animal Biotechnology

Optimization of culture medium for biomass production of Avibacterium paragallinarum serA

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Keywords: Infectious Coryza, optimization methods, Plackett-Burman Design, Box- Benhken Design.

Avibacterium paragallinarum is the etiological agent of Infectious Coryza (C.I.), a disease of the upper respiratory tract of chickens. The prevention of the disease is possible with the use of vaccines and antibiotics. Inactivated biomass is used in the preparation of the vaccine to immunize fowls against the disease; however, the cultivation is difficult because its nutritional requirements are very demanding. In this research, a culture medium was optimized by applying experimental design to produce Avibacterium paragallinarum biomass; the culture medium was designed evaluating three carbon sources, five nitrogen sources and three growth inducers reported by the literature. Two statistical designs were used: a Plackett-Burman design to determine the most significant components in the growth and a Box-Behnken design to define their concentrations. It was determined that Polypeptone, Peptone Protease, Meat Extract, Yeast Extract and chicken serum were the most significant components for the growth of Avibacterium paragallinarum ($p < 0.05$). Nutrient concentrations were optimized using the Box- Behnken design and response surface methodology. Using the optimized culture medium a colony of 2.25x1010 forming units per milliliter (CFU/ ml) was obtained. Components optimization of culture medium allows biomass with high colony count to obtain safe and effective vaccines against inf. Coryza.

Contribution

Section I: Agricultural and Animal Biotechnology

Evaluation of the Newcastle Disease Virus–Specific T Cell Proliferation in Vaccinated Chickens

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Keywords: Flow cytometry, vaccines, proliferation assay

Newcastle disease (ND) is an important viral disease of poultry causing elevated morbidity and mortality. A routine evaluation on the performance of the vaccines against ND is realized through the monitoring of the rising antibody titer in vaccinated chickens. However, a lack of correlation between the specific antibodies for the Newcastle disease virus (NDV) titer and the protection to the virus has been reported. The role of the cell-mediated immune response has also been reported and appears to contribute to decrease the disease and transmission potential, however the techniques that evaluate this arm of the immunity are less established. The purpose of this study is to evaluate the immune cellular response through the detection of NDV-specific memory T cells in vaccinated chickens using the CFSE T cell proliferation assay. In this technique, the cells are labeled with a fluorescent cell staining dye (CFSE) and the proliferation of the fluorescent cells is measured through flow cytometry. In order to standardize this technique spleen mononuclear cells (previously stained with CFSE) were stimulated with the mitogen Concanavalin A. Subsequently, the antigen specific proliferation was evaluated through the stimulation of spleen mononuclear cells from immunized chickens with a recall antigen (RA). The RA was prepared from a velogenic NDV (genotype XII), propagated in embryonated chicken eggs, concentrated by sucrose density gradient ultracentrifugation and inactivated by ultraviolet C irradiation. To evaluate the immunogenicity of the RA and the presence of memory T cells specific to NDV, we established two SPF chickens groups. One group (n=10) was vaccinated at 20 days of age by ocular route with a NDV vaccine strain (LaSota-106 pfu) and the other one of the same age was used as a control group (n=10). On day 20 and 27 post vaccination, five chickens of each group were sacrificed and spleen mononuclear cells were cultured for three days in the presence or absence of the RA. We observed that the index stimulation of CD8+ T cells was higher in vaccinated chickens (1.52 ± 0.24 vs 0.96 ± 0.15, $P<0.01$) on 27 day post vaccination, but not on day 20. We neither observed a difference on the CD4+ T cells. The CFSE T cell proliferation assay can be used to evaluate the cell-mediated immune response in NDV-vaccinated chickens. In addition, this technique could be used as a quality control in order to evaluate the effectiveness of vaccines against NDV.

Trabajos presentados al ***I Congreso Internacional de Biotecnología e innovación (ICBi)***, 9- 12 de julio de 2018, Universidad Nacional Agraria La Molina, Lima, Perú.

Editoras:
Ilanit Samolski Klein
Maria Lucila Hernández-Macedo
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Contribution

Section I: Agricultural and Animal Biotechnology

Post-thawing quality of spermatogonial stem cells (SSC) of alpaca (Vicugna pacos) proliferated in vitro

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Keywords: Cryopreservation of SSC, mitochondrial activity, early apoptosis, apoptosis, flow cytometry.

Peru has the largest reserve of alpaca in the world, which has great economic, cultural and biological importance. Because of the difficulties in reproduction of alpaca, a good quality of post-thawing spermatogonial stem cells (SSC) would allow maintaining genetic diversity and the potential to produce gametes by in vitro differentiation. The goal of this project was to evaluate the post-thawing quality of alpaca SSC proliferated in vitro. For this reason, alpaca testicles from twenty-one adult animals from 2 to 6 years were obtained from local slaughterhouse in Huancavelica city (Peru) and submitted to the Laboratory of Physiology of Reproduction of the FBS (UNMSM). The testicular cells (CT) from each individual, which contain the SSCs, were isolated by enzymatic digestion and evaluated to determine the initial vitality and cell concentration. An aliquot of the isolated CT was cultured, between 15 to 22 days, in STEMPRO medium (n=10) or DMEM (n=11) supplemented with growth factors to induce the proliferation of the SSC (FP). An aliquot of this FP was cryopreserved and analyzed after thawing and named Proliferated-Cryopreserved (PC). The other part of the CT was cryopreserved and cultured post-thawing in the same proliferation medium, called Cryopreserved-proliferated (CP). All samples were evaluated by flow cytometry, the percentage of SSC was determined with the specific marker DBA-FITC and the quality of living cells by the analysis of active mitochondrial potential, apoptosis and early apoptosis. The average percentage of SSC in the FP cells was 7.1% ± 1.2, which had a significant difference with the SSC of the CP cells (1.36% ± 0.4), but not with the PC cells (5.53% ± 1.2). The percentage of cells with active mitochondrial potential of the FP and PC showed no significant differences (79.6% ± 1.8 and 70.9% ± 5.1) and low percentage of apoptosis (9.47% ± 1.3 and 7.27% ± 1.82 respectively), while they differed statistically from the percentage of cells with active mitochondrial potential of CP (63.2% ± 4.7), which additionally, showed a higher percentage of apoptosis (19.3% ± 4.32). Furthermore, the three groups showed a low percentage of early apoptosis without difference among them. These results suggest that the in vitro pre-freezing proliferation of SSCs maintains the proportion and quality of post thawing cells unlike proliferation post- thawing.

Contribution

Section I: Agricultural and Animal Biotechnology

Development of downstream purification processes of recombinant glycoprotein G obtained from insect cells: a proof of concept

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Keywords: Bioreactor, Tangential Filtration, Affinity Chromatography, Workflow

The wide range of scientific and commercial applications of recombinant proteins is demanding an interest in the development of downstream processing (DPS) involved in the scale-up production. This study developed a proof of concept based on downstream purification processing using the workflow of harvest, tangential filtration, affinity chromatography and desalting. Three-liter Sf9 insect cells, cultivated in the bioreactor BIOSTAT B plus, produced histidine-tagged glycoprotein G (gG) from the supernatant culture at a concentration of 0.22 mg/mL. For the cell clarification, the tangential filtration was performed on the SARTOFLOW Advanced equipment using a 0.45 ?m Sartocon slice cassette at a flow rate of 20.5 l/h, and a Sartocon slice cassette of 5 kD was used for the protein concentration at a flow of 2.8 l/h. The aforementioned processes have been carried out with Sartorius equipment. Four-hundred milliliter retentate was obtained as a result of tangential filtration allowing the supernatant volume at 7.5-fold reduction. One-hundred milliliter retentate was incubated with a nonionic surfactant, Nonidet P-40, at 2% (V/V) for 60 min. and used for protein purification. The immobilized metal-ion affinity chromatography (IMAC) technique was used on AKTA Pure using a HisTrap Excel column, both carried out with General Electric equipment. Fractions went through a desalting process using a Spin-X UF-20 5K WMCO concentrator (Corning). All the processes were performed at room temperature. The design of this downstream purification processing workflow allowed the gG protein production with 84.6% of purity and 1.87 mg per each 100 mL of retentate. Further process improvement is necessary to evaluate the protein production efficiency and yield and implement a step of intermediate purification (ion exchange chromatography or hydrophobic interaction chromatography) to increase the purity level. The development of this downstream purification processing makes it possible to cover the demand for recombinant proteins used in future tests, regarding its function and application, and to understand the structure or generation of monoclonal antibodies for the development of immunological methods.

Trabajos presentados al ***I Congreso Internacional de Biotecnología e innovación (ICBi)***, 9- 12 de julio de 2018, Universidad Nacional Agraria La Molina, Lima, Perú.

Editoras:
Ilanit Samolski Klein
Maria Lucila Hernández-Macedo
Gretty Katherina Villena Chávez

Contribution

Section I: Agricultural and Animal Biotechnology

Bradyrhizobium sp. with high potential for phosphorus solubilization in Lupinus ass ballianus c.p Smith "jera"

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Keywords: acid soils, biodiversity, fertilizers

Legumes are very extractive in phosphorus (P) from soil; some grow up in acid soils like lupines, being microorganism's dynamics what allow its availability spite of limiting conditions. The aim of this research was to determinate if Bradyrhizobium sp. presents a higher melting potential of phosphorus in Lupinus ass ballianus c.p, Smith "jera" rhizosphere. Raised hypothesis was if Bradyrhizobium sp. presents a high ability to melt soil's P this could be done with phosphoric fertilizers. The study area were the slopes near the Parinacochas lagoon (3750 masl), Ayacucho. Very little of the floristic diversity of this area is known. Rhizosphere samples (L. ballianus) were obtained and physico-chemical and microbiological soil characterization analysis were determined. The results were: sandy loam texture, gravimetric moisture 13 %, pH 4.7, organic matter 5.23 %, P available 2.3 mg.Kg-1, microbial population: bacteria 6.8 x 106 CFU / g of dry soil (g.d.s.); fungi 1.2 x 105 CFU/g.d.s. and actinomycetes 1.65 x 105 CFU/g.d.s. Bradyrizobium sp. strains were isolated from active nodes of L. ballianus roots and cultured in Pikoskaya medium pH 5.5, choosing the strain with the highest phosphate solubilizing efficiency (PSE), strain "C". The P solubilization capacity was quantified in vitro inoculating 108 CFU "C"/mL for phosphate rock (PR), triple superphosphate (TSP) and diammonium phosphate (DAP). Also was added a treatment of phosphate solubilizing rhizosphere bacteria for PR (T) with 108 CFU /mL. It was found that the solubilization activity of P (ppm) for Bradyrhizobium sp. "C" was: PR (1.38), DAP (1.03), ST (0.73), however, with T (0.31). These activities of Bradyrhizobium sp. "C" were higher than total soil bacteria activity.

Contribution

Section I: Agricultural and Animal Biotechnology

Response of Andean Tarwi to inoculation with Bradyrhizobium strains isolated from a wild lupine

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Keywords: Lupinus, Bradyrhizobium, rhizobacteria

The plant called "Tarwi or chocho" (Lupinus mutabilis Sweet) is a legume edible grain native to the Andes, valued for its high protein content and resistance to pathogens. The cultivation of this legume is maintained in different production systems from Ecuador to Chile and the northeast of Argentina and its great genetic variability is enhanced by the presence of about 70 wild species. There is bound to be an interspecific cross between L. mutabilis and wild lupins, and the evidence is the great morphological variation and certain properties related to pathogen resistance. Furthermore, the species that grow at higher altitudes would have cold-resistant genes potential to adapt the cultivated specie. In addition this lant have the capacity to fix nitrogen through their symbiotic bacteria. Therefore, the present investigation was performed to show if the symbiotic bacteria of wild species are able to nodulate the cultivated specie. In this sense, nodules of Lupinus cf lindleyanus were collected in the zone of Huaraz (Ancash) at 3500 ma sl. 8 slow-growing bacterial strains (6-7 days) were isolated in YEM medium, and three of them were chosen (LSHZ-L1, LSHZ-L3 and LSHZ-L5) for total DNA extraction, amplification of the 16S rRNA gene, and taxonomic identification. According to the EZtaxon database the strains had a percentage of similarity of 99.64% with Bradyrhizobium cytisi; 99.71% with B. ottawaense and 99.78% with B. canariense respectively. The inoculation assay was carried out in Tarwi seedlings from Aija (Ancash-Peru); the test was performed under greenhouse conditions for 70 days, in which the temperatures fluctuated between 17-25°C. The substrate vermiculite and sand in a ratio of 3:1 were used in 1L pots. A completely randomized design was applied with 5 treatments (3 strains, N+ control and N- control) with 12 repetitions. The results showed that the three inoculated strains were able to nodulate L. mutabilis. Regarding the growth of the plants, strain LSHZ-L1 increased the length of the aerial part and the number of branches respect to N- control. However, the three strains studied increase the dry weight of the plant compared to the N- control. It is necessary to continue with this study to have a bank of strains with potential to improve the nitrogen fixation and yield of L. mutabilis at the field within a sustainable management. Funded by Project 177-FONDECYT 2015

Trabajos presentados al ***I Congreso Internacional de Biotecnología e innovación (ICBi)***, 9- 12 de julio de 2018, Universidad Nacional Agraria La Molina, Lima, Perú.

Editoras:
Ilanit Samolski Klein
Maria Lucila Hernández-Macedo
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Keynote conference

Section II: Industrial and Environmetal Biotechnology

Biorefinerias basadas en microalgas para la producción de compuestos bioactivos

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Keywords: bioactivos, microalgas, biomasa

A pesar del gran potencial de los productos derivados de las algas, su implementación a nivel mundial, sigue siendo limitada debido principalmente a los costes desfavorable de producción. Por lo tanto, las microalgas se están aplicando solo en nichos de mercado como complementos alimenticios y productos cosméticos. Sin embargo, tienen un gran potencial como fuente de compuestos bioactivos debido a su alta productividad en comparación con otras fuentes de biomasa, y la posibilidad de ser producido utilizando solo agua marina y desechos (aguas residuales y gases de combustión) como fuente de nutrientes. El costo de la producción de microalgas es actualmente alto. Sin embargo, por utilizar las aguas residuales como fuente de nutrientes, mejorar la productividad y la solidez de los sistemas de producción, y maximizando los ingresos de la microalga completa, se demuestra que los sistemas económicos viables son técnicamente alcanzables con las tecnologías actuales más avanzadas. El proyecto SABANA tiene como objetivo desarrollar una biorefinería sustentable basada en microalgas de hasta 5 hectáreas, mediante el uso de agua marina y recuperar más del 90% de los nutrientes de las aguas residuales, al tiempo que produce un valor agregado como bioestimulantes, bioplaguicidas y aditivos para alimentos acuícolas, además de biomasa valiosa para biofertilizantes y alimentos acuícolas (hasta 70 t / ha · año). El resultado es un proceso de cero emisiones. La producción de alimentos para animales terrestres no se tiene en cuenta porque la regulación prohíbe la utilización de desechos de animales terrestres para producir alimentos para animales terrestres. SABANA se basa en la amplia experiencia demostrada de los socios en la producción de microalgas en agua de mar, y en la utilización de aguas residuales y estiércol de cerdo como fuente de nutrientes y la demanda de mercados de este tipo de productos y procesos de tratamiento de aguas residuales. Las microalgas se utilizarán para recuperar nutrientes (carbono, nitrógeno y fósforo) de las aguas residuales (aguas residuales de usos urbanos, centrarse en la digestión anaeróbica de lodo activado y estiércol de cerdo de la producción ganadera), evitando la necesidad de usar fertilizantes químicos que presenten una alta presión de sostenibilidad sobre el medio ambiente, mientras al mismo tiempo, obtener un beneficio del tratamiento de estas aguas residuales utilizando menos energía que la asociada a procesos de tratamiento convencionales. Se producirán y evaluarán cepas marinas y de agua dulce, aunque las cepas marinas serán priorizadas. La utilización de microalgas para el tratamiento de aguas residuales permite alcanzar energía (procesos positivos), que consumen menos de la mitad de la energía requerida en los tratamientos convencionales, a la vez que reducen el costo del tratamiento de aguas residuales a menos de la mitad de los sistemas convencionales, y aumentar la sostenibilidad del proceso mediante el ahorro de emisiones de gases de efecto invernadero (CO2, N2O). Mejora de la biología e ingeniería de los sistemas de producción permitirán lograr procesos estables de producción a escala industrial, maximizando la productividad y producción de biomasa estable a bajo costo para cumplir con los requisitos industriales. La biomasa producida contiene productos de alto valor, bioestimulantes, bioplaguicidas y aditivos alimentarios que serán extraído por procesos de extracción suaves y energéticamente eficientes. Estos productos son muy demandados por los mercados, como se ha demostrado por el hecho de que las grandes empresas que participan activamente en este mercado en crecimiento. Los reguladores del crecimiento de las plantas se prevé que el mercado alcance un valor de 1.910 millones de USD en 2020, mientras que el mercado de bioplaguicidas debería alcanzar los USD 6,60 mil millones para 2020, y para la pre mezcla de alimentos acuícolas se prevé que alcance los 10,26 mil millones de USD para 2020. La biomasa sobrante contiene tanto como 40% de proteínas y 30% de lípidos además de carbohidratos, serán utilizados para producir biofertilizantes por hidrólisis enzimática y alimentos acuícolas para la acuicultura porque este sector necesita un suministro estable, seguro y permanente de aceite de pescado y proteínas baratas para el desarrollo de los peces. Los biofertilizantes globales se espera que el mercado alcance los 1,88 mil millones de dólares en 2020, mientras que el mercado de pre mezclas se estima que alcance los 10,26 mil millones de USD para 2020, el requisito de que el aceite y la proteína sean inocuos para la alimentación es una cuestión fundamental para la sostenibilidad en producción acuícola. Cuando se utiliza agua dulce y fertilizantes artificiales, el costo de producir microalgas es alto (> 5 € / kg de masa seca), pero al usar aguas residuales y gases de combustión, este costo de producción puede reducirse en un orden de magnitud (<0,5 € / kg). SABANA producirá microalgas recuperando hasta 90% de nutrientes (C, N y P) de efluentes de aguas residuales, abriendo así nuevas vías para obtener beneficios del tratamiento de aguas residuales La biomasa de microalgas producidas se evaluará para garantizar los requisitos de seguridad y calidad (microbiología, composición bioquímica, bioactividad, nocividad). Tecnologías de cosecha con baja potencia el consumo (<0.1 kWh / m3) se desarrollará, luego la biomasa será procesada por leve / eficiencia energética métodos de extracción (<0.5 kWh / kgdw) para obtener extractos de valor agregado (bioestimulantes, bioplaguicidas y alimentos acuícolas) aditivos), y biomasa residual adecuada para ser utilizada para producir biofertilizantes y alimentos acuícolas, a tasas de producción hasta 30 t / mes.

Trabajos presentados al ***I Congreso Internacional de Biotecnología e innovación (ICBi)***, 9- 12 de julio de 2018, Universidad Nacional Agraria La Molina, Lima, Perú.

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Ilanit Samolski Klein
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Gretty Katherina Villena Chávez

Keynote conference

Section II: Industrial and Environmetal Biotechnology

Bioethanol: the Brazilian experience

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Keywords: bioethanol, enzymatic hydrolysis, sugarcane bagasse

The use of bioethanol as a biofuel in Brazil goes back to the second world war, when the importation of gasoline and diesel from the USA was severely reduced. To continue running the cars and trucks, some sugar mills in the São Paulo State started to convert the engines to run in ethanol. This was mainly done to continue the transportation of sugarcane from the field to the mills. Only in 1975, following the oil crash, the Brazilian government started an official program given incentives to car owners to convert their engines from gasoline to bioethanol. The subsidies given to the bioethanol industry in the first years of the program, and the easy acceptance by consumers of cars running on bioethanol, allowed a fast increase of this biofuel production. The government not only allowed the new engines to run on pure bioethanol, but also added 20% of this biofuel to gasoline. New sugar mills were built with the capability to produce both, sugar and bioethanol, representing a major boom to the sugarcane business. Major progresses were observed in the sugarcane industry, with the average productivity reaching 80 tons/ha. The state of São Paulo became the main sugarcane producer, being responsible for almost 60% of the whole production, with the northeast and central states of Brazil responding to the other 40%. The success of the bioethanol program remained until the end of the 90's, when the price of gasoline started to be highly competitive with bioethanol, and the sudden cancelation of the subsidies to the sugarcane industry, by the central government. Such miscalculated action, led to a sudden reduction of bioethanol production, frustrating consumers, which switched back to gasoline. In 2003 a new development in the car industry, allowed the production of engines that could run on either bioethanol or gasoline, or a mixture of both. With this new flex engines, consumers quickly adopted the new technologies, and another boom in the sugar industry started. This time without subsidies. Today, the flex engines are well accepted in Brazil and the content of bioethanol in gasoline was raised to 27%, without any complain by consumers. The price of gasoline and bioethanol is the key to the success of this program, and the experience is that the market should regulate the prices. This way, consumers can choose either gasoline or bioethanol. The Brazilian experience of bioethanol is highly successful and is now leading to a new era, with the perspectives of producing bioethanol not only from the sugar fermentation, but also from the fermentation of sugars derived from the enzymatic hydrolysis of sugarcane bagasse, the so called E2G (second generation bioethanol). It is estimated that using the bagasse as a source of sugars, the bioethanol could be increased by an extra 30%, approximately. Today the first-generation bioethanol production in Brazil is around 27 billion litres, which could reach up to 35 billion litres, with the addition of E2G, without the need to expand the sugarcane plantation area. One major concurrent for bagasse is bioeletricity. Depending on the price of bioelectricity, the sugar mills prefer the production of bioelectricity by burning bagasse in the steam turbines, instead of using for cellulosic bioethanol. The presentation will focus on the new perspectives of bioethanol production and the development of hybrid engines, hydrogen production from bioethanol, the competition with bioelectricity and the development of the new alchoolchemical industry, or the "green chemistry industry".

Keynote conference

Section II: Industrial and Environmetal Biotechnology

Challenges in the Implementation of Agro?Industry by-Products based Bio-Refineries

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Keywords: Agro?Industry, Bio-Refineries, Lignocellulosic material

Agro-Industry by-products are an important source of renewable raw materials that can be reused and recycled for the production of energy and added value compounds providing an answer to the challenges of a Circular Economy. However, so far, the use of these by-products has been focused on the production of energy (mainly biofuels from the cellulose fraction or thermal energy) and not under a bio-refinery approach. The implementation of Agro?Industry by-Products based Bio-Refineries will contribute for the creation of a Circular Economy - processes efficiency and economics will be improved, new products generated for application in energy, cosmetics, food, pharmaceutical and chemicals as well as new business opportunities and more jobs. Lignocellulosic material is the main component of several agro-industry by-products but other complex structures including starch and pectin also exist as well as simple molecules such as soluble sugars, proteins and phenolic compounds. The recalcitrant nature of the lignocellulosic fraction demands that pre-treatments are applied for its fractionation for further transformation. The other components do not have as strongly linked structure as the lignocellulosic fraction being easier to fractionate. On the other hand, these soluble compounds that are straightforward to fractionate may degrade at high temperatures and pressures or prolonged times. Green and innovative processes are required to replace conventional extraction processes that are energy consuming, use solvents and large amounts of water producing undesirable residues. The main challenges related with the processing of different agro-industry by products for the development of bio-refineries will be addressed with a particular focus on the processing of the lignocellulosic fraction and on the development of new and greener technologies for the extraction of valuable compounds.

Trabajos presentados al ***I Congreso Internacional de Biotecnología e innovación (ICBi)***, 9- 12 de julio de 2018, Universidad Nacional Agraria La Molina, Lima, Perú.

Editoras:
Ilanit Samolski Klein
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Gretty Katherina Villena Chávez

Keynote conference

Section II: Industrial and Environmetal Biotechnology

Present and Perspectives of Industrial and Environmental Biotechnology in Peru

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Keywords: algae biotechnology, fisher waste, waste treatment.

Biotechnology is the most important technology and the highest level of innovation in the world, use living organisms to obtain a useful good or service for human being. Biotechnology and the sustainable use of biodiversity, specifically genetic resources and their derivatives represent a unique opportunity to improve competitiveness and contribute significantly to the country's socioeconomic development, based on the conquest of new markets for high-value-added products that are intensive in innovation and development. Its promotion implies a fast growing alternative, which can translate into significant economic and social gains. However, currently the national scientific and technological production is scarce and fails to respond to the many challenges that this development implies. Based on successful experiences in other countries, examples of how biotechnology would favor each priority sector are postulated, however applications in Peru, even using advanced technologies, do not go beyond small-scale efforts, often only at an academic level. Peruvian companies do not use biotechnology to improve their products or processes, nor do they see an industrial level. Different types of biotechnology have developed over the time: Ancestral people began with the traditional one; doing fermentation with microorganisms, also inducing artificial selection, and directed mating to obtain the domestication of some species of flora and fauna. The conventional one is based on the scientific method and includes techniques such as genome sequencing, reproductive technologies or embryom transfer. Lastly, the modern biotechnology uses genetic engineering to obtain genetically modified organisms that is transgenic. In sense, biotechnology and its applications have a key role in development of a country. Blue Technology is called to the applications of biotechnology in aquatic environments (marine and inland waters). It deals with the exploitation of aquatic organisms in order to create new products that favor the fishing sector, therefore the human being. Even at an early stage of development, its applications are promising for aquaculture, health care, cosmetics and food products. The development of the fishery industry in our country at an artisanal and industrial level generates a large amount of waste and losses in the management, storage, distribution and commercialization, which represent around 29 million tons of waste worldwide. Peru has a wide marine diversity, it has around 800 species of fishes, 900 species of invertebrates (mollusks, crustaceans and echinoderms) and 210 species of algae, generating interest throughout the world. In 2014, the extraction of hydrobiological resources from the sea was about 5 million tons of fish and 650 thousand of invertebrates and 20 thousand tons of algae, in the other hand, the extraction from inland area was about 85 thousand tons of fish. The waste generated of all the hidrobiological resources is 30-50% of their weight. It is necessary to use the tools of biotechnology to be able to eliminate these solid and liquid wastes, converting them into new products to be used in the industry, thus reducing environmental pollution. Peruvian Technological Production Institute (ITP), that belongs to the Ministry of Production has begun using biotechnologies technics with fish and mollusk waste. For fish waste (skin, scales, viscera, gills, spines) they have done a "biological silage", which is a conservation method based on acidification, produced by lactic acid bacteria (BAL) and the hydrolysis of proteins produced by proteolytic enzymes. They reach their highest activity when the pH = 4, inhibiting the growth of putrefactive and pathogenic bacteria. Scallop (Argopecten purpuratus) is the main bivalve mollusk, which 98% of the total volume is exported, mainly to United States and Europe, and the rest 2%, is consumed inside the country. The edible part of this species is the adductor muscle and the gonads that corresponds the 20% of the total weight, the remaining 80% is considered solid waste, this is composed of 70% valves and 10% mantle, viscera and gills. Because of its economic importance, this species is cultivated in several places of the coast of Peru, but in greater quantity in Piura (the northern part of Peru). These waste are left in the dumps, generating severe environmental problems in Sechura area, accumulating annually around 25,000 MT of shell waste. Currently there are several projects to use this subproduct. ITP prepares the biological silage (EB-RCA) of pasty consistency, brown color and pleasant smell, obtained from the fermentation of fishing by-products and the addition of cane molasses and lactic acid bacteria (BAL). This product is used in animal feed for its good quality protein and also helps to reduce a problem of environmental pollution. On the other hand, pilot experiments using biotechnology have shown that calcium carbonate from empty valves can be used to make concrete for construction. Students at the Fishery Faculty of UNALM have done research in some area of biotechnology. It is: (1) Use of paiche skin (Arapaima gigas) to obtain collagen. (2) Obtaining peptides with antioxidant capacity by enzymatic hydrolysis of cooking liquor from anchovy canned (Engraulis ringens). (3) Obtaining gelatin from perico skin (Coryphaena hippurus) and characterization of its physicochemical properties. (4) Elaboration of sweet flake from the ground mantle of pota (Dosidicus gigas) with quinoa (Chenopodium quinoa). (5) Preparation of liquid biofertilizer using byproducts of trout processing (Oncorhynchus mykiss). Biofuels have been obtained with microalgae as well as products for the cosmetics industry, at pilot levels in the laboratory. Currently, a more economical methodology is sought for obtaining these products at an industrial level. With macroalgae, in the case of the tropical algae Caulerpa filiformis, which inhabits the northern part of the country, it is a resistant species that due to its type of vegetative reproduction, has invaded cooler temperatures in the coastal zone, apparently due to the presence of the spores in the shells of the shells that moved from north to south for their cultivation. C. filiformis grows in abundance competing with the species native to the area, currently without a commercial value; however, a potential use has been found as a source of active principles and it has been possible to obtain new products such as biofertilizers by fermentation with lactic bacteria. A liquid biofilm was developed through the silage process of a microbial consortium of lactic acid bacteria to decontaminate the eutrophication caused by the "green tide" product of the large populations of Ulva lactuca (Chlorophyta), in the tourist spot the Poza de la Arenilla – Callao. Transcriptomic analysis of the brown algae Macrocystis integrifolia (Phaeophyceae), showed that there is the differentially expressed genes between individuals from the intertidal - 0m depth (Mi0m) and subtidal - 10m depth (Mi10m). 9,519 unigenes expressed differentially between Mi0m and Mi10m among which 271 upregulated and 264 downregulated genes are significantly altered. To find out the possible molecular mechanisms involved in the stress responses, manually was classified and examined several genes that exhibited major significant changes in the stress conditions (Mi0m) compared to the Mi10m control. At the laboratory or pilot level, there are investigations related to industrial and environmental biotechnology in the fisheries and aquaculture sector. However, it must continue with the applications of these at an industrial level. For this, it is essential to form more human capital and have a greater commitment to the sectors that pollute in some way. This requires the support of the Ministry of the Environment as well as the Ministry of Production.

Trabajos presentados al ***I Congreso Internacional de Biotecnología e innovación (ICBi)***, 9- 12 de julio de 2018, Universidad Nacional Agraria La Molina, Lima, Perú.

Editoras:
Ilanit Samolski Klein
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Contribution

Section II: Industrial and Environmetal Biotechnology

Culture-dependent and -independent analysis of bacterial communities' composition in petroleum-contaminated and uncontaminated areas in Nieva, Amazonas

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Keywords: Bacteria, Metagenomic, NGS, Hydrocarbons, Jungle

The oil industry is one of the pillars of the Peruvian economy but it faces challenges due to chronic and eventual hydrocarbon spills which threaten the environmentHydrocarbon-degrading and tolerant microbial communities, particularly bacteria, are usually present in these petroleum contaminated areas and display mechanisms for eliminating hydrocarbons from the environmentThe identification of these microorganisms is therefore crucial for bioremediation purposesHowever, the culture of environmental microbes is hampered by a large number of cells that are not readily cultivatedRecently, culture-independent metagenomics approaches are being successfully applied, opening up access to untapped genetic resourcesIn this context, the aim of this study was to characterize bacterial communities, by culture-dependent and independent techniques, from hydrocarbon-polluted soil and water due to the oil spill in Nieva, Amazonas, august 2016The culture-independent analysis was based on Illumina Next Generation Sequencing targeted to the 16S rRNA gene using metagenomic DNA extracted from hydrocarbon contaminated and uncontaminated soil and water samplesSequences were processed and analyzed using QIIME1.9.1softwareThe culture-dependent analysis was performed on bacterial isolates in Trypticase Soy Agar and Minimum Salt Medium supplemented with 1% petroleumIsolated bacteria were characterized by partial sequencing of the 16S rRNA gene (universal primers, Lane 1991) and BLAST database analysisMetagenomic analysis showed 139 operational taxonomic units (OTU) at the level of the genus for contaminated soil and 161 in contaminated waterSimilar analysis carried out on uncontaminated soil and water led to establishing a very different bacterial community composition with 160 and 183 OTUs, respectivelySome genera were identified as biomarkers of hydrocarbon contamination due to their high proportion in contaminated samples, like Acinetobacter and PseudomonasThe culture-dependent method allowed the isolation and identification of 6 and 7 bacterial strains from contaminated soil and water samples, respectivelyPseudomonas and Proteus genera were found in both sample types; Serratia and Morganella in soil; Enterobacter, Klebsiella, and Acinetobacter in waterIt is interesting that some cultivable bacteria isolated through the culture-dependent method were detected in a very low prevalence through the metagenomic analysis, showing the biases related to traditional culture- dependent methodsMetagenomics is consequently the method to be selected for the assessment of the impact of contamination as well as for assessing in situ bioremediation processes through monitoring of pollution-degrading bacteria.

Contribution

Section II: Industrial and Environmetal Biotechnology

Optimization of the environmental parameters of the fermentation of kluyveromyces marxianus NRRL-Y-7571 for the production of inulinase

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Keywords: yacon extract, Plackett & Burman

The inulinase (2.1 - ?-D fructan fructanohydrolase (EC 3.2.1.80)) an enzyme that catalyzes the hydrolysis of inulin to high purity fructose syrup, in a single reaction stepIt also has activity to catalyze the fructooligosaccharides synthesisThe overall objective of the study was to determine the optimal values of the environmental parameters for inulinase production, during the Kluyveromyces marxianus NRRL-Y-7571 fermentation, in raw brothThe experimental strategy of optimization was based in four sequential stepsthe Plackett & Burman design, the rotational central composite design, the surface response methodology and the optimized conditions validationIn the first stage, it worked with nine independent variablesSource of carbon30 – 60 % (v/v); source of nitrogen (NH4)2SO4 5 – 10 g/L; growth factor, type of carbon sourceextracts of yacon and asparagus; metal ion; inoculum size; initial pH; temperature and agitation speedIn the second stage was optimized through the complete factorial design for three variables selected in the first stageThe statistical results were obtained using the Design Expert and Statistica programs respectivelyThe experiments were carried out in an orbital shaker, in batch cultures in 250 mL flasks containing 50 mL of mediumThe inulinase and invertase activities were determined by measurements of initial velocity on a standard solution of 10 g/L inulin and 20 g/L sucrose, respectivelyThe optimum values of environmental parameters were26,2 % v/v yacon extract, 0,386 g/L MgSO4.7H2O and 4,3 % v/v inoculum size, corresponding to a predicted maximum inulinase activity of 2,2 U/mL and invertase activity of 93,6 U/mLIn the validation, activities were achieved, inulinase 2,32 U/mL and invertase 78,3 U/mL, confirming that the predicted model of optimization was adequate to describe the experimental designIt was concluded that using an optimization experimental plan was possible to determine the optimal environmental parameters for a producing high of inulinase by Kluyveromyces marxianus NRRL-Y-7571.

Trabajos presentados al ***I Congreso Internacional de Biotecnología e innovación (ICBi)***, 9- 12 de julio de 2018, Universidad Nacional Agraria La Molina, Lima, Perú.

Editoras:
Ilanit Samolski Klein
Maria Lucila Hernández-Macedo
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Contribution

Section II: Industrial and Environmetal Biotechnology

Evaluation of physicochemical and microbiological characteristics of tocosh during fermentation

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Keywords: andean food, fermentation-putrefaction, Lactobacillus.

Tocosh is the product of a fermentation-putrefaction process, during which physicochemical changes occurIt is an energetic food due to its origin, considered as functional by the Andean inhabitants, its consumption continues increasing even as a drink called serumIn this research, physicochemical characteristics and predominant microbial growth were evaluated during fermentation of tocoshTwo varieties of potatoes, Yungay and Peruanita, were selected, washed, weighed and fermented in pots of mud and straw in order to simulate the natural conditions of tocosh productionMoisture, pH and acidity percentage were determined as intrinsic factors of the development of viable aerobic mesophilic microorganisms (ICMSF, 2000), molds and yeasts (ICMSF, 2000) and lactic acid bacteria (de Man, J.C, Rogosa M., Sharpe M.E., 1960), Lactobacillus acidophilus were also identifiedThese counts were carried out every 15 days during a two-month periodIn addition, the amylolytic (halos of hydrolysis) and proteolytic activity (Nessler test) in the substrate was determinedThe experiment was repeated three timesResults were processed by one-way analysis of varianceDuncan's test at $p < 0.05$ were considered as significantThe moisture increased 22.16%, the pH decreased from 5.5 to 3.6 and the acidity increased from 0.088 to 0.485% lactic acid, these conditions allowed the development of Tocosh-associated microorganismsThe viable mesophilic microorganisms increased from 18 x 103 to 36 x 106 cfu g-1, the fungi and yeasts increased from 24 x 10 to 44 x 103 cfu g-1 but decreased at the end of the stage to 50 x 10 cfu g-1The lactic acid bacteria (LAB) increased from 15 x 103 to 66 x 108 cfu g-1Lactobacillus acidophilus, one of the species in LAB group, increased from 102 to 36 x 104 cfu g-1The highest increase of LAB was at the end of the fermentation from 45 to 60 daysThere is no significant difference between the characteristics of the potato varieties in all the processAmylolitic activity, halo of hydrolysis from 9 mm to 13 mm was observed, due to pH and acidity variation and the proteolytic activity (oxidative deamination and decarboxylation) was detected due to ammonia formation (Nessler reagent positive test) as well as, biogenic amines, probably produced during the tocosh fermentation process.

Contribution

Section II: Industrial and Environmetal Biotechnology

Studies on the microbial degradation of cyanideA functional, structural and bioinformatic approach.

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Keywords: CynD, cyanide dihydratases, Bacillus, dynamics simulation, 16s rRNA sequencing.

Cyanide is a toxic compound used in industries including mining, plastics and agriculturalThe usefulness and toxicity of cyanide is based on its capacity to form metal complexes; this feature allow cyanide to remove metals from enzymes such as cytochrome oxidases producing cellular asphyxiaThereby, cyanide in effluents has to be decreased before it reaches the environmentPhysical and chemical treatments are using for this but with several disadvantagesAn alternative approach is the bioremediation which has been used in some industriesHowever, more studies are necessary to improve biotreatments to position these as the best decision for the industriesThere are several bacterial species described to degrade cyanide but little is known about its metabolic pathways, regulation mechanisms or structure of enzymes involvedThe goal of this project is understand how some Bacillus species respond to the presence of cyanide in the environment and how the enzymes responsible for the degradation of cyanide workOur studies include isolation and genome sequencing of native cyanide-degrading Bacillus strains; genome editing of bacterial operons involved in cyanide metabolism; cloning and purification of cyanide-degrading enzymes; structural modeling and molecular dynamics simulationsWe isolated three native cyanide-degrading strains; identified them as Bacillus subtilis, Blicheniformis and Bpumilus using 16SrRNA sequencing and MALDI-TOF, and sequence its genomes using next-generation sequencing (Ilumina-MiSeq)Also, we were able to clone and purify CynD (Cyanide dihydratase) from Bpumilus strain and showed that it is activeOur structural modeling and molecular dynamics simulations of CynD revealed that two regions in the c-terminal of CynD are very flexible in the monomerIn conclusion, Bacillus species seems to be good candidates to study the cyanide metabolism in bacteriaThe high activity showed by the purified CynD enzyme from Bacillus pumilus allow us to propose it as a protein to be use in cyanide degradation although more studies are necessary to determine the best conditions for thisThe flexibility shown by the c-terminal region of CynD monomer lead us to hypothesize that it is involved in the oligomerization processAlso, we conclude that in order to increase the probability of crystallize this protein for structural studies we have to remove this region.

Trabajos presentados al ***I Congreso Internacional de Biotecnología e innovación (ICBi)***, 9- 12 de julio de 2018, Universidad Nacional Agraria La Molina, Lima, Perú.

Editoras:
Ilanit Samolski Klein
Maria Lucila Hernández-Macedo
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Contribution

Section II: Industrial and Environmetal Biotechnology

16S rDNA NGS analysis shows microbial community associated with the generation of acid mine drainage in a gold mine, Peru.

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Keywords: Metagenomics, microbial diversity, sedimentation ponds, mine tailings.

Acid mine drainages are characterized by their extremely acid pH, high concentrations of sulphates and heavy metals constituting one of the main environmental problems related to mining activitiesThe objective of this study was to characterize by metagenomics the microbial community present in gold mine acid drainagesThe samples were obtained from two sedimentation ponds, PS-1 and PS-2 (pH 1.99 and 2.47, respectively), two infiltration zones of these ponds, ZI-1 and ZI-2 (pH 2.02 and 2.90, respectively) and from a natural creek adjacent to the sedimentation ponds location of acid mine drainage (pH 2.95)DNA of each sample was extracted and sequenced by NGS directed to V4 region of the 16S rDNA gene using the Illumina MiSeq systemThe sequences obtained were processed and analyzed by QUIIME softwareThe microbial community associated with the sedimentation ponds and zones of influence is closely related and represented by members of genera Metallibacterium, Leptospirillum, Acidiphilium and Acidibacillus (PS-1 and ZI-1)Leptospirillum, Sulfobacillus, Ferroplasma, Acidiphilium, Thermoplasma, Metallibacterium, Ferrimicrobium, Gammaproteobacteria - uncultured bacterium, Ferrithrix, Acidisphaera and Acidithiobacillus (PS-2 and ZI-2) which are widely reported as iron-sulfur oxidizing chemolithotrophic microorganisms.In addition, we found very low abundances of sulfate-reducing bacteria, such as Desulfosporosinus, potential microorganisms for bioremediation of these zonesLikewise, it was determined that although the natural creek receives a direct influence of acidic discharges, the microbial community was represented mainly by members of the genera Gallionella, Sideroxydans, Rhodanobacter, Granulicella, Acidimicrobiales-uncultured bacterium, Limnohabitans, Acidocella, Mucilaginibacter, AcidithrixThese results showed that the predominant microbial community in acid mine drainages is represented by microorganisms adapted to acid environmental related to the oxidation sulphured minerals compounds present in a gold mine and their wastes

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Contribution

Section II: Industrial and Environmetal Biotechnology

Evaluation of a bioremediation system for heavy metals based in on fungal biomass from santuario nacional del Ampay

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Keywords: Basidiomycetes, metal-ligand bonds, bioremediation

This study assesses a bioremediation system designed for treating water polluted whit heavy metals, by using dried fungal biomass from four strains obtained from the Santuario Nacional del Ampay in Apurimac, a region with one of the highest mining activities but also are the most impoverished and geographically small regions in PeruA laboratory scale packing porous bed system was developed for treating water polluted with lead, cadmium and copper, first Fungal strains from the genera Geastrum, Higrosporus, Cantharellus y Ectomicorriza were isolated and identified by morphologyThe system biosortion capacity was them quantified for heavy metals using air-acetylene flame atomic absorption spectrophotometry with the standard calibration techniqueAll tested fungi showed moderate to high adsorption of heavy metalsIt was observed that the four fungal strains had different metal retention capacities¸amongst which the isolate belonging to the Cantharellus y Ectomicorriza genus showed the best retention percentage of all heavy metals studied, with an average of 100% y 100 % for lead; 100% y 87.5% for Cadmium; and 100% y 56.5% for copper, respectively, while the isolate belonging to the Geastrum genus had the worst retention of the three metals studied with an average of 0.6%; 69.4% and 1.6% for copper, lead and cadmium correspondingly; The functional groups responsible for metal uptake on biomass (the metal-ligand bonds) of the four fungi were analyzed by FTIR spectroscopy, spectra was obtained in the range of 400–4000 cm1Primary aliphactic amines (RNH2) were identified as the causing agent of cadmium, while copper binding, amide I (CO) and cyanide (CN-) were identified as causing agents for lead bindingAbove results show that this system based on fungal biomass can be successfully used for heavy metal bioremediation from water contaminated with multiple heavy metals.

Trabajos presentados al ***I Congreso Internacional de Biotecnología e innovación (ICBi)***, 9- 12 de julio de 2018, Universidad Nacional Agraria La Molina, Lima, Perú.

Editoras:
Ilanit Samolski Klein
Maria Lucila Hernández-Macedo
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Contribution

Section II: Industrial and Environmetal Biotechnology

Potential application of commercial enzyme preparations for the production of isomaltooligosaccharides from maltose

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Keywords: transglycosylation activity, hydrolysis activity, ?-glucosidase, panose

Isomaltooligosaccharides (IMOs) are glucose oligomers containing one or more ?-(1,6) glycosidic bonds, although they are marketed as a mixture of glucosyl saccharides with both ?-(1,6) and ?-(1,4) bondsIMOs are emerging prebiotics and exhibit unique physicochemical properties for the food industryThey are produced from maltose by means of ?-glucosidases (EC 3.2.1.20) transglycosylation reactionsHowever, few ?-glucosidases exhibit the required level of transglycosylation for industrial application because several of these enzymes catalyze the maltose hydrolysis instead of the synthesis of IMOsTherefore, we performed a screening of available commercial enzyme preparations for the potential production of IMOs from maltose and determined the effect of temperature on the transglycosylation and hydrolysis activities of the selected enzyme preparationUsing maltose as substrate, the activities were determined by the initial reaction rates of panose formation and glucose releasedThe reaction mixture consisted of 1.9 mL of 300 g/L maltose in 50 mM sodium acetate buffer at pH 5.0 and 0.1 mL of either enzyme or diluted enzyme using the same bufferThe mixture was incubated at 50 °C and stirred at 150 rpmThe samples were analyzed for their carbohydrate composition by high-performance liquid chromatography (HPLC) The effect of temperature on the activities was studied in the range from 35 to 60 °C while the other reaction conditions were constant as the previously mentioned valuesThe experiments were performed in duplicateThe enzyme preparation called Cellulase DS was selected from six enzyme preparations, since it exhibited the highest transglycosylation activity (464 ± 7.1 UI/g) and ratio of the synthesis and hydrolysis activities (1.1 ± 0.06)In addition, this enzyme is highly selective for the panose synthesis compared to the enzyme called Transglucosidase L, which is used on an industrial scaleAs expected, the transglycosylation and hydrolysis activities significantly increased as the temperature increased, but the hydrolysis was more sensitive to temperature than the synthesisThus, the ratio of the synthesis and hydrolysis activities was higher at 35 °C (R =1.25 ± 0.039)The results indicate that there are commercial enzyme preparations exhibiting a secondary transglycosylation activity, which can serve as a source of ?-glucosidase for the enzymatic production of IMOs from maltose.

Contribution

Section II: Industrial and Environmetal Biotechnology

Proteomic analysis of the cyanotrophic strain Alcaligenes aquatilis after its exposure to cyanide by MALDI TOF / TOF.

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Keywords: Cyanide, bioremediation, nitrilase, exoproteome, enzyme

Cyanide leaching is the main process used in the mining industry for the extraction of gold and silver in Peru and worldwideCyanide is a highly toxic and harmful compound to the environment; however, there are microorganisms with the ability to degrade this compoundThe cyanotrophic strain Alcaligenes aquatilis isolated from mining areas affected by cyanide in the La Libertad region, was studied due to its high performance to resist and degrade cyanideIn order to examine the physiological responses of cyanide degradation by this bacterium, a bottom-up proteomics approach by proteolytic digestion of proteins prior to analysis by MALDI TOF / TOF mass spectrometry was applied, in addition a genomic identification of cyanide degrading enzymes by endpoint PCR was doneFor the proteomic study Alcaligenes aquatilis was cultured in a minimal medium with 200 ppm of sodium cyanide (NaCN), the intracellular and extracellular proteins were extracted and migrated in SDS-PAGE gel for separation by molecular weight, the differential bands were cut, processed and analyzed in the MALDI TOF / TOFFrom the genomic DNA extracted from this microorganism, the endpoint PCR was carried out with primers target to the NHase gene (nitrilase)Alcaligenes aquatilis cultivated in minimal medium with cyanide as the sole source of nitrogen, managed to degrade it in 24 hoursOn the other hand, enzymes involved in the cyanide degradation, such as cyanase, nitrilasa and cyanate hydratase were identified by MALDI TOF / TOF; moreover, transmembrane proteins involved in the transport of cyanide and cyanide compounds as MFS cyanate transporters and ABC cyanate transporters were identified in the intracellular samplesIn addition, proteins such as the siderophore synthetase that participate in the biosynthesis of siderophores were identified, these are key for the resistance of the strain to cyanideThe transcription regulator CynR, also identified, fulfills the function of modulating the expression of the enzyme cyanase in the presence of cyanideBesides, the nitrilase enzyme was found in the exoproteome samplesAt the genomic level, amplification of the NHase (nitrilase) gene was achieved by PCRThis work shows that a novel cyanotrophic bacteria has the potential to be used for bioremediation based on its genomic and proteomic characteristics.

Trabajos presentados al ***I Congreso Internacional de Biotecnología e innovación (ICBi)***, 9- 12 de julio de 2018, Universidad Nacional Agraria La Molina, Lima, Perú.

Editoras:
Ilanit Samolski Klein
Maria Lucila Hernández-Macedo
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Contribution

Section II: Industrial and Environmetal Biotechnology

Genomic and proteomic characterization of native coastal bacteria degrading petroleum hydrocarbons

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Keywords: Bioremediation, metagenomic, bacteria, MALDI TOF-TOF, mass fingerprint.

The oil exploration and production activities in Peru generates serious environmental problems by polluting natural ecosystems that are difficult to remedyThe present study was carried out in two petroleum hydrocarbon-polluted coastal areas (Talara in Piura and Zorritos in Tumbes)The total bacterial microbiota of the contaminated soil samples was characterized through culture-independent techniques by metagenomic analysis directed to the v4 region of the 16S rRNA gene, using Qiime bioinformatics software for the metadata analysisFurther, in the present study bacteria were isolated using Mineral Salt Medium supplemented with 1% crude oil and these were identified by partial 16S rRNA sequencing from the aforementioned region, the identification was based on the similarity of the partial sequence with the BLAST database of the NCBIThe isolated bacteria that showed resistance to petroleum hydrocarbons were characterized by mass spectrometry MALDI TOF-TOF shotgun proteomics, considering in particular cellular proteins and peptide mass fingerprintingMetagenomic characterization of the bacterial microbiota revealed the presence of bacterial genera widely reported as degraders of petroleum hydrocarbons such as Marinobacter, Halomonas, Pseudomonas, Acinetobacter and SphingomonasIn addition, an inverse relationship was found between the level of contamination and bacterial diversityA total of 45 bacterial isolates from both areas contaminated with oil has been molecularly identified, being the most representative generaAcinetobacter, Bacillus, Pseudomonas, Halomonas, Stenotrophomonas, ShewanellaLikewise, the peptide mass fingerprinting of some of the members of these genera were determinedPseudomonas putida, Acinetobacter pittii, Acinetobacter sp., Stenotrophomonas sp., Bacillus sp., Serratia marcescensFinally, the ability of the isolated bacteria to degrade hydrocarbons present in diesel was evaluated in vitro, being Acinetobacter sppand Serratia marcescens the strains most efficient for degradationThe petroleum hydrocarbon degrading enzymes from these bacteria were identified through proteomic analysis as alcohol dehydrogenase, aldehyde dehydrogenase, monooxygenase, 2-nitropropane dioxygenase, which are involved in the petroleum hydrocarbons degradationThese results show the suitability of "omic" technologies for the characterization and selection of native coastal bacteria as potential candidates for bioremediation of marine environments contaminated with petroleum hydrocarbons.

Contribution

Section II: Industrial and Environmetal Biotechnology

Isolation and Identification of Mangrove Bacteria Sediment with Potential Biodegradation of Petroleum

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Keywords: Impact; Bioremediation; Hydrocarbons.

The hydrocarbon accumulation, in addition to their residual effect, causes a serious environmental impact, affecting soil and water, and consequently diverse ecosystemsPhysical, chemical and biological methods for the control of this contamination have been applied, although with limited efficiencyIn this context, the use of microorganisms with pollutant degrading potential is investigated as an alternative to reduce the impact of hydrocarbonsHowever, for an adequate biodegradation, an efficient microbiota is necessary, so, the search for microorganisms with these characteristics in poorly exploited environments such as mangroves can provide greater efficiency in the remediation processThus, the objective of this study was to evaluate the potential of petroleum hydrocarbon biodegradation by isolating microorganisms from mangroveFor this, three mangrove sediments were collected from the State of Sergipe, Brazil, and bacteria were isolated in mineral medium (MSM) containing monopotassium phosphate (KH_2PO_4, 0.5g/L), dipotassium phosphate (K_2HPO_4, 0.5g/L), sodium chloride (NaCl, 2.0 g/L), magnesium sulfate heptahydrate ($MgSO_4$ 7 H_2O, 0.5 g/L), manganese (II) sulphate $MnSO_4$ 7 H_2O, 01 g/L, ferrous sulfate heptahydrate ($FeSO_4$ 7H20, 0.01 g/L), ammonium nitrate (NH_4NO_3, 1.0 g/L), using crude oil as the carbon sourceThe cultivation was performed at 27ºC, analyzing bacterial growth by optical density (OD) at 595 nm every 48 h for 15 daysAfter growth, isolates were identified by sequencing the 16S rRNA gene after PCR amplification using the primer set 968F (5'AACGCGAAGAACCTTAC 3 ') and 1401 R (5'CGGTGTGTACAAGACCC3')Isolation assays allowed to obtain six isolates identified as Bacillus aerophilus, Exiguobacterium profundum, Pseudomonas xanthomarina, Proteobacterium, Pseudomonas spand Bacillus sp., which displayed potential for petroleum degradationThe bacterium with the highest potential was Pxanthomarina followed by Proteobacterium sp., Pseudomonas sp., and BaerophilusBoth Proteobacterium spand Pseudomonas sppresented idiophasic growth after 12 days of culture, showing industrial and bioremediatior potential of these isolates due to the metabolic, adaptive and degradation capacity of these bacteria in an oil rich medium

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Trabajos presentados al ***I Congreso Internacional de Biotecnología e innovación (ICBi)***, 9- 12 de julio de 2018, Universidad Nacional Agraria La Molina, Lima, Perú.

Editoras:
Ilanit Samolski Klein
Maria Lucila Hernández-Macedo
Gretty Katherina Villena Chávez

Contribution

Section II: Industrial and Environmetal Biotechnology

Chemical study of the seaweed Caulerpa filiformis (chlorophyta) as a potential source of bioactive principles

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Keywords: polyphenols, antioxidant activity, secondary metabolites, flavonoids, green algae.

Caulerpa filiformis is a marine macroalgae whose growth is in abundanceIn Peru, it is originally distributed on the north coast (Isla Lobos de Afuera and Piura) and recently introduced in the central coast (Ancash, Lima and Ica)Caulerpa species produce secondary metabolites as a defense mechanism against herbivory, having identified polyphenols, alkaloids, terpenoids, among others, which are attributed activities such as antioxidant, anti-inflammatory, inhibition of cell aging, in the prevention of diseases cardiovascular, cancer and othersThe purpose of this research was to identify the main groups of secondary metabolites, determine the total polyphenol content and the antioxidant activity of the methanolic extracts of Cfiliformis, collected in the Sechura Bay (Piura) and the Bay of Paracas (Ica)The samples of the fresh algae were dried and pulverized, from which 5% methanolic extracts were prepared by macerationThe method used for the partitioning of the extracts and the phytochemical screening was the method proposed by Rondina and Coussio (1969)The total polyphenol content of the extracts was determined by the Folin-Ciocalteu method and the antioxidant activity was measured by the ABTS method (2,2-azinobis- [3-ethylbenzothiazoline-6-sulfonic])The results of the phytochemical screening indicated in both samples the presence of carbohydrates, polyphenols, tannins, phenolic oxhydrils, flavonoids, alkaloids, steroids and triterpenesThe total polyphenol content of the Sechura Cfiliformis sample (459.56 mg AG / 100g dry weight) was significantly higher (P <0.05) than in the Paracas sample (424.63 mg AG / 100g dry weight)However, there are no significant differences (P> 0.05) between the antioxidant activity of the Sechura Cfiliformis sample (873.51 ?mol trolox eq./100g dry weight) and the Paracas sample (890.14 ?mol trolox eq./100g dry weight) It is concluded that Cfiliformis is an important source of secondary metabolites with antioxidant activity, which is related to its polyphenol contentIn addition, it was shown that the biochemical expression of the main chemical groups is similar in both samples. FinancingFONDECYT 129-2015"Macroalgae diversity of the central coast of Peru using DNA barcodes,in the perspectives of their potential uses and biotechnological applications".

Contribution

Section II: Industrial and Environmetal Biotechnology

Synthesis of cellulose nanofibers from lignocellulosic materials from industrial waste.

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Keywords: nanocellulose, ionic liquids, pretreatment

Cellulose from lignocellulosic waste is an abundant and available material for obtaining value-added productsEnzymes, nanocrystals and nanofibrils are examples of value-added products obtained from celluloseIn the specific case of nanofibrils and nanocrystals, their low density, high crystallinity, stiffness and traction make them an industrially attractive materialIn addition to these characteristics, other interesting properties, such as non-toxicity, non-allergene, biodegradability and biocompatibility make these materials applicable in the biomedical and pharmacological area as reinforcement in polymeric matrix and for drug delivery, as well as in the area of energy and environment for the manufacture of batteries and airshipsHowever, pre-treatment remains necessary to obtain these nanocellulose materials and in this regard, ionic liquids (IL) are emerging as a promising strategy for cellulose pre-treatment due to being considered an environmentally friendly method, with applications in agro industrial waste bioconversion among othersThe aim of this study is the application of lignocellulosic materials for nanocrystals and cellulose nanofibrils production using IL as pre-treatmentConsequently, 2.5 g of the lignocellulosic material (orange peel) were treated with 25 mL of ionic liquid (Methylimidazolium, THTDP (Br) and THTDP (CH3SO3)) with time and temperature variationsAfter treatment with IL, the sample was filtered and the solid fraction obtained was stored for extraction efficiency analyzesThe liquid fraction from the process was subjected to dialysis to obtain the crystalsThe samples were subjected to physical-chemical characterizations (FTIR, TG, AFM, MET)According to the results, it was possible to verify the influence of time on the extraction performanceThe average weight yield of the extraction with ionic liquids was more efficient between 90-150 minat range of 80 and 100 °CAfter the lignocellulosic material treatment, were determinate the depolymerization efficiency and subsequent extraction of the cellulose nanocrystalsFrom this, a yield of 42.5% was obtained when methylimidazolium was usedFor THTDP (CH3SO3) the yield was 51.4% and for THTDP (Br) a yield 56.5% was observedThis statement can be explained by the relationship between the productivity and extraction, as well as, when have a lower mass percentage a higher extraction rate was observed after treatmentTherefore, it was possible to conclude that time is a limiting factor in the process performanceThe crystalline polymer confirmation was evidenced by the FTIR and AFM techniques, showing the efficacy of chemical treatments with IL in the lignocellulosic material depolymerization, as well as its yield, which shows the production capacity of cellulose nanocrystals.

Trabajos presentados al ***I Congreso Internacional de Biotecnología e innovación (ICBi)***, 9- 12 de julio de 2018, Universidad Nacional Agraria La Molina, Lima, Perú.

Editoras:
Ilanit Samolski Klein
Maria Lucila Hernández-Macedo
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Contribution

Section II: Industrial and Environmetal Biotechnology

Bioelectricity from cellulolítics microbes in cell of microbial fuel

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Keywords: Microbial fuel cell, cellulolytic microorganism, bioelectricity

It is increasingly necessary to find new sources of energy that constitute an alternative to current energy sourcesBy making use of microorganisms in Microbial Fuel Cells, MFC, which are devices composed of two chambers, an anodic and a cathodic, divided by a proton exchange membrane, electricity can be generatedThe objective of this research was to generate electricity by establishing the maximum voltage, power density, current density and polarization curve in microbial fuel cells (MFC) using carboxymethyl cellulose degrading cellulolytic microbesIn the process, a common microbial fuel cell was used, which is a two-chamber systemIn one of the chambers called anodic an inoculum of the cellulolytic microbial strain RCC01 and 1% carboxymethylcellulose was placedIn the other chamber called cathodic, distilled water was placed and aerated at a rate of 1 volume of aeration per volume of the cathodic chamber per minuteThe experiment was carried out at 26 °C for 44 hThe data collection was with a Multitester digital multimeter at 0, 7, 12, 16, 20, 26, 35, 39 and 44 h for each evaluation, in the resistance values of 0 (assuming as air resistance for this value), 10, 20, 50, 200, 500, 1000, 2000, 10000, 40000 and 100000 ohmsA maximum voltage of 203 mV was obtained without using current resistance, at room temperature and at 20 h of experimentationLikewise, a polarization area at 16 h of experimentation in which evidencedin the upper limit, a higher limit value of the curve at 20 h corresponding to a power density of 0.655575 mW / m2 and of current density of 2.35344 mA / m2Also was observed at the lower limit, a lower limit value of the curve obtained at 12 hours corresponding to a power density of 0.22183 mW / m2 and current density of 2.225 mA / m2.

Contribution

Section II: Industrial and Environmetal Biotechnology

Evaluation of Chenopodium album L(Liccha) phytoremediation capacity in contaminated water with lead and cadmium.

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Keywords: Underbrush, phytoremediation, lead and cadmium

Metals are not biodegradable and persist in the environment, polluting ecosystems such as waterNowadays, the toxicity of lead (Pb) and cadmium (Cd) in water is increasing significantly due to the massive industrial release accumulated in the environment, affecting human health causing teratogenic and lethal carcinogenic damage There are several physicochemical methods for the removal of heavy metals from water, however, most of these are expensive and not feasible; currently, the development of new remediation technologies is being sought, inexpensive and compatible with the environment; such is the case of phytoremediationThis research aims to evaluate the phytoremediating capacity of Chenopodium album (Liccha) in waters contaminated with lead and cadmiumFirst, the propagation of Chenopodium album was carried out by cuttings up to a growth of 7 cm; to then, be adapted in a 25% nutrient solution of Hoagland containing 52.500 mg.L-1 of potassium, 47.500 mg.L-1 of nitrogen, 37.500 mg.L-1 of calcium, 17.500 mg.L-1 of sodium, 11.250 mg.L-1 of magnesium, 8.750 mg.L-1 of phosphorus, 0.250 mg.L-1 of iron, 0.125 mg.L-1 of manganese and boron, 0.038 mg.L-1 of zinc, 0.025 mg.L-1 of copper and 0.013 mg.L-1 molybdenum; under environmental conditionsPhytotoxicity tests were carried out by triplicate for 20 days, analyzing 3 concentrations of 5, 10 and 15 mg.L-1 of lead (Pb) and cadmium (Cd) respectivelyThe degrees of toxicity were characterized and thus, the optimal concentrations of bioaccumulation were determined (greater tolerance and accumulation of metals under study), being 10 mg.L-1 of lead and cadmium; at the maximum concentration of each metal, plants showed symptoms of chlorosis and early senescenceFinally, the bioaccumulation test was performed by triplicate; determining the accumulated concentrations in the radicular and aerial parts of the plants, every 5 days for 30 days; using the ICP - OES inductively coupled plasma spectroscopy technique, after validation of the analytical methodThus, a maximum accumulation was determined at 15 daysfor lead (Pb) 1741.316 ± 5.255 mg.Kg-1, with a final growth of 40 cm; and for cadmium (Cd) 10049,884 ± 4.284 mg.Kg-1, with a growth of 10 cmIn conclusion, Chenopodium album has a high bioaccumulation capacity of lead and cadmium; this plant can be used for the phytoremediation of highly polluted water with these metals; whether they are mine or industrial tailing waterThis is a sustainable and a very low cost technique, since the Liccha is a fast growing herb with few nutritional requirements.

Trabajos presentados al ***I Congreso Internacional de Biotecnología e innovación (ICBi)***, 9- 12 de julio de 2018, Universidad Nacional Agraria La Molina, Lima, Perú.

Editoras:
Ilanit Samolski Klein
Maria Lucila Hernández-Macedo
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Contribution

Section II: Industrial and Environmetal Biotechnology

Prospecting of mangrove bacteria with organochlorine compound remediation potential

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Keywords: degradation, microorganisms, pollutants.

Organochlorine compounds have been widely used in agricultural pest control and as inputs in timber and chemical industriesOne of these compounds is hexachlorobenzene (HCB), which is restricted in many countries due to its liposolubility and recalcitrance, responsible for environmental impacts and human and animal healthIn view of this, biological methods employing microorganisms are presented as sustainable tools to remedy contaminated areas by these compoundsIn this context, mangroves represent a microorganism source with potential applications for the industrial sector, although in environmental control, these microorganisms are still poorly studiedThereby, the objective of this study was to isolate bacteria from mangrove sediments to evaluate their potential for HCB degradationFor this, mangrove sediment samples, collected from the State of Sergipe, Brazil, were resuspended in 0.85% NaCl and after incubation (180 rpm/50 min), bacterial suspension serial dilutions were seeded in minimal salt media (MSM) and Luria-Bertani mediumIsolated microorganisms were afterwards seeded in MSM containing 4.0 mM HCB as carbon source and cultured at 28°C, and their growth was evaluated spectrophotometrically at 540 nm every 24 h for 3 daysMicroorganisms were also characterized by the Gram staining methodFrom mangrove sediments, 50 bacterial isolates were obtained, of which only nine displayed HCB biodegradation potential, exhibiting OD540 nm values between 0.2 and 1.6Overall, the majority of the bacterial isolates showed a growth over 72 h, indicative of the use of their biochemical machinery to degrade HCB and to use metabolites produced from their degradation as carbon sourceThe microbiological characterization identified eight Gram-positive and one Gram-negative bacteria, with cocci and rod morphologiesMost of the nine bacterial isolates exhibited endospores, which indicates their biotransformer potential through the action of enzymes contained in these structures, secreted under conditions of environmental stressTherefore, results display the bioremediation and industrial potential of these bacteria due to their adaptive metabolic capacity in the presence of HCB.

Contribution

Section II: Industrial and Environmetal Biotechnology

Biotechnological treatment for printed office paper designed with thermostable enzymes of Geobacillus thermoparaffinivorans CB-13

Castellanos, R.*; Zúñiga, A.; Espinal, D.; Naquiche, A.; Salazar, I.; Yllanes, M.; Ramírez, H.; Salazar, F.

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Keywords: test sheet, residual ink, pulp, enzymatic cocktail

The biotechnological treatment with bacterial thermostable enzymes for the deinking of office paper was performed with an enzymatic cocktail comprised of amylases, cellulases and lipases thermostable produced by Geobacillus thermoparaffinivorans (CB-13) from the geysers of Calientes-Candarave (Tacna, Peru)Three variables were considered (pulp concentration, time of enzymatic treatment and enzyme concentration) to evaualted the residual ink released and quantified by spectrometry at 300 nm, 60 °C and pH 7In a completely randomized block design (DBCA) with interaction was used, evaluating cellulase, amylase and lipase concentrations at 10, 15 and 20 % (v/v), pulp concentrations at 0.8; 1 and 1.2 % (w/v) and enzymatic activity from 0 to 200 minThe highest released ink values were obtained at 1 % pulp concentration for amylase and lipase while 1.2% for celluloseA high enzymatic activity was observed at 40, 60 and 180 min with an enzyme concentration of 20 % for amylase, cellulase and lipase respectively, which were used for the deinking processThe enzymatic activity methods for amylases, cellulases and lipases were Bernfeld, Ghose and YasapanDetermination of the total amount of soluble protein in the supernatant was measured at 595 nm acording to BradfordThe enzymatic activity specific for amylases, cellulases and lipases were 21.1, 1.5 and 3.9 U/mg respectively, and then the pulp pretreatment was carried out with SDS and H20, with residual ink values of 0.159 and 0.200 mg/mL respectively, which did not present a significant differenceNext, the pulp pretreated with 0.5 % SDS and 60 % (v / v) of amylase enzymes, cellulases and lipases (ratio 111) was evaluated, obtaining the best value of 1.731 mg/mL of residual ink for pulp treated with SDS and enzymeFinally, the test sheets were prepared and the properties of the paper formed by the pulp and paper laboratory service of the Forestry Production Center of the National Agrarian University La Molina were determinedThe values reported for the pulp treated with SDS and enzyme were 86.7 º of whiteness (TAPPI T452-om98), 91.6 % opacity (TAPPI T452-om96) and strain elongation (TAPPI 404 cm-92)Thermostable enzymes obtaining a test sheet with 92.23 % whiteness, 61.5 % tension elongation, achieved the de-inking of the office paper and a total opacity compared to a commercial sheet.

Trabajos presentados al ***I Congreso Internacional de Biotecnología e innovación (ICBi)***, 9- 12 de julio de 2018, Universidad Nacional Agraria La Molina, Lima, Perú.

Editoras:
Ilanit Samolski Klein
Maria Lucila Hernández-Macedo
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Contribution

Section II: Industrial and Environmetal Biotechnology

Bioprospecting thermophilic bacteria for biotechnological production of thermostable enzymes

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Keywords: KeywordGeyser, phylogenetic analysis, enzymatic activity

Bioprospecting was carried out toward new thermostable enzymes evaluating the enzymatic activity of bacterial strains isolated from the geysers of Calientes, Candarave (Tacna, Peru)A total of 40 geysers were evaluated, with temperatures of 55.3 to 87.7 °C and samples of sediment enriched in liquid médium were taken, obtaining 120 strains at 60 °CThe microorganisms were molecularly identified by isolating 16S rRNA genes, which were sent to commercial company Macrogen Inc., to determine their sequencingEach identified bacterial strain was induced with the substrates starch, carboxy-methylcellulose, olive oil and pectin for the expression of termostable enzymes, performing a qualitative screening by visualization of hydrolysis ring at 60 °CTwenty nine strains were selected asamylolytic (CB-13, CB-16, CB-19, BT-2, FT-3, FT-4, FT-8), cellulolytic (CB-4, CB-13, CB-16, FT-4), lipolityc (CB-13, CB- 14, CB-16, FT-3, FT-5, FT-8, TM-8, TM-10, TM-13, GT-5) and pectinolytic (CB-13, CB-16, CB-19, CB -28, FT-3, FT-8, TM-1, TM-13)From the bacterial strains obtained, the production of the afore mentioned enzymes was carried out for the determination of enzymatic activity at 60 °C and were the strains with the highest activity for amylasesGeobacillus thermoparaffinivorans CB-13 (14.47 U / mL), Geobacillus thermoleovorans CB-16 (9.48 U / mL); cellulasesGeobacillus kaustophilus FT-4 (0.048 U / mL), Geobacillus thermoparaffinivorans CB-13 (0.028 U / mL); lipasesGeobacillus thermoleovorans FT-5 (0.62 U / mL), Anoxibacillus kualawohkensis GT-5 (0.2 U / mL) and pectinasesGeobacillus thermoparaffinivorans CB-13 (0.180 U / mL), Geobacillus thermoglucosidasius TM-13 (0.203 U / mL)The phylogenetic analysis established a closeness between the bacterial species identified belonging to the Domain Bacteria, Phylum Firmicutes, Class Bacilli, Order Bacillales, Family Bacillaceae and Genus Geobacillus (Gthermoglucosidasius, Gparaffinivorans, Gthermoleovorans, Gkaustophilus, Gstearothermophilus, Gtoebii), Genus Anoxibacillus (Akualawohkensis, Apallidus, Aflavithermus, Arupiensis), and Genus Bacillus (Bhalodurans, Boceanisediminis, Bfirm, Bflexus)In the bioprospecting of thermophilic bacteria for the biotechnological production of thermostable enzymes, amylases, lipases, pectinases and cellulases have been obtained, which through recombinant DNA technology can be a product of industrial interest.

Contribution

Section II: Industrial and Environmetal Biotechnology

Efficiency of biodegradation of ammonia by an isolated microbial consortium in a bioreactor typeRunoff filter

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Keywords: pollution, environmental, efficiency, ammonia.

Environmental pollution is a current worldwide problem, due to the various toxic waste and effluents emissions frequently discharged into the environment, affecting natural resources, the ecosystem and human healthIn Peru, eutrophication problems have been occurring in Lake Titicaca (since August 1998), caused mainly by domestic waste discharged, emissions from canned fish factories, dairy products, petrochemical products, among othersFaced with this problem, aimed at the objective of the research was to evaluate the ammonia biodegradation efficiency by a microbial consortium, using a "Runoff Filter" bioreactor (Shuller & Kargi, 1995), at ammonia concentrations of 12.5 mg / l, 25 mg / l and 50 mg / l, with flow rates of 12 ml / h, 24 ml / h and 36 ml / h; as well as, design and build the type bioreactor "Runoff Filter" that will serve as a means for such evaluationThe research was carried out using bioremediation as an alternative for the treatment of ammonia effluents, considering the advantages that this technology provides such as its low costs, reduced energy requirements, easy operation and the use of biological methods for transformation or degradation of toxic compounds (Revah & Christen 1997)These methods usually use microbial consortiums isolated from pollution sources, taking into account that in nature the degradation processes are usually carried out by microbial consortia allows obtaining high levels of efficiency, yield or productivity, which is the main objective of these processesThe microbial consortium was isolated from a Wastewater Treatment Plant (Lambayeque) and inoculated in "Runoff Filter" bioreactorFrom this consortium, were isolated 6 Gram positive bacteria with morphology of cocci (2), coccobacilli (1) and pleomorphic bacilli (3)These strains were reported as Pseudomonas, Actinomycetes and StreptomycetesLikewise, the amount of residual ammonia at 12.5 mg / l and a flow rate of 12 ml / h represented the maximum biodegradation efficiency (97.33%), concluding that a lower concentration of ammonia increases the efficiency of its biodegradationThis results, allowed verifying that this process is an alternative for the treatment of ammonia effluents, since the amounts found are below the contamination standards for this compound, which indicates that this technology is a viable alternative for the treatment of domestic and industrial effluents.

Trabajos presentados al ***I Congreso Internacional de Biotecnología e innovación (ICBi)***, 9- 12 de julio de 2018, Universidad Nacional Agraria La Molina, Lima, Perú.

Editoras:
Ilanit Samolski Klein
Maria Lucila Hernández-Macedo
Gretty Katherina Villena Chávez

Contribution

Section II: Industrial and Environmetal Biotechnology

Identification of cellulolytic and xylanolytic thermotolerant bacteria from Chancos hot spring and their ability to degrade agriculture waste

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Keywords: Callejón de Huaylas, Bacillus, thermotolerant cellulases, baggasse, quinoa, wheat

The high demand of energy faced to global warming has increased interest to develop processes to produce energy by clean and renewable technologies, such as biofuel from lignocellulosic biomassIn this context, the aim of this research was isolate and select thermotolerant cellulolytic and xylanolytic bacteria from Chancos hot spring (Callejón de Huaylas, Ancash – Peru), and evaluate their ability to degrade agriculture wastesBacteria isolation was performed using fresh samples, ex situ enrichment and in situ baiting, culture in basal salt medium at 50°C and pH 6.5Congo red staining method was performed for selection16S rRNA gene was used for taxonomic identificationEndoglucanase and xylanase activities were quantified to determine the best strain; then, optimum temperature and pH, and thermal stability of its crude enzyme extract were characterizedThe ability to degrade agriculture wastes (wheat stems, quinoa stems, and sugar cane bagasse) was evaluated in submerged cultureAlso, saccharification of these waste, using only crude extracts were evaluatedQuantification of reducing sugars and enzymatic activities were carried out by using DNS method31 bacterial strains were isolated and 14 showed hydrolysis halo on CMC and xylan platesAccording to 16S rRNA gene analysis, selected strains were identified as Bacillus subtilis or BlicheniformisBsubtilis DCH4 showed the highest endoglucanase and xylanase activities, its optimum temperature and pH for endoglucanase and xylanase activities were 45°C - 5 and 55°C - 6, respectivelyIn submerged culture using agriculture waste, Bsubtilis DCH4 showed ability to degrade cellulose, achieving better results with peptone as nitrogen sourceThe enzymatic extracts of cultures with sugar cane bagasse and quinoa showed the highest endoglucanase and xylanase activitiesFinally, it was found that the presence of cellulolytic and xylanolytic thermotolerant bacteria in Chancos hot spring, and Bacillus subtilis DCH4 showed ability to degrade agricultural residues either in culture or when only their enzymes were usedLikewise, the agricultural residues evaluated can be used both as a carbon source for obtaining fermentable sugars and for production of xylanolytic and cellulolytic enzymesBacteria isolated would be useful for bioprocesses and clean technologies such as production of second generation bioethanol.

Contribution

Section II: Industrial and Environmetal Biotechnology

Design of a production medium with waste agroindustrials optimizing the capacity of mineralization of calcium by Bacillus Pseudofirmus.

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Keywords: mineralization, self-healing, concrete spores.

The biotechnological potential of the bacterium Bacillus pseudofirmus is based on its ability to self-healing concrete, by calcium mineralization process and the sealing of microcracks in concrete, which, being part of a specific formulation, this avoids corrosion internThe aim of this work is to standardize the growth, yield and productive capacity of BpseudofirmusThe growth was standardized in 3 culture media, (1) ASWM4 medium with calcium ions, (2) national base medium (Compound of molasses, flour and salts) and (3) balanced base medium, national medium with acid hydrolysis processCell yield and growth rate were determined by microbial kineticsThe capacity of production of insoluble calcium was evaluated by means of the indirect redox titration method, to demonstrate the process of induced calcium mineralizationThe balance made to the medium '2' result in a rapid initial growth, reaching the maximum growth point of 7.7x108 spores/mL in 30 h compared to 72 h in the medium '3' with a longer period of adaptation and slow growth1.2x108 spores/mL and 34 h in the medium '1' (4.6x108 spores/mL)The growth rate bacterium in the medium '2' was 0.34 h-1, with a cellular yield of 56.77% and evidencing a 10.24% w/v of insoluble calcium in suspension, compared to 8.23% w/v of the medium '1' and 9.34% p/v of the medium '3'Comparatively, the calcium mineralization proportion of the medium '2' was only around 1 to 2% higher than the others media evaluated; and this formation may be due to the high pH value of the medium, where the CO2 in the air reacted with OH- in the medium resulting in the CO-2 formation, which reacted additionally with Ca2+ to form insoluble CaCO3The national base medium was more effective among the media evaluated for the production of cellular biomass of Bpseudofirmus, which includes the bacteria cellular growth, its metabolic capacity of CaCO3 production, which could be used to scale in industrial processes of spores with up to 75% efficiency and also can be used as an active principle of repairing biomaterials for microcrack seals, concrete mixtures and integral formulations.

Trabajos presentados al ***I Congreso Internacional de Biotecnología e innovación (ICBi)***, 9- 12 de julio de 2018, Universidad Nacional Agraria La Molina, Lima, Perú.

Editoras:
Ilanit Samolski Klein
Maria Lucila Hernández-Macedo
Gretty Katherina Villena Chávez

Contribution

Section II: Industrial and Environmetal Biotechnology

Mixed culture of Aspergillus niger and Trichoderma reesei for the production of cellulases by Surface Adhesion Fermentation with Bolaina Blanca residues

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Keywords: Mixed culture, solid state fermentation, cellulolytic enzymes.

Bolaina Blanca is a tropical tree from the Amazon of Peru, Ecuador, and BrazilWood processing residues of this species are being thrown into rivers and forestsHowever, these residues could represent a source for enzymes productionPrevious studies have indicated that mixed systems of Aspergillus niger and Trichoderma reesei can be more productive than individual strainsIn this context, the establishment of partners strains for Solid State fermentation (SSF) is needed to develop sustainable technologies for cellulase production with wood residuesTherefore, the aim of this study was to determinate inoculum conditions for mixed culture establishment on bolaina residuesHence, inoculum time of each strain was evaluated by using three native strains of Aniger and two native strains of Treesei (LMB-HP34, LMB-TM4.04, LMB-TM4.05, LMB-HL1, and LMB-HL2)In order to evaluate strain compatibility and simultaneous growth, confrontation cultures were performed with cellulase production agarTwo inoculum conditions were comparedIn the first experiment Aniger and Treesei were co-inoculated; in the second, Treesei was inoculated 24 h after AnigerThe results indicated that mixed cultures can grow cooperatively when granting a 24 h growth advantage to AnigerSubsequently, SSF flask cultures were prepared to evaluate the same inoculum conditionsFor this purpose, every flask contained 7.5 g of a chemically pretreated substrate and 6 g of soy cakeHumidity was regulated up to 80% with salt solution ($CaCl_2$ 0.03%, $MgSO_4.7H_2O$ 0, 03% and KH_2PO_4 0.2%)Cellulase activity (endoglucanases and total cellulases) showed that by inoculating Treesei 24 h later, the mixed cultures were able to secrete a greater amount of cellulases compared to the individual culturesThe results indicate that the optimal time of inoculation in mixed cultures is Treesei after 24 h of Aniger for the production of cellulases.

Contribution

Section II: Industrial and Environmetal Biotechnology

Evaluation of hydraulic residence time in a MBBR bioreactor for the treatment of effluents from the tannery industry

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Keywords: Removal, COD, BOD5, water resource, effluent

The environmental problem on water resources is growing every day, so one of the industries with great impact on this is the tannery industry, which uses a large volume of water along with various toxic chemical inputs and harmful to the environment for its activitiesThe effluents produced by this industry present different characteristic according to the stage of the tanning process, where the most outstanding are a high content of organic and inorganic matter, a pH between 3 and 12, salts, metals among othersAt present there are several alternatives for the domestic and industrial wastewater treatment that have been developed to reduce and mitigate the impact on the environmentThus in the present research work the Time of Hydraulic Residency (HRT) in the biological treatment of the effluents produced in the tannery sector has been evaluated using a MBBR bioreactor (Moving Bed Biofilm Reactor), in which microorganisms are developed in the biofilm form on inert structures that increase the surface area of contact per unit volume, allowing greater efficiency for the treatment of pollutants compared to other technologiesFor the development of the work began with the implementation of a bioreactor of 2.5 l of work volume in closed state until the development of native biofilm on Kadnees K3 type supports with constant aeration of 8 l/minAfter the biofilm generated, the removal of pollutants for hydraulic residence times of 4, 8 and 12 h in a continuous state was evaluated, monitoring the Chemical Oxygen Demand (COD) and Biochemical Oxygen Demand (BOD5)In addition the pH and Oxide Reduction Potential (ORP) during 30 d was evaluatedAt the end of the tests, removal percentages of up to 96% of COD and BOD5 were achieved for a HRT of 12 h, in addition was obtained an output pH of 8.4 and -32.3 mV for the ORP on average for both casesAs can be observed with the bioremediation process in the MBBR bioreactor, it has been possible to reduce the contaminants (organic and inorganic matter) present in the effluents of the leather tanning industry, this being a technology with great potential for eco-friendly application.

Trabajos presentados al ***I Congreso Internacional de Biotecnología e innovación (ICBi)***, 9- 12 de julio de 2018, Universidad Nacional Agraria La Molina, Lima, Perú.

Editoras:
Ilanit Samolski Klein
Maria Lucila Hernández-Macedo
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Contribution

Section II: Industrial and Environmetal Biotechnology

Assesment of biomass in mixed fungal biofilm cultures of Aspergillus niger and Trichoderma reesei through quantification of genomic DNA content

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Keywords: diphenylamine, lignocellulolytic enzymes, mixed biofilms cultures.

Production of lignocellulolytic enzymes with mixed biofilm cultures of filamentous fungi has many advantages over single cultures, probably due to the genetic regulatory responses that such interaction impliesProduction of cellulases with mixed cultures of Aniger and Treesei shows that enzymatic titers of total cellulases, endoglucanases and ?-glucosidases can be higher in comparison with individual culturesAlthough mixed cultures constitute a promising fermentation system, it has not been adressed how much of this enzymatic synergism depends on a certain proportion of fungal mixed biomass and what are the effects of growth rates and culture time on this proportionTo accurately perform a specific measurement of biomass, it is feasible to take advantage of the natural differences between the genomic DNA (gDNA) sequences of each fungus in order to quantify and correlate it with biomass weightHowever, this extrapolation assumes a direct relationship between gDNA and biomassTherefore, a method is required to verify whether this condition is met in the proposed culture systemTo this end, mixed fungal biofilms were formed by adding separately 1.5 % (v/v) of 1x106 spores per milliliter of Aniger ATCC 10864 and Treesei QM6a on a polyester support in relation 0.27 cm2/mL with the flask culture volumeAfter washing the non-adhered spores, the flasks were incubated at 28 °C and 120 rpm for 24, 48, 72, 96, and 120 hoursBiomass collected from mixed biofilms was used to calculate dry weight and to measure the amount of total gDNA by spectrophotometric method using the diphenylamine reagentFinally, both datasets were comparedIndirect quantification of biomass by total gDNA quantification shows a statistically significant correlation to direct quantification of biomass by dry weight when analyzed by the chi-square goodness-of-fit test, which indicates that there is a direct relationship between both methodsIn conclusion, it is possible to perform a subsequent extrapolation of the amount of biomass of each fungus in the mixed biofilm system from the gDNA quantification.

Contribution

Section II: Industrial and Environmetal Biotechnology

Evaluation of mining tailings as a source of sustainable energy through microbial fuel cells

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Keywords: Bioelectrochemical, power density, current density, voltage.

In the current context of the growing need for energy worldwide and environmental considerations that these entail, is it currently looking for technologies within the framework of renewable energiesAmong these we have bioelectricity which is produced in Microbial Fuel Cells (MFC); in this case the MFCs usesubstrates for degradation or conversion using the exchange of electrons and protons for mediated by electrogenic microorganismsOn the other hand, the mining industry, source of great economic activity, generates waste byproducts that are little used as tailings, which are mixtures of inorganic compounds, minerals, water, rocks and earth, but which can be used as a source of sustainable energy in bioelectrochemical systemsThus, in the present work, tests were performed in MFCs of H-type 700 ml capacity for each cell, connected with external resistances from 100 to 12000 ? and saline bridges, as well as open-circuit cells with graphite electrodesMining tailings were used as a substrate, which have high concentration of inorganic compounds and heavy metals for the production of bioenergy by processes of oxide reduction mediated by chemotrophic microorganismsWas determinated the power densities, current densities and voltage within the system for 700 h and the voltage produced by the cells was monitored with a Keysight 34972ª voltage data acquisition equipmentAfter the analysis, favorable results were obtained in the production of energy, where voltages of up to 0.24 V were obtained in open circuit cells, besides maximum densities of power and current 72 mA/m2 and 25 mW/m2 respectively for an external resistance of 4.7 k?Therefore, according to the results obtained, it has been possible to demonstrate the energy production from mining tailings in MFCs, which with future optimizations of the design parameters and materials could increase the power generated in order to produce low energy cost and environmentally friendly using waste by-products.

Trabajos presentados al ***I Congreso Internacional de Biotecnología e innovación (ICBi)***, 9- 12 de julio de 2018, Universidad Nacional Agraria La Molina, Lima, Perú.

Editoras:
Ilanit Samolski Klein
Maria Lucila Hernández-Macedo
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Contribution

Section II: Industrial and Environmetal Biotechnology

Identification of candidate genes neutral-alkaline cellulases from Aspergillus fumigatus LMB-35Aa

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Keywords: endoglucanases, biofilms, real-time PCR, Aspergillus fumigatus

Cellulases represent one of the main groups of industrial enzymesParticularly, neutral-alkaline cellulases have acquired more interest due to their applications in various industries such as food, textiles, brewery as well in the paper industryDespite its high demand, most of commercial cellulases have an optimum pH at acidic range, making it indispensable the search for neutral-alkaline cellulases producing microorganisms, as well as the identification of the genes responsible for this activityIn that sense, the objective of this study was the identification of potential candidate genes of neutral-alkaline cellulases from the strain Aspergillus fumigatus LMB-35Aa, isolated from a soil sample from the Tingo María-Perú forestBiofilm fermentation was carried out at 28°C and 37°C for 72 h and the endoglucanase activity, specific activity and enzymatic yield on biomass at pH 4.8 and 7.6 was quantifiedThe culture at 37°C showed an increase of 40% in specific activity and 30% in enzymatic yields at pH 7.6, with regards to the culture at 28°C, which could indicate a higher level of neutral-alkaline cellulase genes expression at 37° CThis was verified by expression analysis of 11 putative endoglucanase genes by real-time PCR (qPCR) comparing both temperaturesThe results showed that 3 of the 11 genes evaluated (Afu7g06150, Afu3g03950, Afu6g01800) could be involved in the neutral-alkaline activity of the strain, exhibiting 3 to 4 fold more expression levels at 37°C in relation to 28°CIn conclusion, 3 potential genes of neutral-alkaline cellulases were identified

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Contribution

Section II: Industrial and Environmetal Biotechnology

Screening of components for a cellulase production media for Aspergillus fumigatus in a deep-well plates as a submerged fermentation system.

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Keywords: Aspergillus fumigatus, bioprocess, optimization, experimental designs

Experimental designs have been used continuously in research and bioprocesses because they can be adopted during different stages within an optimization strategy to obtain an improvement in product yields, reduction of process variability, reduction of time and costs, the screening of variables of interest, etc., Therefore, its application had been recognized as a more efficient alternative to the traditional approach, such as one factor-at-a-time methodThe objective of this study was to determine the main factors with significant effect on the response variable, evaluated as endoglucanase activitySubmerged fermentation cultures were carried out in deep-well plates with Aspergillus fumigatus, inoculated with 3% v/v of an inoculum with 106 spores/ml in 0.8 ml of culture medium per well, this was replicated five times per experimental run (14 experimental runs in total)Then, endoglucanase activity was quantified by determination of reducing sugars with DNS methodSignificant effects on endoglucanase activity showed that carboxymethylcellulose, urea and peptone have the stronger effectHowever, the use of a better culture system would favor a decrease in experimental errorIt is concluded that a selection process such as the one that was carried out, would allow the design and formulation of culture medium, as an important step in the optimization of enzyme production bioprocess

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Contribution

Section II: Industrial and Environmetal Biotechnology

Evaluation of different variables in the production of lignolytic enzymes from Guazuma spresidues with Trametes polyzona LMB-TM5

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Keywords: Solid State Fermentation, Bolaina residues, lignolytic enzymes, de-lignification, Trametes polyzona.

The wood industry in Peru is mainly developed in the Amazon rainforest, generating large volumes of waste during the sawing processThe use of Bolaina wood, a specie used for reforestation, had generated large amounts of waste, causing a negative impact on the environment, polluting rivers, forests and with harmful effects for the public healthThe use of these residues for the production of enzymes or biofuels, would not only reduce the negative environmental impact, but also could represent an important economic source for the small producersThe present

Trabajos presentados al ***I Congreso Internacional de Biotecnología e innovación (ICBi)***, 9- 12 de julio de 2018, Universidad Nacional Agraria La Molina, Lima, Perú.

Editoras:
Ilanit Samolski Klein
Maria Lucila Hernández-Macedo
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study was focused on the production of ligninolytic enzymes with Trametes polyzona LMB-TM5 by Solid State Fermentation (SSF) with Bolaina residuesThe cultures were carried out in flasks with 5 g of residues and mineral saltsTwo nitrogen sources were evaluatedsoybean cake and ammonium nitrate, with different concentrations of copper (2, 3, 4 and 5 mM) to induce laccase activityWe also, evaluated the effects of particle size (0.5, 2.0 and 3.35 mm), inoculum size (5, 10, 15 and 20%, v/v) and incubation timeAccording to the results, the production of lignolytic enzymes was higher soybean cake as nitrogen source, with a particle size of 0.5 mm, 5 mM of copper sulphate and 15% (v/v) of inoculums (containing 150 mg of biomass)The highest activity achieved for laccase, manganese peroxidase and lignin peroxidase were 7.35, 72.1 and 1553 IU L-1, respectively, supporting that Trametes polyzona LMB-TM5 holds a high capacity to produce a variety of lignolytic enzymes by SSF with Bolaina residues.

Contribution

Section II: Industrial and Environmetal Biotechnology

Comparison of the production of colored pigments by filamentous fungi in submerged fermentation and surface adhesion fermentation

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Keywords: surface adhesion fermentation (SAF), biofilms, coloured pigments, filamentous fungi.

Coloured pigments come from natural or synthetic sources and have many applications in food, pharmaceutical and textiles industries, among othersPlants are the most commonly sources of natural coloured pigments, however natural colorants obtained from fungi have many advantages, because they can be grown in reactors without depending on the time of harvest and have a higher extraction yieldIn the biotechnological production of secondary metabolites, the submerged fermentation system (SF) is the most usedHowever, Surface adhesion fermentation (SAF) with biofilms (BF) or the solid-state fermentation (SSF) constitutes a biologically improved systemIn this sense, the objective of the present work was to compare the production of fungal coloured pigments in SF, SSF with inert support and BFFor production in SSF and BF, two media were used, potato dextrose and Czapek broth, with glucose and sucrose as a carbon source, respectivelyFor the production of coloured pigments the strains LMB-HP37 (Penicillium mallochii), LMB-HP33 (Penicillium maximae), LMB-HP19 (Penicillium mallochii), LMB-HP43 (Talaromyces brunneus) and LMB-HP14 (Talaromyces wortmannii) were previously selected by microbial bioprospectingBased on growth and production characteristics, experiments were performed in Czapek mediumFor SSF, perlite was used as a growth support, and polyester cloth for biofilmsColoured pigments production yields were considering the pigment unit (PU) as a function of biomass, which showed significant differences between SF and SAFThe strains LMB-HP37, LMB-HP43 and LMB-HP19 produced colorants in the three culture systems, unlike LMB-HP33 and LMB-HP14 whose have not produced any pigment in SSFHowever, it was observed that strain LMB-HP14 has a higher yield value in BF using Czapek broth, reaching values ??of 41.07 PU/gThe strains LMB-HP37 and LMB-HP43 have the highest production yield in submerged fermentation (40.75 and 36.02 PU/g respectively)While in SSF, 492.67 and 783.48 PU/g were obtained for the same strains, respectivelyTherefore the coloured pigment production yield between strains is different in the three cultivation systemsOf all the strains tested, LMB-HP37 and LMB-HP43 showed the highest yield in the three systems.

Contribution

Section II: Industrial and Environmetal Biotechnology

Tolerance of lignolytic basidiomycetes to chlorpyrifos and metamidophos under in vitro conditions

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Keywords: organophosphorus insecticide, xenobiotic compounds, biodegradation, fungi.

The presence of toxic residues of pesticides in agriculture could cause an irreversible effect on the quality of the soil, water and harvested products, so efficient efforts must be taken for their degradationSeveral studies have demonstrated the ability of white rot fungi to degrade and mineralize lignin by an extracellular enzymatic system; and because this metabolic capability they can be used for biodegradation of xenobiotic compoundsTen strains of basidiomycetes were isolated and / or reactivated from fruiting bodies collected in Choquequirao (Cusco) and from the Fungal Culture Collection of Laboratory of Mycology and Biotechnology (LMB)A factorial statistical design was used in which the type of organophosphorus insecticide (chlorpyrifos and methamidophos), the concentration of the insecticide (500, 1000 and 5000 ppm) and fungal strains were tested as factors, giving rise to 60 treatmentsControl were used in each case from which the percentage growth inhibition (PGI) was calculatedThe PGI was expressed as the relationship between the diameter of the control colony compared to the different treatments at 96 h of incubation, 28 °C ± 1°C by duplicate in Malt Extract Agar solid mediumThe analysis of variance showed significant differences at 95% reliability for the three factors tested as well as their interactionsThe main effects calculated from the marginal means show that, among the type of pesticide, chlorpyrifos (59.35% ± 17.17) was more toxic than methamidophos (13.86% ± 16.67) and therefore the one with the highest PGIAlso was observed that when the concentration of the insecticide increases, the percentage growth inhibition of the mycelium of the fungus increases, obtaining 25.89% ± 21.85 (500 ppm), 33.5% ± 27.04 (1000 ppm) and 50.43% ± 30.42 (5000 ppm), respectivelyRegarding the strains tested, the LMB-BAS 18 (30.51% ± 29.76) and LMB-E1 (30.77% ± 33.68) showed a lowest PGIAccording with these results, the simple effect of each factor and its levels were analyzed, resulting

Trabajos presentados al ***I Congreso Internacional de Biotecnología e innovación (ICBi)***, 9- 12 de julio de 2018, Universidad Nacional Agraria La Molina, Lima, Perú.

Editoras:
Ilanit Samolski Klein
Maria Lucila Hernández-Macedo
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that PGI for each type of insecticide at 500 ppm was 44.71% ± 0.7 with chlorpyrifos and 0% with methamidophos for LMB-BAS 18 and 45.8% ± 0.7 with chlorpyrifos and 0% with methamidophos for LMB-E1Therefore, these strains could be selected for biodegradation of pesticides.

Contribution

Section II: Industrial and Environmetal Biotechnology

Sodium Alginate from the kelps Macrocystis pyrifera, Macrocystis integrifolia and Sargassum sp(Phaeophyceae)

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Keywords: Macrocystis, Sargassum, alginate, kelps.

The cell wall of brown algae consists mainly of fucoidan, laminarian and alginateAlginates represent almost 45% of the dry weight of the brown algaeIn this work, three brown algae species, Macrocystis pyrifera, Macrocystis integrifolia and Sargassum has been chosen to obtain sodium alginate. The alginate extraction procedure comprised three stepsa) hydration, b) acidic pre- extraction and c) alkaline extractionBiomass powder was hydrated in to 0.1% formaldehyde solution during 12 h and then washed several timesThe acidic pre extraction was carried out with HCl 0.1N at pH 4 with constant agitationFor the alkaline extraction sodium carbonate solution at 10% was added to adjust pH to 10Samples were kept in a water bath at 80°C with constant agitation during 2 h, the pulp obtained was diluted with hot water and then vacuum filteredAlginate was precipitated with 96%, ethanol and dried up to 45°C during 12 hTo evaluate the gelling capability, a 3% aqueous solution (w/v) of alginate was allowed to drop on 0.5 M of calcium chloride solutionThe yields obtained with respect to the initial dry biomass of Macrocystis pyrifera, Macrocystis integrifolia and Sargassum sp., were 22% 33%, and 16%, respectively; According with these results, Macrocystis integrifolia presents the higher yieldFurther experiments will be performed in order to optimize an extraction protocol of alginate from brown algae.

Contribution

Section II: Industrial and Environmetal Biotechnology

Cellulases production by Trametes polyzona on orange waste as substrate

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Keywords: Cellulase, submerged fermentation, HPAC pretreatment.

Orange industry is one of the most important crops in the Peruvian marketAround 50% of the raw material consisting of peels, seeds and membranes are thrown away because of are considered wasteConsequently, disposal of orange waste by industry lead to serious environmental problemsHowever, lignocellulosic biomass from agroindustrial waste is an abundant renewable biological resource so that it can be used to produce secondary products in order to solve those environmental problemsCellulases are the group of hydrolytic enzymes responsible for release of sugars in the bioconversion of lignocellulosic biomass into a variety of value-added productsThe present study was aimed to evaluate the utility of orange waste, to produce cellulases with Trametes polyzona in submerged fermentation (SmF)It was also examined whether it was appropriate or not to use chemical treatment so that enzymatic activity can be facilitatedSubstrate pretreatment was made with hydrogen peroxide-acetic acid (HPAC) (1:1 v/v) solution at 80ºC for 1hTotal cellulase activity was determined by DNS methodMaximum titers of total cellulase activity were 0.244 U/mL and 0.542 U/mL in 5 days with pretreated and non-pretreated orange waste respectivelyAccording to these preliminary results, non-pretreated orange waste could be used as substrate for higher cellulase production in submerged fermentation with Tpolyzona

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Contribution

Section II: Industrial and Environmetal Biotechnology

Gene expression analysis of cellulases in mixed fungal biofilms of Trichoderma reesei and Aspergillus niger

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Keywords: Synergism, mixed cultures, cellulolytic enzymes, real-time PCR.

Trabajos presentados al ***I Congreso Internacional de Biotecnología e innovación (ICBi)***, 9- 12 de julio de 2018, Universidad Nacional Agraria La Molina, Lima, Perú.

Editoras:
Ilanit Samolski Klein
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A remarkable advance is being achieved in the discovery of new bioactive products and in the optimization of production processes of several metabolites through the co-culture of microorganismsPrevious studies show that the mixed fungal biofilms formed by Trichoderma reesei and Aspergillus niger are highly efficient for cellulolytic enzymes productionAlthough the genetic mechanisms that regulate the cellulase production in other individual systems have been described, the mechanisms that lead to the synergism in mixed biofilms are not deep knownIn this sense, the present work aimed at the analysis of differential expression of cellulases in mixed biofilms with respect to their individual culturesFor this, total RNA was extracted from mixed biofilms of Treesei QM6a and Aniger ATCC 10864 which were cultured in two stagesa first stage of pre-growth in presence of glucose 2% (w/v) and a second stage of cellulase production in presence of carboxymethylcellulose 1% (w/v), then cDNA was synthesized for q-PCR analysisThe Aniger genes analyzed by qPCR were two cellobiohydrolases (cbhA and cbhB), two endoglucanases (eglA and eglB), two ?-glucosidases (bglA and bglB) and two regulators (creA and xlnR)For Treesei a cellobiohydrolase (cbh1), a endoglucanase (egl1), two ?-glucosidases (bgl1 and bgl2) and two regulators (cre1 and xyr1) were analyzedThe differential expression analysis between mixed and individual cultures proved an increase of 53.6 folds for cbhB of Aniger in mixed biofilm with respect to its individual; and an increase of 55.5 folds for cbh1, 14.3 folds for egl3, 68.7 folds for egl1, 18.3 folds for bgl1 and 25.3 folds for bgl2 of Treesei in mixed biofilm with respect to its individualThese results correlate with endoglucanase quantitative activity76.88 U/L, 0 U/L and 104.5 U/L for Aniger, Treesei and mixed cultures respectivelyFrom these results, it is possible that there would be communication between fungal strains to achieve a synergistic expression of cellulase genesIt could not be considered as an enzymatic activity complementation, since the expression of ?-glucosidases of Aniger were downregulated in the mixed cultures and a higher expression was achieved for Treesei genes

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Contribution

Section II: Industrial and Environmetal Biotechnology

Production of fermentable sugars from lignocellulosic residues with Aspergillus niger

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Keywords: Aspergillus niger, chemical treatment, fermentable sugars

The production of second generation bioethanol presents different challenges; among them is the pre-treatment of lignocellulosic material used as raw materialIn order to use these residues with a better performance, a previous treatment is required for the delignification and reduction of the cellulose crystallinitySeveral chemical and physical treatments are used currently, whose effectiveness depends on the residues usedIn this context, the objective of this work was to obtain fermentable sugars through two chemical treatments, Alkalyne treatment with 2.4% NaOH at 120oC, and acid treatment with acetic acid and hydrogen peroxide (HPAC) (11 v / v) at 80 oCTwo agro industrial residues were evaluatedsugarcane bagasse and rice husk with a particle size between 0.5 to 2 mmFor fermenting sugars production, liquid cultures with a cellulolytic fungal Aspergillus niger ATCC 10864, containing 2 % (w/v) of sugarcane bagasse or rice husk and Resse and Mandels medium were performed at 28 °C and 175 rpmReleased sugars were quantified each 24 h by DNS methodThe results show that the chemical treatment with acid acetic-hydrogen peroxide (HPAC) was better for both substratesWith this treatment, Aniger was able to produce 65% more fermentable sugars than substrates without treatment at 24 h of culture with 0.994 g / L in sugar cane bagasse and 100% more in rice husks (0.802 g/L)From these, the treatment with acetic acid-hydrogen peroxide would be the better, not only for the percentage of released sugars, but also because reduces water consumption compared with alkaline methodBy other hand, main advantage of sugar cane bagasse is related to low cost and availability

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Contribution

Section II: Industrial and Environmetal Biotechnology

Cosmeceutical potential and bioactive compounds of commercial algal extracts

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Keywords: antioxidant capacity, algae,

Cutaneous aging is a complex, progressive and irreversible biological process, which includes two processesintrinsic aging and photo-agingThe latter can be defined as a set of macroscopic, microscopic, cellular and molecular skin changes, products of chronic and cumulative solar irradiationWithin the spectrum of radiation, UVB rays are the main cause of photo-damageThis damage is produced, in part, by the production of reactive oxygen species (ROS) that generate the oxidation of cellular constituentsMoreover, UV rays indirectly stimulate the transcription of metalloprotein genes, which degrade collagen and proteins of the dermal extracellular matrixIn this sense, the cosmeceutical industry seeks to prevent or reduce photo-aging by inhibiting the mechanisms UV rays actionIn the present study, the presence of bioactive compounds and the cosmeceutical potential of different species of continental and oceanic algae, including Chlorophyta, Phaeophyceae, Rhodophyta and cyanobacteria (7 macroalgae and 3 microalgae) and 4 different extraction processes (P1, P2, P3, P4) proportionated by PSW SA were evaluated in vitro testsThe antioxidant capacity was analyzed by ABTS and the total phenols by the Folin Ciocalteu methodFurther, enzyme inhibition was evaluated on Collagenase from Clostridium histolyticum (ChC – EC.3.4.23.3) and Porcine pancreatic elastase (PE – E.C3.4.21.36)The IC50 was found using the method proposed by Thring et

Trabajos presentados al ***I Congreso Internacional de Biotecnología e innovación (ICBi)***, 9- 12 de julio de 2018, Universidad Nacional Agraria La Molina, Lima, Perú.

Editoras:
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al(2009)Finally, 10 samples were selected according to their industrial application to perform photoprotection and MTT analysisPrimary Dermal Fibroblast (HDFa) ATCC® PCS-201-012™ cells were pre-incubated with the extracts and exposed to UVB irradiation at 50mJ/cm2 after that viability cell was measured by MTTWithin the different processes, the P3 shows the highest values of antioxidant capacity and total phenols, obtaining values of 23.9 mmol TEAC / 100g and 3828 mg AGE / 100gIn the enzymatic assays, the differences between the processes and the evaluated species were not observedHowever, all the extracts inhibit the enzymes evaluatedFinally, the photoprotection and MTT results allowed the establishment of AR2P3, AR2P4 and AV1P1 samples as those with greater cell viability valuesThe results obtained allow affirm that the AR2P3, AR2P4 and AV1P1 samples present a cosmeceutical potential and can be applied as inputs in the industry.

Contribution

Section II: Industrial and Environmetal Biotechnology

Identification and characterization of cellulases from Aspergillus niger ATCC-10864 obtained in three fermentation systems

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Keywords: endo-1,4-?-glucan glucanohydrolase, electrophoresis, gel-polyacrylamide, molecular mass, zymogram.

Cellulases are a multi-component system, consisting of three types of enzymesendo-1,4-?-glucan glucanohydrolase EC 3.2.1.4; cellobiohydrolase 1,4-?-D-glucan EC 3.2.1.91 and ?-glucosidase EC 3.2.1.21These act synergistically allows to obtain glucose from the hydrolysis crystalline celluloseCellulases are currently the third most important industrial and commercial enzymes in the world, due to their application in the processing of cotton, paper recycling, extraction of juice fruit and vegetable, extraction of oils and pigments from plant, preparation of detergents, biofuel production, brewing, food and feed industry and agricultureCellulases are produced by different microorganisms (bacteria, fungi, actinomycetes, aerobes and anaerobes)Each of these organisms can produce different types of cellulases which differ in their mode of action, biochemical properties, activity and stability at acid or alkaline pHThe objective of the research was to identify the cellulases produced by Aspergillus niger ATCC 10864 in submerged fermentation (FS), fermentation in biofilms (FB) and fermentation in solid state (FES)The fermentative process was carried out for 120 h and the FS liquid culture medium was contained the following composition in g l-1KH2PO4, 2; CaCl2.2 H2O, 0.3; MgSO4.7H2O, 0.3; urea, 0.3; (NH4)2S04, 1.4; peptone 1; lactose, 10; Tween 80 0.2 % (v/v) and (in mg l-1) FeSO4.7H2O, 5; MnSO4.2H2O, 1.6; ZnSO4.7H2O, 1.4; CoCl2.6H2O, 2In FB medium a support of inert perlites with liquid substrate similar to FS and in FES a pearlite support with a humidity of 74% was usedThe cellulolytic extract obtained was partially purified by ultrafiltration, then analyzed by SDS-PAGE electrophoresis and zymography to identify the extracellular cellulasesZymograms with cellulolytic extracts of FS, FB and FES show the highest expression of EGs at 72 h in the three fermentation systems; in FS, five endoglucanases (EGs) were expressed with molecular masses of 21 to 54 kDa, in FB eight of 20 to 80 kDa and in FES eight of 23 to 54 kDaThe EGs with the highest hydrolytic activity were 38 to 54 kDaThese molecular mass values ??of EGs are similar to the results obtained by other researchers with both extra and endocellular EGsIt is concluded that in the FB and FES systems, a greater number of subunits of EGs are expressed, since the growth of the fungi adhered to the solid surface is closer to its natural conditions, therefore the secretion of enzymes resembles more the secretion in natural stage.

Contribution

Section II: Industrial and Environmetal Biotechnology

Effect of cellulases in the extraction of red drop chilli carotenoids (Capsicum baccatum), Oxapampa province - Peru

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Keywords: Cbaccatum, carotenoids, cellulases, Fbiofilms, hydrolysis

The fruits of Capsicum baccatum contain a set of hydrocarbon and oxygenated carotenoids accumulated in the pericarp, including ?-carotene, ?-cryptoxanthin, zeaxanthin which are responsible for the yellow-orange color; while capsanthin and capsorribin give it the red color of the fruitCarotenoids are considered as natural ingredients that are used in the food industry, cosmeceutical, food industry for poultry and fish, etc., as well as their antioxidant properties and as pro vitamin A are used as chemopreventive molecules in the pharmaceutical industrySpecies of Capsicum baccatum and its varieties grow and develop in the tropical zones of the country, being a potential for carotenoidsThe importance and demand of these natural compounds have led to the development of new biotechnological proposals, one of them being the application of enzymes, as a viable alternative, safe and compatible with the environmentThe objective of this research was to improve the extraction of carotenoids from the fruit of the native chili fruit (Capsicum baccatum) using cellulases as pretreatment, followed by a leaching with organic solventsThe chili fruits were conditioned (dried, ground and sieved) and previously the Aspergillus niger ATCC 10864 cellulases production was carried out in fermentation in biofilms (FB)The cellulase extracts were used to hydrolyze the cellulose from the red drop chili pericarp, a substrate ratio was usedenzyme (1:15) and (1:20); agitation of 170 and 190 rpm for 2 and 4 h and 35ºCOnce the hydrolyzed cake was obtained, the carotenoids were extracted with organic solvents and the carotenoid content of the oil extracts was quantified by HPLCThe highest yield of oleoresin was 11.435%, with a significance of $p < 0.05$, it was obtained under substrate conditionsenzyme

Trabajos presentados al ***I Congreso Internacional de Biotecnología e innovación (ICBi)***, 9- 12 de julio de 2018, Universidad Nacional Agraria La Molina, Lima, Perú.

Editoras:
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1:20, 4 h of hydrolysis and stirring at 190 rpmIn addition, 8356.247 ?g / g of total carotenoids were obtained in the E8 treatmentIt is concluded that the application of cellulolytic enzymes improves the extraction of carotenoids from 8 to 10%, compared with conventional methods, since cellulases break the molecular structures of cellulose and allow a better release of the carotenoids distributed in the cell wall of the pericarp of red drop chili.

Keynote conference

Section III: Molecular Tools Applied to Biotechnology

New tools for multiplexed immunofluorescence: More colors and more dimensions, faster and easier

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Keywords: multiplexed immunofluorescence, Transparent Tissue Tomography, immunotherapy antibodies

While advances in flow cytometry allow a dozen cellular features to be detected at once, cell-cell interactions are lost in dispersed tissue, obscuring dynamic processes such as differentiation and immune response. Answering this challenge, novel 3D immunodetection methods are revolutionizing understanding of interactions and function of the diverse cell types that make up normal organs and malignant tumors. Much of the recent progress has come from pioneering studies of the brain, but 3D methods such as CLARITY may not match the dense cellularity and complex geometry of other organs. We have developed Transparent Tissue Tomography (T3) as a simple, gentle, fast and robust method to examine 4 to 6 targets at once in 3D. Briefly, tissues are lightly fixed, cut into thick sections, stained with cocktails of fluorescent probes, rendered transparent with fructose and imaged by confocal microscopy. Thereby, T3 enables imaging cytometry, where multiple features of cells including surface and intracellular proteins and messenger RNAs can be mapped in situ. Importantly, T3 is non-destructive, allowing the tissue to be fixed and sectioned for immunohistochemistry or dispersed for flow cytometry. We used T3 to map the distribution of parenchyma, vasculature and infiltrate in normal tissues and tumors. As a practical application, we examined T3 as a tool to improve prediction and monitoring of response to cancer immunotherapy. We also applied T3 to tracking delivery of immunotherapy antibodies to tissue (pharmacokinetics), binding to their targets, and measuring local effects on anti-tumor immune response (pharmacodynamics). Thereby, T3 provides a new and practical tool for research and clinical translation.

Keynote conference

Section III: Molecular Tools Applied to Biotechnology

Breaking Chromatin, Mutagenic Binding, and Other Oddities of CRISPR/Cas9 Genome Editing

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Keywords: Nucleosomes, dead Cas9 (dCas9), R-loop, Mutations

The bacterial Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) phage defense system has proven to be a powerful tool for genome editing in eukaryotes. The most common CRISPR enzyme used in genome editing is the CRISPR-associated Cas9 protein from Steptococcus pyogenes. Cas9 generates targeted DNA double strand breaks (DSBs) at genomic loci that are homologous to the 20 nucleotide long guide segment of the Cas9-bound CRISPR RNA (crRNA). Cas9 targeting and endonuclease activity also requires a short protospacer adjacent motif (PAM) immediately adjacent to the guide target. Steptococcus pyogenes Cas9 recognizes an 5'-NGG-3' PAM sequence. Cas9 recognizes a target sequence in DNA by first binding to the PAM sequence. Following PAM recognition, Cas9 unwinds the target DNA to promote the pairing of the guide segment of the crRNA with the complementary DNA strand. This results in an R-loop between the target DNA strand and the crRNA. R-loop formation between the crRNA and target DNA induces a conformational change in the Cas9 protein that triggers target DNA cleavage, which is exploited to induce random or targeted mutations at the targeted locus in genome editing applications. Unlike phage DNA, eukaryotic DNA is packaged with histone proteins into nucleosomes, the fundamental building block of eukaryotic chromatin. Nucleosomes and higher order chromatin structures are known to inhibit other DNA modifying and cleaving enzymes. Hence, a key question is to what extent nucleosomes and chromatin alter Cas9 activity and specificity when targeting eukaryotic genomes. To address this question, we tested how the packing of the DNA target into a nucleosome affected Cas9 endonuclease activity in vitro. As a substrate, we used a 289 bp DNA fragment containing the strong '601' nucleosome positioning sequence. This DNA fragment was efficiently cleaved by Cas9 when targeted by a complementary single guide RNA (sgRNA). However, when this DNA fragment was packaged with histone proteins into a nucleosome, Cas9 cleavage of target sites within the positioned nucleosome was greatly diminished. In contrast, a guide RNA targeting the accessible linker region adjacent to the nucleosome was able to direct efficient cleavage of the substrate. This result indicates that the packaging of the DNA target into a strongly positioned nucleosome inhibits Cas9 activity. However, a guide RNA (sgRNA3) targeting the edge of the nucleosome was able to direct Cas9 to efficiently cleave the nucleosome substrate, even though most of the guide RNA target was located within the nucleosome boundary. The PAM motif for this particular guide RNA was located outside of the nucleosome. Hence, we hypothesized that this particular guide RNA was able to direct efficient cleavage of the nucleosome substrate because the PAM was located in accessible linker DNA, as opposed to within the nucleosome core. To test this hypothesis, we tested a new guide RNA (sgRNA4), which also targeted the nucleosome edge, but in the opposite orientation as sgRNA3, so that the PAM of sgRNA4 was located within the nucleosome core. Cas9 activity was significantly inhibited on the nucleosome substrate when the PAM motif was inside the nucleosome (sgRNA4). In contrast, Cas9 efficiently

Trabajos presentados al ***I Congreso Internacional de Biotecnología e innovación (ICBi)***, 9- 12 de julio de 2018, Universidad Nacional Agraria La Molina, Lima, Perú.

Editoras:
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cleaved the nucleosomes substrate when the PAM was located outside the nucleosome in the accessible linker DNA (sgRNA3). These results indicate that nucleosome packaging primarily inhibits Cas9 activity by preventing recognition of the PAM. Hence, PAM accessibility appears to be a critical determinant of Cas9 activity in chromatin. However, our data suggest Cas9 can efficiently catalyze subsequent steps, including target DNA unwinding and guide RNA invasion, in the context of a nucleosome. An important question is to what extent nucleosomes and other chromatin structures inhibit Cas9 activity in cells. Subsequent studies by other groups confirmed our finding that strongly positioned nucleosomes inhibit Cas9 activity in vitro (and potentially in cells). However, less strongly positioned nucleosomes had a smaller impact on Cas9 activity in vitro. Moreover, ATP-dependent chromatin remodeling enzymes, which can move or evict nucleosomes, stimulated Cas9 endonuclease activity in nucleosomes. Alternatively, Cas9 may cleave a DNA locus when it becomes transiently accessible during DNA replication or transcription. Hence, it seems likely that Cas9 can efficiently cleave DNA targets located in strongly positioned nucleosomes and/or heterochromatin in cells through one or more of these mechanisms. We have examined how chromatin affects the Cas9 specificity by characterizing Cas9 activity in vitro at both on-target and off-target sites in nucleosomes. Cas9 targeted by sgRNA3 is able to efficiently cleave an on-target site assembled into a nucleosome because its PAM is located in accessible linker DNA. However, mismatch-containing guide RNAs were not able to direct efficient Cas9 cleavage of the nucleosome substrate at this same site, even though they could direct efficient cleavage of a naked DNA substrate. Presumably this is because the guide RNA-target DNA mismatch prevents Cas9-induced target DNA unwinding and guide RNA invasion specifically in the context of the nucleosome. We hypothesize that while Cas9 can efficiently induce R-loop formation between the guide RNA and the target DNA at on-target sites in nucleosomes, this becomes energetically unfavorable at mismatch-containing off-target sites. Hence, nucleosomes and other chromatin structures may enhance Cas9 specificity by inhibiting Cas9 activity at off-target sites. It would seem that modulating chromatin structure and the activity of chromatin remodeling or modifying enzymes could be a potentially important method for reducing background mutations during Cas9-mediated genome editing. Finally, we have investigated whether DNA binding alone by Cas9 is mutagenic. Many groups are using 'dead' Cas9 (dCas9), which lacks endonuclease activity, for a variety of different biotechnology applications, including altering the expression of specific genes, and altering epigenetic marks (DNA methylation, histone acetylation, etc.) at specific loci. It is commonly assumed that dCas9 targeted to a DNA locus is not mutagenic, since it lacks endonuclease activity. However, we speculated that the dCas9-induced R-loop might induce mutations, since R-loops are known to be mutagenic in other contexts. To test this hypothesis, we targeted dCas9 to the yeast CAN1 gene. CAN1 encodes an arginine permease, and is commonly used as a mutation reporter in yeast. The natural product canavanine, which is a toxic arginine analog, inhibits growth in wild-type yeast. In contrast, can1 mutant yeast are able to grow on canavanine-containing media (i.e., they are canavanine resistant), since canvanine is not transported into the cell in a can1 mutant. Our data indicate that targeting of dCas9 to the CAN1 gene can significantly increase the frequency of canavanine-resistant mutants in yeast. This effect was primarily associated with guide RNAs targeting the non-transcribed strand of the CAN1 gene, for reasons we do not yet fully understand. Sequencing of the CAN1 gene in canavanine-resistant isolates revealed that dCas9-induced mutations tended to cluster within or near the guide RNA target. The mutation spectrum for one of the dCas9 and guide RNAs combinations suggested that cytosine deamination rates are elevated on the non-target strand in the dCas9-induced R-loop, since many of the mutations at this target were mutations at cytosine nucleotides on the nontarget DNA strand, which adopts single-stranded DNA conformation in the dCas9-induced R-loop. The rate of spontaneous cytosine deamination to uracil is known to be greatly elevated (~100-fold higher) in single stranded DNA (ssDNA), such as ssDNA that forms in R-loops. Consistent with this hypothesis, the dCas9-induced mutation frequency was significantly increased (~8-9-fold) in a yeast strain lacking a key uracil repair protein (ung1?). Sequencing of the CAN1 gene in this strain revealed that most of the dCas9-induced mutations were cytosine-to-thymine mutations on the nontarget strand in the dCas9-associated R-loop. Taken together, our data indicate that dCas9 binding alone can induce mutations at the guide RNA target, likely in part through a R-loop mediated mechanism involving cytosine deamination. These findings suggests that dCas9 targeting may induce unexpected background mutations at the guide RNA target. We also tested whether dCas9 can induce mutations at a mismatch containing off-target site in the Can1 gene. Our data indicate that the dCas9-induced mutation frequency was reduced when targeted by a mismatch-containing guide RNA, although the mutation frequency was still significantly higher than background. Notably, mutation frequency was significantly elevated in a repair defective (ung1?) strain. These data suggest that dCas9 (or Cas9) binding can also stimulate mutagenesis at off-target sites, particularly in cells with lower or inhibited uracil repair activity. Since Cas9 binds at many more DNA sites than it cleaves, these findings may have important implications for the origin of a subset of background mutations during Cas9-mediated genome editing.

Keynote conference

Section III: Molecular Tools Applied to Biotechnology

Characterization of (Meta) genomes structures and dynamics using high-throughput chromosome conformation capture data

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Keywords: Metagenome, bacteria, meta3C

Metagenome sequence analysis relies principally on compositional approaches, which hypothesise that sequences sharing similar characteristics (GC%, codon bias, coverage-covariation, etc.) Although these approaches have generated important results, they remain somehow limited and do not allow the full characterization and understanding of the genetic composition of a complex microbial population. Contact genomics, which aimed at exploiting the 3D physical signature of genomes to solve their sequence, has the potential to alleviate or improve some of these caveats. To explore the genomic content of bacterial population at a new level of resolution we have recently developed meta3C, a derivative protocol of the chromosome conformation capture (3C) assay that aims at deciphering the average 3D organization of a genome. Using controlled mixes of bacterial or yeast species, we showed that the frequent collisions experienced by DNA molecules sharing similar cellular compartment can be measured through meta3C and conveniently used to assemble larger scaffolds of the genomes present in a metapopulation. Here i will present data obtained from different complex mixes of species of the gut microbiota of mouse. Meta3C allow unveiling hundreds of genomic compartments, hence species. Moreover, meta3C allow to

Trabajos presentados al ***I Congreso Internacional de Biotecnología e innovación (ICBi)***, 9- 12 de julio de 2018, Universidad Nacional Agraria La Molina, Lima, Perú.

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link phage sequences to their bacterial host and provide a convenient way to study interactions between genomic entities in a complex population. I will discuss the different way to explore this network and the results in light of the promising potential of the approach for future applications.

Keynote conference

Section III: Molecular Tools Applied to Biotechnology

Whole Genome Sequencing in Genomic Medicine

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Keywords: human genome, diversity, medicine

Accessing information about individual human genome variability has become affordable, and genetic information is increasingly important for medical decision making and health care related questions. Each human genome harbors approximately 5 million variants in comparison to the human genome referent sequence. The majority of this information is rare, occurring in less than 1% of the population, underscoring the unique constitution of each human genome. A portion of this rare variation may include pathogenic variants that influence health outcomes. Determining the pathogenic nature of rare genetic variation is based on segregation information within families. In the absence of segregation information, many variants are classified as being of unknown significance, indicating insufficient knowledge surrounding that variant. The American College of Medical Genetics and Genomics has identified 59 genes as being medically actionable. Pathogenic variants in medically actionable genes are returned to patients since this data can inform health care decisions. The medically actionable gene list includes both cancer related genes and genes linked to cardiovascular conditions. We examined findings in an ethnically diverse cohort of patients seen at Northwestern in Chicago. We specially studied the medically actionable genes and found that those of non-European backgrounds were more likely to have variants of uncertain significance compared to those of European descent. These findings underscore the need to establish larger human genome sequence cohorts so that more can be learned about the diversity of the human genome. Furthermore, under some conditions it may be reasonable to return results regarding variants of uncertain significance.

Keynote conference

Section III: Molecular Tools Applied to Biotechnology

Enzymatic degradation of pesticides, from mycology to nanotechnology

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Keywords: Pesticides degradation, ligninolitic enzymes, Fungi

Three decades ago, the World Health Organization estimated that three million cases of severe pesticide poisoning occurs each year, resulting in 220,000 deaths, occur each year. In addition, pesticide poisoning is the preferred suicidal method in rural areas. It is estimated that pesticide self-poisoning accounts for about one-third of the world's suicides, conservatively estimating a plausible range of 234,000 to 326,000 deaths from pesticide self-poisoning each year worldwide. In the past two decades, we were exploring the catalytic capacity of ligninolitic enzymes, mainly from fungal sources, for the pesticide transformation. Lignin, manganese, and versatile peroxidases, as well laccase from white-rot fungi were tested for the transformation of halogenated and organophosphorus pesticides. In fungal cultures, most of organophosphorus pesticides are effectively transformed by the cytochrome P450 enzymes. The use of cytochrome P450 for biomedical and environmental purposes was studied. Unfortunately there is not an effective treatment for severe organophosphorus pesticide intoxication. Some specific enzymatic treatments have been proposed, including direct enzyme injection, liposome and erythrocytes carriers, PEGylated preparations and extracorporeal enzymatic treatments. Nevertheless, no enzymatic treatments are currently available. A non-immunogenic enzymatic bioconjugate based on cytochrome P450 was assayed for organophosphorus pesticide transformation. Enzyme therapy is an alternative approach to inactivate pesticides in the bloodstream, transforming them into less toxic metabolites. A variant of cytochrome P450 (CYPBM3 F87A) from Bacillus megaterium was chemically modified with polyethylene glycol. The PEGylated enzyme showed enhanced pesticide transformation activity when compared with the unmodified protein. The transformation rates were higher than those obtained with the unmodified enzyme for all six pesticides transformed. The specific activity of PEGylated preparation for parathion and dichlorophen was up to 9-times higher than these obtained with the unmodified enzyme. In addition, the bioconjugate showed good catalytic activity in blood serum and innocuousness on immune cells. The potential use of PEGylated CYP as a detoxification strategy for pesticide poisoning is demonstrated and discussed. Finally, for environmental applications laccase and versatile peroxidase were encapsulated in chitosan nanoparticles in order to increase they lifetime under real bioremediation conditions. In addition, enzyme immobilization in innovative copper nanocages was performed and the catalytic properties were determined and discussed.

Trabajos presentados al ***I Congreso Internacional de Biotecnología e innovación (ICBi)***, 9- 12 de julio de 2018, Universidad Nacional Agraria La Molina, Lima, Perú.

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Contribution

Section III: Molecular Tools Applied to Biotechnology

Genetic diversity and population structure of Persea Americana Mill. at northwestern Mexico

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Keywords: Avocado, microsatellites, local genotypes.

Mexico is the main avocado producer and exporter. The area of avocado culture in Mexico consists of 175,939 Ha accounting for a production capacity of about 1.5 million t/year. Most avocado orchards in México are located in temperate areas and dedicated to the production of the Hass commercial variety. However, Hass avocado is not able to grow well in hot weather and low elevation or sea level soils, thus, avocado production in lowlands is limited. In this sense, local avocado germplasm is an invaluable resource for selecting and developing new varieties for their utilization in lowlands with high temperature. There is a large and unexplored stock of adapted avocado genotypes at northwestern Mexico. This represents an outstanding opportunity to explore new materials of local genotypes for both local and international avocado breeding programs, focusing mainly in selecting heat-tolerant genotypes with potential for commercial production both as a fresh product or as a source of material for further industrial applications. This research aims to study the genetic diversity and population structure of avocado (Persea americana Mill.) from local genotypes in northwestern Mexico. Fifty-nine local trees plus an avocado Hass control were genotyped by ten microsatellite loci analyzed by capillary electrophoresis. The genetic diversity averaged 10.5 alleles by locus, PIC value around of 0.68 and He ranging from 0.9 to 0.4. These values suggest a high genetic diversity. Population genetic structure clustered the 60 individuals into 3 groups. Our findings support previous studies of genetic diversity and suggest a high genetic diversity for local avocado genotypes in northwestern Mexico. Despite the high diversity, low levels of heterozygosity were observed in the populations, which suggest the presence of inbreeding.

Contribution

Section III: Molecular Tools Applied to Biotechnology

Bioinformatic analysis of polyphenoloxidase coding genes in avocado (Persea americana Mill).

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Keywords: Browning, Avocado, Gene.

The present work was focused on characterizing and identifying through the use of bioinformatic tools the genes coding for PPO in the genomes of Persea americana var. Drymifolia and Persea americana var. Hass. A search in the transcriptome and genome sequences of Persea americana var. drymifolia and var. Hass, it was possible to identify genes coding for PPOs. To achieve this, the annotation of the genomes was first carried out using AUGUSTUS and Maker-P. The BLAST algoritm and the annotated genomes of five plant species, distributed in different clades or phylogenetic orders were used as model genes in order to search ortologues in the predicted avocado coding sequences (CDS). Once those putative PPO proteins were identified in the avocado, the presence of the characteristic domains was confirmed, using the Pfam database (Bateman et al., 1999). Subsequent phylogenetic analyzes were performed using a maximum likelihood (ML) framework and the SeaView v2.4 software. In order to support the branches, the probability was estimated by performing a test (aLRT). The 3D structures of the identified PPOs in the avocado genome (P. americana var. Drymifolia and / or Hass var.) were modeled using the Swiss-Model (Liu et al., 2015). The superposition of the proteins was done using the SWISS-PDB viewer v4.1.0 program (Liu et al., 2015). Six genes coding for PPO were identified in Hass and three in Drymifolia. The number of PPO members in avocado is relatively similar to the species that present darkening. The PPO coding sequences are phylogenetically close to those of Musa acumta grouping within the same phylogenetic group. The polyploidy level of the analyzed species could direct the number of members in the PPO family, causing the loss and/or gain of genes in some species. The identified PPO sequences showed high similarity in the characteristic PPO domains, presenting variable regions in the copper binding domain B, thus possibly affecting the enzymatic activity but the Copper binding domain A resulted highly conserved. Only one of the identified sequences in Hass presented a slight difference in its tertiary structure and this was also observed in the corresponding phylogenetic analysis. This is the first report of the avocado PPO at the genetic level. Credits/ SIP Project 2018446: Propagation of non-commercial avocado and obtaining raw extracts with potential biological activity.

Trabajos presentados al ***I Congreso Internacional de Biotecnología e innovación (ICBi)***, 9- 12 de julio de 2018, Universidad Nacional Agraria La Molina, Lima, Perú.

Editoras:
Ilanit Samolski Klein
Maria Lucila Hernández-Macedo
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Contribution

Section III: Molecular Tools Applied to Biotechnology

Bioinformatic analysis of orthologs sequences to HMGR, VTE1 and VTE5 in avocado (Persea americana) genes

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Keywords: Avocado, α-tocopherol genes, β-sitosterol genes

The avocado fruit is of great importance worldwide, highlighting that Mexico is the main producer and exporter of the Hass variety. Avocado is a fruit characterized by the accumulation of lipids in the mesocarp. In the same way, previous studies report the accumulation of α-tocopherol and β-sitosterol in the fruit. This accumulation is dependent on the avocado variety, tissue-specific and is affected by the stage of development of the fruit. From a molecular perspective, a few genes related to the levels of accumulation of these metabolites have been identified. In this sense, the VTE1 and VTE5 genes, for α-tocopherol and the HMGR gene, for β-sitosterol, have been identified in different plants, including Arabidopsis, maize, tomato, potato and olive, as key participants during the biosynthesis of these metabolites. However, this information has not been elucidated in avocado, so, the objective of this work was to perform a bioinformatic analysis in order to identify and analyze the orthologous sequences of the VTE1, VTE5 and HMGR genes in avocado. The following methodological strategy was as follows: First, a compilation of the sequence of previously reported genes in model plants were built. Second, a local database was created using the Bioedit software with an avocado transcriptome and from this, local BLAST was performed to identify the orthologous sequences. Third, in order to verify that the identified sequences contain the typical traits of the proteins of interest, a search for conserved domains and ORF was carried out. For the VTE1 gene, three sequences were identified in the avocado transcriptome, which have the complete domain PLN02828, which belongs to the cl14571 superfamily of tocopherol cyclase. Likewise, avocado sequences were identified with high identity to the model VTE5 genes, with the conserved domain COG0170, which belongs to the family of phytol kinases. Finally, two unigene sequences were identified that could code for the HMGR gene, both sequences presented an ORF and in addition, they contain the motifs of substrate binding and NADP (H) binding which are highly conserved according to previous reports in other plants. By means of this first bioinformatic analysis for the identification of orthologous sequences to the VTE1, VTE5 and HMGR genes, it can be concluded that these genes are present in avocado and that they probably have more than one isoform, however, a more in-depth study is necessary perform to corroborate the presence of these genes in the avocado transcriptome. Credits: Project SIP: 20180446, "Propagation of non-commercial avocado and obtention of raw extracts with potential biological activity"

Contribution

Section III: Molecular Tools Applied to Biotechnology

Plasmid library construction through in vivo cloning in the yeast Saccharomyces cerevisiae

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Keywords: plasmid library, homologous recombination, gap-repair cloning

Plasmid libraries include a collection of cloned fragments with different sequences that can be used in several types of screenings. This work describes the construction of a plasmid library using an expression vector and in vivo cloning in the yeast Saccharomyces cerevisiae. The constructed library allows expression of oligopeptides of semi-random sequences of 18 amino acids. Each inserted fragment includes random nucleotides and sequences that code for tryptophan or tyrosine, so that the expressed oligopeptides have the sequence "Z-X-X-Tyr-X-X-X-Tyr-X-X-X-Tyr-X-X-X-Tyr- (Tyr or Trp)-Tyr" (where "Z" = Leu, Pro, His, Gln, or Arg and "X" is any amino acid. The fragments to be cloned were obtained by PCR using semi-random oligonucleotides and other primers. The library was constructed co-transforming yeast cells with the digested plasmid and the PCR products. The plasmids of several selected transformants were amplified, purified and subjected to nucleotide sequencing. This analysis demonstrated that 50% of the plasmids included fragments of the expected semi-random sequences. On the basis of these results it is possible to conclude that in vivo cloning in Saccharomyces cerevisiae is an efficient method for the construction of plasmid libraries.

Trabajos presentados al ***I Congreso Internacional de Biotecnología e innovación (ICBi)***, 9- 12 de julio de 2018, Universidad Nacional Agraria La Molina, Lima, Perú.

Editoras:
Ilanit Samolski Klein
Maria Lucila Hernández-Macedo
Gretty Katherina Villena Chávez

Contribution

Section III: Molecular Tools Applied to Biotechnology

Aptamer-based biosensors for the direct detection of infectious microorganisms

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Keywords: SELEX, Biomarkers, Malaria, Tuberculosis

Infectious diseases generate severe morbidity and economic losses annually, affecting millions of humans. Tuberculosis and Malaria are part of the most studied infectious/tropical diseases. They reported about 1.3 million and 445000 death globally for 2016. Although the clinical symptoms of both diseases are well characterized, point-of-care tests remain a challenge. In this work, an in vitro evolution technique was used to identify and develop aptamers for a new biosensing platform for rapid diagnostic of Malaria and Tuberculosis. First, we employed bioinformatic and bibliographic tools to identify abundant, well conserved biomarkers, three proteins from M. tuberculosis and 3 proteins for Plasmodium spp. were selected as targets. These targets were recombinantly produced using E. coli systems and high affinity ssDNA binders were identified by Systematic evolution of ligands by exponential enrichment (SELEX) and enrichment analysis from Next-Generation Sequencing (NGS) data. The ssDNA aptamer candidates obtained were chemically synthetized including a fluorescein reporter and the binding capacity of the resultant fluorescent aptamers to their respective target was probed by pull down assays using target-immobilized magnetic particles. For each aptamer two controls were tested in parallel as an affinity indicator, a no protein control and a non-specific protein control. Higher fluorescent signal in presence of the specific target that the controls was considered as an indicator of binding. Fluorescence imaging was used as a second technique for the assessment of aptamer to target binding. From 29 initial ssDNA aptamer candidates for Plasmodium spp. and M. tuberculosis, 9 showed differential binding to their biomarkers. The combination of combinatorial biology with next-generation-sequencing analysis provides a solid workflow the rapid identification of biosensors. The identified aptamers isolated in this work could be used to develop a direct detection assay in biological samples.

Contribution

Section III: Molecular Tools Applied to Biotechnology

Genetic screening for proteins that confer resistance to UV radiation or oxidants in Saccharomyces cerevisiae

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Keywords: overexpression, UV radiation, hydrogen peroxide, null mutant.

Because of its location and topography, Peru is one of the countries with highest levels of UV irradiation and therefore, it is crucial to develop more efficient sunscreens and antioxidants. Towards this end, this work was started to identify proteins that protect yeast Saccharomyces cerevisiae cells from exposure to UV irradiation or the oxidant hydrogen peroxide. The strategy followed relied on the availability of a high copy plasmid genomic library that includes all the genome of S. cerevisiae divided in fragments of 6 to 8 Kb. The plasmid used in the library is stably kept in high numbers in each cell (around 200 copies), thus allowing overexpression of the harbored genes. For the screen, yeast cells were transformed with the plasmid library and the transformants were assayed for increased resistance to UV irradiation or exposure to 10-20 mM hydrogen peroxide. The assay conditions were adjusted to obtain the optimum times of exposure. Several resistant colonies were initially identified and after further assays. In order to better characterize these selected resistant colony and improve the resolution of the UV and oxidant protection assays, three deletion mutants were constructed: rad9, rad24, and yap1. The rad9 and rad24 mutants exhibit high sensitivity to UV irradiation and deletion of YAP1, causes high lethality in the presence of oxidants such as hydrogen peroxide. It is expected that the assays with these mutants will allow a better characterization of the selected plasmids and improved the efficiency of the genetic screens. The plasmids of the six selected colonies were recovered and amplified in E. coli, purified and submitted to nucleotide sequencing. Comparison of the obtained sequences demonstrated that the fragments included in the plasmids were different, each having from 3 to 4 open-reading frames.

Trabajos presentados al ***I Congreso Internacional de Biotecnología e innovación (ICBi)***, 9- 12 de julio de 2018, Universidad Nacional Agraria La Molina, Lima, Perú.

Editoras:
Ilanit Samolski Klein
Maria Lucila Hernández-Macedo
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Contribution

Section III: Molecular Tools Applied to Biotechnology

An in vitro assay to identify NAD+, FAD or CoA as components of the capping structures of RNA

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Keywords: NudC, RNA capping

The flow of genetic information, as explained in the widely accepted central dogma of molecular biology, states that gene expression starts from DNA and then progresses to RNA and finally, to proteins. Each step is carefully regulated by complex cellular machineries, which guarantee the accurate progression of all events and the coordinated communication between cellular compartments. The passage from DNA to RNA (Transcription) is successfully accomplished after the synthetized RNA molecule undergoes a series of modifications that are necessary for its maturation. A key modification in eukaryotic organisms is the incorporation of an m7G cap structure at the 5'- end of each RNA molecule. This cap provides a layer of epitranscriptomic regulation and is involved in several features such as transcriptional elongation, splicing, translation, and mRNA stabilization. This type of capping modification was until recently, the only one identified but a few others have been reported, not only in eukaryotic organisms but also in prokaryotes. Interestingly, the RNA polymerase itself was discovered to introduce well-known enzyme cofactors as the first nucleotides in some transcription initiation events, for several genes. This new type of RNA processing was termed "cofactor capping". Up to this point, the consequences of this novel type of RNA capping in regulation are not understood, which makes the study of this topic of great importance. Cofactor capping of RNA has been reported in bacteria, and includes, nicotinamide dinucleotide (NAD+), flavin adenine dinucleotide (FAD), and coenzyme A (CoA). So far, it has only been possible the identification of NAD-RNA in eukaryotic organisms. Aiming to unravel the enigma of the possible presence of other coenzymes on the RNA 5' end in human cell lines, we set to establish a high-throughput method based on the use of the NAD+ decapping enzyme NudC. This enzyme was tested for its decapping properties to other types of cofactor-RNAs because of its hydrolytic activity on the pyrophosphate that links NAD with RNA. We cloned and expressed NudC-His6 fusions and showed that the purified enzyme effectively cleaves NAD+-, FAD-, and CoA- capping of in vitro synthesized RNA.

Contribution

Section III: Molecular Tools Applied to Biotechnology

Comparison of the photoprotective effects of commercial sunscreens using yeast cells survivability to UV irradiation

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Keywords: Sun-protection factor, SPF

Ultraviolet radiation (UV) is a fraction of the electromagnetic spectrum with wavelengths from 10 to 400 nm. On the earth surface, solar UV radiation consists mainly of UV-A (320 to 400 nm) and UV-B (295 to 320 nm), since penetration through the atmospheric ozone layer drops substantially the UV radiations of lower wavelengths. Nowadays, the deleterious effects of this natural physical agent are well recognized and the most commonly used protection method is the application of sunscreens. These products are categorized according to their "sun protection factor" (SPF), which provides a measurement of their effects against UVB rays and is determined on trials with human volunteers. Unfortunately, there is a great confusion in the meaning of the term SPF, which results in the inadequate use of sunscreens and the preference of products with the highest SPF values. Previous work in our laboratory resulted in the optimization of an assay that allows comparison of the photoprotective effects of sunscreens with different SPFs. This simple assay relies on the evaluation of the survivability of yeast cells subjected to UV irradiation, using serial dilutions. For the current work, this assay was used to compare the photoprotective effects of ten different commercial sunscreens with SPFs from 15 to 100, using both UVB and UVC irradiations. These assays were run using a wild-type strain and a rad9 deletion mutant, which is highly sensitive to UV radiation. Plates containing rich media were spotted with 400-, 2000- and 10000-fold dilutions of cultures of both strains. After an hour, each plate was covered with a cellophane sheet divided into four quadrants, each of which had been spread with a 2 mg/cm2 layer of sunscreen. For some of the cellophane sheets, a piece of aluminum foil was placed on one of the quadrants to provide a shield against UV radiation (positive control), and another was left without sunscreen nor aluminum foil (negative control). Subsequently, each cellophane-covered plate was treated with an adequate dose of UVB or UVC radiation. Our results demonstrate that under the conditions followed in the assays, no difference can be observed in the photoprotective effects of sunscreens with SPFs from 50 to 100. Since the sunscreens of higher SPFs have a considerable higher price, our results demonstrate the futility of paying extra money for sunscreens with high SPF. Accordingly, with respect to sunscreens, instead of considering their SPFs or brand names, it is more important to take into consideration their adequate usage: Generous and thorough application on the skin 20 minutes before exposure and re-application every two hours (or earlier depending on physical activity, amount of body sweat, contact with water, etc.).

Trabajos presentados al ***I Congreso Internacional de Biotecnología e innovación (ICBi)***, 9- 12 de julio de 2018, Universidad Nacional Agraria La Molina, Lima, Perú.

Editoras:
Ilanit Samolski Klein
Maria Lucila Hernández-Macedo
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Contribution

Section III: Molecular Tools Applied to Biotechnology

Identification of small peptides targeting pknG from Mycobacterium tuberculosis by structural and combinatorial biology approaches

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Keywords: Protein kinase G; X-ray crystallography; Phage display; Mycobacteria; Tuberculosis

The ability of Mycobacterium tuberculosis to hijack and survive inside the macrophage greatly contributes to its pathogenicity, latency and persistence. Evidence suggests that these bacilli induce alterations in the intra-phagosomal environment and inhibit phagosome maturation thus, allowing bacterial survival. The eukaryotic-like serine/threonine kinase pknG, encoded in M. tuberculosis genome, has been suggested to be essential for mycobacterial survival within macrophages, precisely by avoiding phagosome-lysosome fusion. Thus, inhibition of pknG activity could reduce bacterial fitness within the phagosome, making this kinase an appealing target for drug development. The presented study aims to develop high-affinity peptides for pknG by using the atomic structure of the kinase and combinatorial heptapeptide libraries as a source of potential binders. pknG wild-type (pknG-WT) and the rubredoxin-kinase domains (pknG-RK) were expressed for 16 hours at 18°C in Escherichia coli BL21. Both proteins were purified by affinity chromatography followed by size-exclusion chromatography. While pknG-WT did not result in the formation of crystals, pknG-RK was successfully co-crystallized with ADP by the hanging drop vapor diffusion method at pH 7.5 using PEG 4000 as precipitant. X-ray crystallography was used to solve the atomic structure of pknG-RK complexed with ADP at 1.85 Å resolution. The structure was determined by molecular replacement and refined to a free R-value of 0.196 and a work R-value of 0.269. The phage display (PhD) method was used to identify potential peptides that interact with pknG-WT and -RK. Three cycles of selection were performed, including a negative and positive selection per cycle. 108 peptide-displaying phages were used as an initial library. Selection stringency was conditioned by target concentration (55 – 200 nM), binding time (15 – 60 minutes), and washing steps. An increment trend of the eluted phages was observed from round 1 to round 3 (103 to 1010 PFU/mL). This multidomain protein represents a family of novel molecular targets that can be exploited for the discovery of new antibiotics for latent tuberculosis. High- throughput combinatorial methods based on the guidance of structural data will permit to rapidly discover novel therapeutic molecules that could reduce mycobacterial survival and pathogenicity by facilitating lysosomal delivery.

Contribution

Section III: Molecular Tools Applied to Biotechnology

Aptamers as inhibitors of bacterial protein synthesis

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Keywords: translation initiation, ribosome, SELEX, protein synthesis, antibiotics

The continuous appearance of antibiotic-resistant bacteria requires equally continuous efforts to identify novel inhibitory compounds. The ribosome is the target of a large number of commercially available antibiotics that arrest bacterial cell-growth or cause bacterial death. Thus, the ribosome can be surmised as an appealing target to further develop new antibiotics. Targets of known antibiotics include the 30S and 50S ribosomal subunits where multiple translational factors act in the different phases of protein synthesis. In the initiation phase, the action of initiation factors (IFs) is required to assemble a 70S initiation complex, ultimately leading to the formation of the polypeptide chain. Inhibiting the formation of the initiation complex could be achieved by constraining the action IFs. An opportunity to obtain potential inhibitors is using combinatorial biology techniques. We use: i) Systematic Evolution of Ligands by EXponential enrichment (SELEX), a combinatorial biology technique that selects high-affinity binding molecules (aptamers), for initiation factors, and; ii) in vitro inhibition assays to assess their potential as inhibitors. Purified initiation factor 1 (IF1) and initiation factor 3 (IF3) from Escherichia coli were used as targets for aptamers selection from a randomized library. Ten potential aptamers were identified and subsequently chemically synthesized, five molecules for IF1 and five for IF3. Fluorescent derivatives of the aptamers were used to test binding to their respective target by pull-down assays using target-immobilized magnetic particles. The assay included a control without protein (nonspecific binding to beads) and a non-targeted protein as a specificity indicator. The fluorescence associated to the magnetic beads was considered as a binding indicator. Out of the ten initial molecules, four aptamers showed binding to IF3 while only one for IF1. Then, we used Microscale Thermophoresis (MST) to determine the dissociation constants (KD) of the aptamer-target complexes. All four IF3 aptamers showed KD values in the range of 100 to 400 nM, while the IF1 aptamer did not showed any binding by MST. Furthermore, the inhibitory property of one IF3 aptamer was tested during the formation the 30S initiation complex, showing an apparent inhibitory constant of 200 nM. Altogether, the presented work provides solid bases to use combinatorial biology schemes for the development of new inhibitors of the protein synthesis apparatus.

Trabajos presentados al ***I Congreso Internacional de Biotecnología e innovación (ICBi)***, 9- 12 de julio de 2018, Universidad Nacional Agraria La Molina, Lima, Perú.

Editoras:
Ilanit Samolski Klein
Maria Lucila Hernández-Macedo
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Contribution

Section III: Molecular Tools Applied to Biotechnology

Bacteria lactic acid resistant to biliary salts and low pH in stool of newborns characterized with 16SrRNA

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Keywords: Newborn, BOX-PCR, Enterococcus

Recent research points to probiotics as beneficial microorganisms because they can be very effective in the prevention and treatment of many diseases and even better if these microorganisms belong to the native microbiota. The objective of this study is to characterize molecularly strains with probiotic potential isolated from stool of human neonates. Sixty feces samples of neonates were evaluated (0-3 days) and it were subjected to enrichment in Man Rogosa and Sharp (MRS) broth at 37° C / 24h. The strains were isolated and selected on MRS agar with bromine blue phenol and subjected to in vitro tests for tolerance to bile salts, resistance to low pH and antimicrobial activity against Escherichia coli ATCC25922, E. coli ATCC35218, Salmonella enterica and Listeria inocua by diffusion agar assay. Molecular characterization of isolates was carried out by BOX-PCR and 16S rRNA gene sequencing. A total of 48 strains were isolated and all of them showed resistance to pH 3 and 0.3% bile salts; only three strains showed antimicrobial activity against E. coli ATCC25922, one strain against E. coli ATCC35218, five strains against L. inocua and all of the strains against S. enterica. From all the strains, two BOX-PCR profiles belonging to Lactobacillus and Enterococcus genus were obtained. Nine strains (C52, C61, C71, C112, C16 2, C192, C20, C35, and C42) showed 100% of similarity to Lactobacillus plantarum ATCC 14917T [ACGZ01000098] and two strains (C15 and C40) showed 99.93% and 99.80% of similarity, respectively, to Enterococcus faecium CGMCC 1.2136T [AJKH01000109]. In conclusion, the Lactobacillus plantarum ATCC 14917 and the Enterococcus faecium CGMCC are the ones that have probiotic potential.

Contribution

Section III: Molecular Tools Applied to Biotechnology

Design and evaluation of DNA vaccines against the Tilapia Lake Virus (TiLV) in Oreochromis niloticus from Peru

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Keywords: aquaculture, gene expression, plasmid, neuroaminidase

Since its first apparition in Israel in 2009, the Tilapia Lake Virus (TiLV) has strongly affected Nile Tilapia aquaculture worldwide with cumulative mortalities ranging from 10% to 90%. First detected in Ecuador and Colombia, the presence of this emergent pathogen has been recently confirmed in Peru by SANIPES after being associated with O. niloticus massive mortalities. The production of a DNA vaccine has appeared as an interesting alternative to prevent mortalities and further spreading of the disease. The DNA vaccine named pTiLV-4 consists of a plasmid encoding putative neuroaminidase gene under the control of a cytomegalovirus (CMV) promoter. The prediction of the three-dimensional structure of the putative viral capsid proteins of TiLV such as hemagglutin and neuraminidase was carried out by a modeling analysis of biomolecules, where was evidenced structural homology between the TiLV fourth segment and the neuroaminidase of influenza virus C. The synthetic gene was cloned into a plasmid vector pCMV and the construction was confirmed by enzymatic digestion and sequencing. Tilapias of approximately 40g had been injected with polyethylenimine+pTiLV-4 or polyethylenimine alone and muscle tissues were collected at 8h, 16h, 24h and 72h to monitor putative neuroaminidase gene expression in vivo. The evaluation of the expression was carried out by RT-qPCR, finding a maximum expression at 16h. The next step will consider challenging experimentally vaccinated O. niloticus against TiLV suspensions prepared from infected tissues collected in Peru to evaluate the efficiency of the DNA vaccine.

Trabajos presentados al ***I Congreso Internacional de Biotecnología e innovación (ICBi)***, 9- 12 de julio de 2018, Universidad Nacional Agraria La Molina, Lima, Perú.

Editoras:
Ilanit Samolski Klein
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Contribution

Section III: Molecular Tools Applied to Biotechnology

OMICs; applications in pathogen diagnostic of aquatic species cultivated in Peru

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Keywords: Aquaculture, disease, Genomic, metagenomic, proteomic, metabolomic.

The generic term "omics" is a group disciplines of molecular biology that focus on the analysis of structure and function of the genetic material, expressed genes, proteins and low molecular weight metabolites. Moreover, these techniques also allow the study of complex mix of microorganisms obtained from environmental samples or may be used to discover unknown molecules. Their applications in modern biology is probably the major breakthrough of the last decades thanks to the growing availability of analytical high-throughput technologies. Since 2007, Omics tools have been successfully applied to the study of known and emergent pathogens, generally associated with mortalities in cultivated species such as the whiteleg shrimp (Litopenaeus vannamei), Peruvian scallop (Argopecten purpuratus) pustulose ark (Anadara tuberculosa), rainbow trout (Oncorhynchus mykiss), Tilapia (Oreochromis niloticus) and pirarucu (Arapaima gigas). PCR based techniques have been extensively used for the development of disease prevention programs in L. vannamei, for confirmatory diagnostic of numerous bacterial fish pathogens, but also for the identification by sequencing of specific DNA fragments of previously unreported pathogens in Peruvian bivalves (malacoherpesvirus, Perkinsus chesapeaki, P. beihainensis.) and fish (TiLV). The use of metagenomics allowed the understanding of complex pathogenic patterns by evidencing variations in diversity and abundance of circulating bacteria in shrimps and bivalves. Shotgun proteomics tools promise an exciting future for disease diagnostic by permitting an instantaneous scanning of multiple unknown pathogens without the need of previous genetic information or even of indications of their presences. Proteomic and metabolomic tools have also been applied to characterize pathogenic and antagonist microbial molecules in cultures and cocultures. Actually, it is even possible to visualize and quantify microbial molecules directly from colonies grown on agar or from tissues by Mass Imaging. Altogether, the availability of such spectacular techniques contributes to the advancement in aquatic pathogen diagnosis, and it can be hoped that they will allow the Peruvian aquaculture industry to prevent productive and socio-economic losses caused by infectious diseases.

Contribution

Section III: Molecular Tools Applied to Biotechnology

Difference in long range communication of the second PDZ domain of protein tyrosine phosphatase

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Keywords: Coevolution analysis, allostery, thermodynamic-structural method.

The second PDZ domain of Protein tyrosine Phosphatase from human (hPTPE1) and its homologous from Mus musculus (mPTPE1) are involved in the regulation of cell apoptosis. Malfunction of this protein perturbs apoptosis regulation and increases the risk of generating tumor cells, benign and malign (cancer). The human and mouse variants of this protein are very similar, identity percentage equal to 93%, identical folding, however, their specificity profile for ligands are different. To date, it is well known that this protein possess an allosteric mechanism to regulate its function, however, it is still unknown which residues are involved in this mechanism, and if the difference between the human and mouse variants only involves conformational changes or includes also changes in the protein dynamics. The present work uses computational tools, coevolution analysis and a hybrid thermodynamic-structural method (COREX algorithm), to get insights into regions or residues involved in the stability and long range communication mechanism of this protein. Results suggest the difference between hPTPE1 and mPTPE1 would be associated to two residues V40 and Q43 in the human protein, or I40 K43 in the M. musculus protein, consequently, these two residues would be responsible for their difference in their specificity profile.

Trabajos presentados al ***I Congreso Internacional de Biotecnología e innovación (ICBi)***, 9- 12 de julio de 2018, Universidad Nacional Agraria La Molina, Lima, Perú.

Editoras:
Ilanit Samolski Klein
Maria Lucila Hernández-Macedo
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Contribution

Section III: Molecular Tools Applied to Biotechnology

Specific allele PCR and RFLP as molecular techniques for the diagnosis of anticonvulsant resistance in epileptic patients

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Keywords: CYP2C9, Genetic Polymorphism, Allele

Single nucleotide polymorphisms (SNPs) constitute an important tool in biotechnology and molecular biology in the personalized medicine study. The variability between individuals in the response to drugs is a serious problem in clinical practice and in the development of drugs that could lead to therapeutic failure or, even worse, to adverse effects or death in individuals or subpopulations. Cytochrome P450 with its CYP2C9 subfamily, related to drug metabolism, has been linked to resistance to the treatment of epilepsy. The objective of this study was to implement molecular techniques to identify and report single nucleotide polymorphisms (SNPs), contributing to the success of the treatment. For this trial, 50 voluntary epileptic patients (VPE) between 18 and 60 years old, were recruited at the external consultation in the Neurology Service of the Hospital Regional Honorio Delgado Espinoza of Arequipa. Volunteers with not related disease were included in the control group. The samples were taken to the Laboratory of Molecular Biology and Experimental Pharmacology of the Faculty of Pharmaceutical, Biochemical and Biotechnological Sciences of the Santa María Catholic University, where the genotyping of CYP2C9 was analyzed. Initially, genomic DNA extraction of blood cells was carried out using the Silica Columns method, according to the supplier's recommendations. Then the concentrations of the samples, as well as their quality, were evaluated by means of fluorescence and spectrophotometry, respectively. The polymorphism identification was analyzed by PCR-RFLP sequence amplification technique, using the restriction enzyme Sau96I (CYP2C9 * 2) and specific allele PCR and primers designed to recognize the mutated nucleotide (CYP2C9 * 3). In the CYP2C9 * 2 genes, the heterozygous allelic variant CYP2C9 * 1 / * 2 and the homozygous allelic variant CYP2C9 * 2/2 were identified and 4% of the VPEs exhibited the C430T mutation, and 100% the allelic variant * 3 showed A1075C mutation. The results obtained in the present study indicate that 2 VPE with mutation in CYP2C9 * 2 allele and 50 VPE with CYP2C9 * 3 allele mutation, could present a slow metabolism for the antiepileptic drugs Carbamazepine and Phenytoin, being these the most used in the antiepileptic treatment. The techniques with which the polymorphisms were evaluated could become biotechnological tools, used in hospitals, in order to help in the rapid and accurate clinical diagnosis.

Contribution

Section III: Molecular Tools Applied to Biotechnology

In silico analysis of 334 mutation in SLC17A5 explain defects in lysosomal transport of sialic acid

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Keywords: Sialin, Sialic acid, Salla disease, i-Tasser.

Degradation of glycolipids and glycoproteins in the lysosome produce sialic acid and other acid sugars. Sialin (SLC17A5) is a membrane protein that transport the sialic acid from the lysosome to the cytoplasm. Mutations that cause total or partially inactivation of sialin lead to the accumulation of sialic acid in the lysosome generating severe anomalies in the neural development called free sialic acid storage diseases, an early lethal multisystemic condition known as infantile Salla disease (ISSD) or a non-lethal neurological condition known as Salla disease (SD). Some mutations inhibit the localization of the sialin in the lysosome preventing the sialic acid transport. Other mutations do not affect protein location but hamper the sialic acid transport to the cytoplasm also causing the disease. One of this mutation is P334R, in this work we used bioinformatic tools to try to understand how this mutation affect the sialic acid transport mediated by sialin. First, we produced a 3D model of the human sialin protein using i-Tasser server. Then, we used the autodock vina tool from UCSF Chimera software to produce models of the sialin in complex with its substrates Neu5Ac and Neu5Gc. Our models showed that the side chain of P334 is pointed to the channel. Taking in consideration that the sialic acid probably has a deprotonated carboxyl group, the presence of a positive charge in the channel produced by the substitution of a proline by an arginine could be promoting a strong interaction between the sialic acid and this residue hampering the correct excretion of the acid to the cytoplasm. In conclusion, the presence of a negative charge in the channel caused by P334R could be the explanation for the absence of transport in this variant. This information could help to better understand the molecular mechanisms involved in the disease and to develop a new gene therapy.

Trabajos presentados al ***I Congreso Internacional de Biotecnología e innovación (ICBi)***, 9- 12 de julio de 2018, Universidad Nacional Agraria La Molina, Lima, Perú.

Editoras:
Ilanit Samolski Klein
Maria Lucila Hernández-Macedo
Gretty Katherina Villena Chávez

Contribution

Section III: Molecular Tools Applied to Biotechnology

Design of an artificial bioproduction microfluidic system for protein synthesis

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Keywords: bioproduction, microfluidics, protein synthesis

The production of proteins is classically developed in recombinant organisms that include viruses, bacteria, yeasts and fungi. The scalability of the production usually requires expensive equipment and resources such as bioreactors, specialized laboratories and advanced methods in molecular genetics. The synthesis of cell-free proteins makes it possible to produce proteins without cellular requirements, however, their multiplexing can be a limitation for increasing the component production. Microfluidic systems have been proposed to replace devices used in molecular biology that include incubators, thermocyclers, sequencers, among others. The aim of this work is to design and evaluate a portable microfluidic system with specific parameters for cell-free protein synthesis and multiplexing to 30rx. The layers of the device were designed in Rhino v6.0 and AutoCad v21.0, consisting of 04 inputs and 01 output. The system includes a 4 input mixer, a 30 channel multiplexer, and an incubation subsystem. A Laser machine Trotec speedy-100 was used for layer manufacturing in acrylic and PDMS materials. A microfluidic pump was used to evaluate the flow rates in nanoliters of the mixture with the chemical staining technique. Was determined a design that integrates an analogous flow to the synthesis of cell- free proteins in a portable type of continuous multiplexing microfluidics device. Also, was established a flow rates in nanoliters for a type of organic chemical liquid that can include components analogous to plasmid (input 1), gene (input 2), enzyme (input 3), water (input 4) and processes included (1) premixed, (2) multiplexing in 30 rx, (3) incubation subsystem and (4) concentration of product of organic chemical component. The microfluidic system model designed works with an analogous process to the synthesis production of cell-free proteins and could be used to low cost mass production of proteins.

Contribution

Section III: Molecular Tools Applied to Biotechnology

Advances in the selection of frost tolerant genotypes in peruvian potato landraces (Solanum sp.)

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Keywords: potato, abiotic stressor, candidate genes.

Frequency of frost in the Peruvian highlands affect the native potato production and has an effect on farmer's food security. The objective of this research focuses on identifying genes for frost tolerance. Ninety accessions of native potatoes from the Peruvian departments of Puno, Cuzco, Huancavelica, Junín and Cajamarca were previously screened for frost tolerance in fields. Four plants from each accession were planted in pots and treated with low temperatures of -4 and -8°C for 1 h in a cold chamber. DNA isolation was performed for frost-tolerance allelic diversity analysis, using the RAD-seq technology. Twenty candidate genes associated with frost tolerance will be used to design primers for marker-assisted selection and improvement of native highland potatoes. All accessions tolerated the -4 °C treatment and only three the -8 °C (Piñaza, Locka and UNAQP-2350). These three accessions will be used for a differential expression analysis of frost tolerant genes, which will allow finding new genes from Peruvian potato landraces.

Trabajos presentados al ***I Congreso Internacional de Biotecnología e innovación (ICBi)***, 9- 12 de julio de 2018, Universidad Nacional Agraria La Molina, Lima, Perú.

Editoras:
Ilanit Samolski Klein
Maria Lucila Hernández-Macedo
Gretty Katherina Villena Chávez

Contribution

Section III: Molecular Tools Applied to Biotechnology

Bacterial groups associated with the microbial decomposition of Peruvian Anchovy (Engraulis ringens) identified by metagenomic aimed to the 16S rRNA gene

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Keywords: high-throughput sequencing, metagenomic DNA, Illumina bioinformatics, hypervariable region V4.

The Peruvian anchovy is a small pelagic fish 12 cm long, high in protein and polyunsaturated fatty acids Omega 3, considered the most important resource for the Peruvian fishery. This resource is highly susceptible to degradation by microbial action, drastically affecting the quality of its derived products for direct and indirect consumption. The objective of the work was to identify bacterial groups associated with the decomposition of the Peruvian anchovy fishery resource using high-throughput sequencing of the 16S rRNA gene. The methodology used was to evaluate the microbial diversity present in Peruvian anchovy samples incubated under two treatment of controlled temperature conditions 8°C and 28 °C, for 6 h. Samples were taken at the beginning and at the end of each treatment to proceed with the extraction of the metagenomic DNA present in each sample, using the commercial kit Power Soil. The DNA was evaluated through a PCR assay of the 16S rRNA gene. Subsequently, the shipment of 25 ?g of metagenomic DNA was made for sequencing of the V4 hypervariable region of the 16S rRNA gene using the Illumina MiSeq platform. The obtained information was processed and analyzed using QIIME bioinformatic software version 1.9.1, the sequences were filtered according to quality scores Q ? 30, and length ? 250 base pairs. The chimeras were filtered with usearch61, the non-chimeric sequences were assigned to OTUs (operational taxonomic units) with a 97% taxonomic identity using the SILVA v128 database and finally the OTUs with relative abundance less than 1% were filtered. The obtained results indicate the presence of bacterial groups strongly associated with the treatment conditions. In this way, samples stored at 8°C were colonized in 17% of relative abundance by the genus Photobacterium sp. On the other hand, samples stored at 28°C, showed a bacterial variety dominated by the genus Fusobacterium sp. represented in 68% of relative abundance. Another interesting result was to prove the reduction of the relative abundance (51%) of the genus Synechococcus sp., the abundance was reduced by 22% and 50.9% after each treatment 8°C and 28°C respectively. The results allow to conclude the presence of bacteria associated to the microbial deterioration of the Peruvian anchovy resource under the evaluated conditions.

Keynote conference

Section IV: Innovation in Biotechnology

Development of molecular tools by in vitro evolution technologies

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Keywords: Combinatorial Biology, in vitro evolution, Biosensors, SELEX, Phage-display

Pathogenic microorganisms negatively affect the economic development of societies by infecting crops, animals, and people. Directly or indirectly, infectious organisms account for millions of lives lost every year. The current strategies to cope with the infectious threats focus on the specific detection of the causing agent followed by targeted treatment. Available technologies for detecting infectious agents generally require infrastructure and specialized personnel, taking long times between sampling and availability of results. Altogether, the window for treatment application remains reduced, highlighting the importance of developing rapid detection platforms. Here, we use in vitro evolution technologies to isolate synthetic molecules that are capable of recognizing specifically a biomarker for a given pathogen. Two technologies are being deployed: i) Systematic Evolution of Ligands by EXponential enrichment (SELEX) that allow the selection of short single-stranded DNA or RNA, and; ii) Phage Display that allows the selection of short heptapeptides. Besides allowing the identification of high-affinity binders, the latter could also lead to the development of drugs for novel molecular targets. Proteinic biomarkers of Trypanosoma cruzi, Mycobacterium tuberculosis, Plasmodium spp, Escherichia coli, and others were recombinantly produced and isolated to purity to be used with both in vitro evolution technologies. Bioinformatic analysis indicated 132 potential novel molecules from which, 48 % showed experimental evidence of interaction with their respective biomarker. Further biochemical characterization by microscale thermophoresis and rapid kinetics provided dissociation constants in the nanomolar range. Molecules targeting essential bacterial proteins also showed strong inhibitory properties in vitro. Molecular dynamics and structural models of short peptides against a protein kinase from M. tuberculosis showed binding to the catalytic core of the biomarker. Altogether, SELEX and Phage Display are powerful technologies for the development of biosensors, allowing the specific recognition of molecular determinants of pathogenic microorganisms. Additionally, the techniques can also aim to develop new inhibitors of essential reactions of the pathogenic organism. Altogether, the molecules developed here are a small step to cope with the infectious threat at both fronts, rapid detection, and targeted treatment.

Trabajos presentados al ***I Congreso Internacional de Biotecnología e innovación (ICBi)***, 9- 12 de julio de 2018, Universidad Nacional Agraria La Molina, Lima, Perú.

Editoras:
Ilanit Samolski Klein
Maria Lucila Hernández-Macedo
Gretty Katherina Villena Chávez

Keynote conference

Section IV: Innovation in Biotechnology

Uso de NGS e indicadores genómicos para certificar origen acuícola en conchas de abanico

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Keywords: NGS, biotecnología, conchas de abanico

BioAl SAC es una empresa peruana que ofrece servicios biotecnológicos a la industria agroalimentaria, pesquera, textil y ambiental. Además de análisis cotidianos para la certificar la calidad y trazabilidad de productos, desarrollamos soluciones basadas en biotecnología para problemas productivos y/o comerciales. En este contexto, junto con la ONG Ecoceánica y la empresa Seacorp Perú SAC se investigó una estrategia para certificar el origen acuícola de stocks de conchas de abanico (Argopecten purpuratus), para darles valor agregado en un entorno de competidores que realizan pesca ilegal y mercados extranjeros que exigen garantías de sostenibilidad. Se usaron dos enfoques: (i) desarrollar marcadores genómicos que permitan identificar SNPs diagnóstico para trazar individuos a sus poblaciones de origen, incluyendo criaderos y (2) establecer valores poblacionales referenciales entre poblaciones silvestres y poblaciones de criadero. Para ello, se colectaron individuos de poblaciones silvestres a lo largo del litoral peruano y el norte de Chile, e individuos de poblaciones en cautiverio (reproductores y progenie del criadero Seacorp). Se prepararon bibliotecas genómicas usando la técnica RAD sequencing y secuenciamiento de alto rendimiento (NGS). La data obtenida con la plataforma Illumina fue analizada con diferentes programas bioinformáticos para determinar la presencia de loci outliers para las diferentes poblaciones, así como indicadores poblacionales como índices de diversidad, coeficiente de endogamia e índices de parentesco. En este estudio, y luego del análisis de 19,088 SNPs identificados en las regiones variables (RAD tags), no se encontró estructuración poblacional entre las poblaciones peruanas, tanto silvestres como en cautiverio. Esto posiblemente se debe a la alta tasa de traslocación histórica de semillas para acuicultura entre las poblaciones peruanas. En contraste, sí se encontró estructuración entre las poblaciones peruanas y la chilena. Respecto a los indicadores poblacionales, se encontró diferencias significativas en los índices de diversidad, coeficiente de endogamia e índice de parentesco entre las poblaciones silvestres y las poblaciones en cautiverio. Como esperado, las segundas exhibieron valores más bajos de diversidad genética y valores más altos del coeficiente de endogamia. Adicionalmente, los individuos de la progenie del criadero de Seacorp exhibieron altos valores de parentesco, consistente con su origen de pocos parentales compartidos. Con ello, se pudo descartar su origen silvestre. En conclusión, si bien no se logró encontrar SNPs diagnóstico robustos para las poblaciones peruanas, sí se logró desarrollar una herramienta confiable para la trazabilidad de stocks acuícolas usando indicadores poblacionales. Más aún, la masificación del uso de NGS y herramientas para la construcción de librerías permite que la aplicación desarrollada sea costo-eficiente para la certificación de stocks temporales para su importación.

Keynote conference

Section IV: Innovation in Biotechnology

Current applications of molecular markers: an opportunity for the development of knowledge and technologies in local genetic resources

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Keywords: molecular markers, microsatelites, NGS, differential gene expression, Arriba flavor

Modern DNA genotyping technologies allow broad genome analysis of cultivated plants, opening the possibility of associating and identifying molecular markers associated to traits of interest. The objective of this presentation is to present how these techniques are currently being used in INIAP from Ecuador in order to develop applied technologies which enhance the use of local genetic resources such as National Cocoa genetic resources. Regarding molecular characterization, INIAP has technology for germplasm genotyping of different crops of interest, mostly plants of importance for research projects and agricultural production. The case of cacao will be focused since this crop is one of the most important for Ecuador and the country is the world's largest producer of fine aroma cocoa with its National variety known for its "arriba flavor". In fact INIAP has one of the most important germplasm banks of cocoa in the world; integrated of several collections of great interest for the breeders and cocoa genetists. However, a lack of an appropriate molecular characterization of this germplasm has limited its potentiation and utilisation. In a first part of the conference the results of molecular characterization of the National Cacao Genotype Collection (CGN) will be presented. DNA genotyping tree by tree with a set of highly discriminating SSR markers allow to identify the type and out types trees of each accesion. Genotyping is an important tool for the management of this germplasm. Additionally, a second study in a collection of old materials of National cacao will be presented. In this case molecular markers are used for the search of clones with higher levels of National genetic purity. Finally the results of genetic identity of commercial National clones will be presented. In this case molecular markers are used for genetic certification of these materials. In a second part of the conference the results of a project regarding the genetic expression associated with aromatic quality in INIAP National clones will be presented. The objective of this study is to characterize the genes expressed in the beans from five commercial clones which come from a rigorous selection process of fine-flavored landraces in Ecuador. Here we used a transcriptome analysis using RNA-seq technology which is carried out at different stages of beans development in each commercial clone (18, 20 and

Trabajos presentados al ***I Congreso Internacional de Biotecnología e innovación (ICBi)***, 9- 12 de julio de 2018, Universidad Nacional Agraria La Molina, Lima, Perú.

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22 weeks) issued from programmed self-pollinations. Mature beans are submitted to microfermentation of 24 and 48 hours completing five different stages for transcriptome analysis for each clone. RNA extractions are taken from a bean pool for the fruits obtained from self-pollinations in each genotype. Next cDNA libraries are obtained for transcriptome sequencing using Next Generation Sequencing (NGS) technology of ILLUMINA-Hiseq. Bioinformatic analyses are performed using the reference genome Criollo (B9761/B2) and the genomic tools available for cocoa genomics. The analysis of genetic expression carried out at the moment allowed us to identify a set of genes expressed in each condition for each genotype; and the quantification of the expression of genes involved in terpenoid biosynthesis pathways and phenolic compounds, both processes are important biochemical compounds in the aromatic quality of cocoa bean. On the other hand, the differential expression analysis has not identify genes associated with these biosynthetic pathways, however the expression of non-annotated genes in Criollo genome were revealed. Based on the results so far, in a second phase of this project, we have integrated biological replicates as well as genotypes of higher genetic contrast. We are also looking to analyze the genetic expression from the pulp tissue which it is presumed that a higher expression of certain monoterpenes genes attributed to the aromatic quality beans of National cocoa. This has not been determined or quantified yet. With this presentation we expect to give a global vision of the status of current application of molecular markers technology in one of the crops of great importance for Ecuador.

Keynote conference

Section IV: Innovation in Biotechnology

Viruses in the cultivation of plantain and banana in Peru and the use of IC-PCR in a participatory strategy for the production of quality seed

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Keywords: Plantain, IC-PCR, episomal, BSV, virus, positive selection

Peru is among the top 10 countries in the world with the highest plantain and banana production and is considered the second among Latin American countries. Banana and plantain crops are characterized as agricultural products of great expansion in the jungle and northern region of the country. On the other hand, Peru is one of the main exporters of organic bananas, whose productions occur along the northern coast. However, Palillo variety (AAB, subgrupo Plantain) is an underutilized crop, despite its high content of carotene (vitamin A), complex B and a long list of minerals. Some viral diseases have caused significant losses in terms of fruit quality and production and have become serious limitations in the growth of the banana and banana industry. Surveys carried out in the Tumbes region, in northern Peru, report that the main viral problem in organic banana production areas is Banana Streak Virus (BSV). The identification of BSV includes several methods such as visual inspection of symptoms, identification of viral particles by electron microscopy from partially purified sap extracts, serological methods (ELISA, Enzyme-Linked Immunosorbent Assay) techniques based on the detection of nucleic acids that include dot-blot hybridization, polymerase chain reaction (PCR), real-time PCR (RT-PCR) and immunocapture-PCR (IC-PCR) [Le Provost et al., 2006], the latter technique is a combination of an ELISA with a PCR. The polymerase chain reaction (PCR) has been used in numerous studies for the rapid, sensitive and reliable detection of BSV. When performed from total Musa DNA it can not discriminate the episomal form of the virus from its sequences integrated into the Musa nuclear genome. In 1998 Harper et al., Proposed a procedure for the episomal detection of BSV where the immunological capture of virus particles is combined with antibodies prepared against a great diversity of isolates and the amplification of DNA fragments by PCR (ICPCR). The main advantage of the IC-PCR method over conventional PCR is that it can differentiate the detection of BSV in relation to false positives due to the integration of the virus in the banana genome [Harper et al., 1999]. This method is currently applied in the sampling and epidemiological surveillance of banana germplasm, produced from quarantine materials and to assess the total extent of BSV infection. In a comparison experiment of three different techniques for the detection of BSV, serological test (Tas ELISA), molecular test (PCR), and IC-PCR test, it was found that the IC-PCR technique was the best with respect to to the previous two for their sensitivity and efficiency. Positive selection was made with the participation of farmers, in Palillo fields of the district of Pichanaqui in Center Jungle. Leaves samples from selected plants were analyzed molecularly by IC-PCR for CMV y BSV, in an efficient, fast and low cost way. Select and negative plants for viruses were multiplied for sprout optimization technique to which the plant tissue formed by the pseudostem and the roots was removed, leaving only the corm, which was disinfected in boiling water for 30 seconds, to be later planted in rustic thermal chambers, where the shoots of the corm produced seedlings more easily, the seedlings were bagged and later transferred to a shed of anti-aphid mesh for acclimation and the seedlings produced were sown in seed fields. The multiplication rate calculated was from 1 to 5, that is, from each corm planted in thermal beds, 5 seedlings were obtained, which in the seed field produced 10 seedlings each. If you start with only 100 corms, you will have 50,000 seedlings at the end of two campaigns in the seed field. Six % of the plants selected for the absence of viral symptoms gave positive for BSV, only two plants were positive for CMV, according to what is found in the organic banana production areas in the north of the country, the main viral problem is banana is BSV. 1280 seedlings were sown in seed fields, from virus-free elite plants. Conclusions: The system allows to obtain elite material free of viruses in the plantain crop efficiently, economically and quickly. The system can be used in other Musa crops such as Manzano (AAB), Seda (Gros Michel, AAA), Isla (AAB, subgrupo Iholena), and other varieties. It is important to involve the farmer in the selection of elite material. Symptomatological evaluation has an efficiency higher than 94% to select plants free of virus in Pichanaqui conditions. The main viral problem in the banana crop in Center Jungle is BSV.

Trabajos presentados al ***I Congreso Internacional de Biotecnología e innovación (ICBi)***, 9- 12 de julio de 2018, Universidad Nacional Agraria La Molina, Lima, Perú.

Editoras:
Ilanit Samolski Klein
Maria Lucila Hernández-Macedo
Gretty Katherina Villena Chávez

Keynote conference

Section IV: Innovation in Biotechnology

Biotechnology as a national Startup

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Keywords: Mission-oriented, policy, Biotechnology, Spin-Off, Startup

Worldwide science is going through a golden age. For the first time in human history, the access to the latest discoveries is not restricted to the academic or scientific community. Nowadays, everyone can query the internet for up-to-date research, technological developments or inventions. The impact that such access can bring to the biotechnological industry is enormous, where every stakeholder could maximize the overall effect in society. Biotechnology in Peru is at its beginnings, despite several limitations, it could benefit from the rapid growth that is characteristic of small entrepreneurships. In a sense, Peruvian biotechnology, as a whole, could be seen as a startup. In this way, we can introduce startup-like tools in the national system to promote rapid growth and competitiveness. For the identification of opportunities, we must be prolific and fast to spot blue oceans (markets where there is a greater potential for development) from the beginning. Such opportunities could greatly benefit from the application of tools of mission-oriented policies for innovation. In Peru, four perspectives may be crucial to find markets with societal impact: i) Attention to endemic problems to Latin America would provide missions with social impact; ii) Focus on the potential of local biodiversity for marketable missions; iii) Application of emerging technologies that have shown a remarkable potential to create a new industry or transform significantly existing ones, and; iv) Market assessment from the beginning of any entrepreneurship. In addition to these, it is necessary to carry out complementary activities: i) Technological forecast to see trends of problems and opportunities; ii) strengthen existing strategic resources (management, professional expertise, infrastructure); iii) Technological surveillance, to monitor the current state of development and to assess timely indicators, and; iv) Funding programs oriented to the biotechnological market, with a foreseen marketable solution for a given opportunity/problem. The Peruvian biotechnology ecosystem is restricted to a few stakeholders, and, this could be taken as both, a weakness or a strength. The latter is appealing, requiring few interventions that could start up a competitive and sustainable biotechnological innovation system.