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Microbial Biotechnology & Vaccine Design

September 17-18, 2018 Lisbon, Portugal

Posters

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Study on the fermentation process of dalesconols A and dalesconols B by marine-derived fungus

Xingchen Wei, Zhenghua Pan and Yanhua Lu
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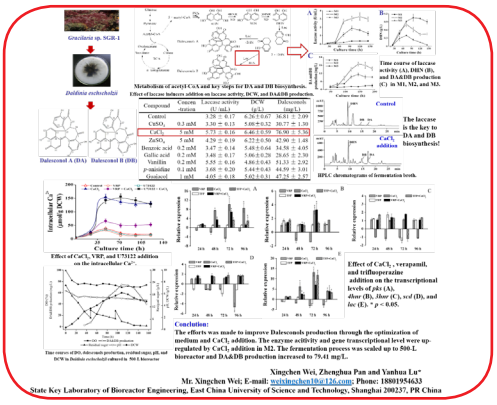
Dalesconols A (DA) and Dalesconols B (DB) are two skeletally unprecedented polyketides isolated from the marine-derived fungus Daldinia eschscholzii IFB-TL01. They are of significant immunosuppressive activity comparable to that of cyclosporine A (CsA), which is a clinically used immune-compromising compound. However, their further pharmaceutical researches have been severely restricted by the low production from submerged fermentation. Allowing for the urgent need in looking for new immunosuppressant, it is quite necessary to improve DA and DB production from the fermentation process. In this work, the effects of different media on fungus growth, Dalesconols biosynthesis and metabolites were firstly tested and compared. Additionally, the detection as well as analysis of four organic acids, intermediates DHN and the laccase activity analysis experimentally confirmed that laccase was the key role on Dalesconols biosynthesis. Based on the biosynthetic pathway of Dalesconols, DA and DB production was supposed to be elevated by the regulation of secondary metabolism in D. eschscholzii. Ca2+ induction was employed to up-regulate of laccase activity and further enhanced Dalesconols production (76.90 mg/L), which was 122.8% higher than that in the control. Ca2+ channel and calmodulin inhibitors were applied to investigate the involvement of calcium/calmodulin signaling in regulating Dalesconols production. The transcriptional levels of Dalesconols biosynthetic genes were up-regulated after CaCl2 addition and down-regulated after inhibitors were added. The results demonstrated that Ca2+ addition induces Dalesconols biosynthesis through up-regulation of Dalesconols biosynthesis genes via regulation of calcium/calmodulin signaling. Then, the fermentation process will scale up to 500-L bioreactor and Dalesconols production reached 79.41 mg/L. The information obtained in this work would be helpful to the large-scale production of DA and DB and other marine-derived secondary metabolites.



Biography

Xingchen Wei is a PhD student in State Key Laboratory of Bioreactor Engineering, East China University of Science and Technology, China. He devotes himself to study on the marine microbial fermentation process, especially marine-derived fungus. His main research involves gene transcription levels, protein expression levels, enzyme kinetics, cell metabolic level and reactor level.

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Transposon insertion sequencing identified a novel horizontal transfer nucleoid associated protein regulating virulence

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Horizontal acquired genes play important roles in bacterial chromosome evolution and they are typically grouped together in blocks termed genomic islands. Transposon Insertion Sequencing (TIS) is a powerful high-throughput genetic technology that facilitates exploration of conditionally essential genes. Using this technique, a horizontal acquired gene named enrR was identified in *Edwardsiella piscicida*, which is one of the chief infectious threats for farm-raised fish. The mutant of enrR can decrease the expression of whole T3SS and T6SS gene islands, thereby attenuate its virulence in host. With comprehensive analysis of its function by ChIP-seq and RNA-seq, we discovered an interesting phenomenon that the working areas of protein EnrR in genome are closed to GIs (genomic islands) and EnrR can repress their expression by binding to their nearby regions. We finally identified EnrR as a nucleoid associated protein with non-specific DNA binding ability and DNA aggregation ability *in vitro* and its binding ability with DNA is dependent on the length of DNA fragments. As a horizontal transfer regulator, EnrR can also enhance virulence in Salmonella. Our study discovered a universal significant horizontal-transferred virulence activator, which deepens our understanding for bacterial virulence evolution.

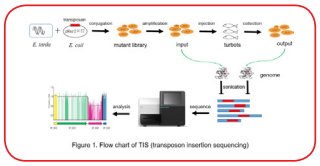

Figure 1. Flow chart of TIS (transposon insertion sequencing)

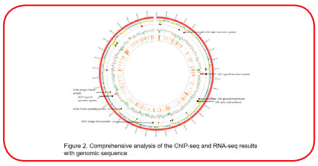
Figure 2. Comprehensive analysis of the ChIP-seq and RNA-seq results with genomic sequence

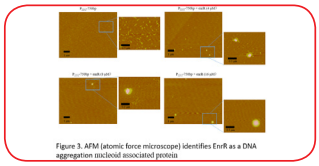
Figure 3. AFM (atomic force microscope) identifies EnrR as a DNA aggregation nucleoid associated protein

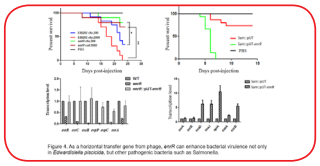
Figure 4. As a horizontal transfer gene from phage, enrR can enhance bacterial virulence not only in Edwardsiella piscicida, but other pathogenic bacteria such as Salmonella.

Biography

Ruiqing Ma is a PhD student of East China University of Science and Technology focusing on marine pathogenic microorganism. He devotes to identify new virulence regulator using sequencing technology including TIS, ChIP-seq and RNA-seq, which is used for developing new attenuated live vaccine. His project is focused on Edwardsiella piscicida that is a bane for aquaculture industry.

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Evaluation of fecal indicator bacteria in a National Park river to asses human impact on the conservation of a protected area

Blanca Perez-Uz[1], Elena Alonso Fernandes[2] and Mercedes Martin-Cereceda[1]
[1]Universidad Complutense de Madrid, Spain
[2]Centro de Investigaciones Biológicas, Spain

Statement of the Problem: The main aim of National Parks is the conservation of outstanding natural resources which must be compatible with other social, cultural and educational purposes. Recreational activities involve an increasing avenue of visitors which cause difficulties to balance tourism with preservation during certain times of the year. The Guadarrama Protected area was recently (2013) erected as a National Park (Madrid). The administration of the park requires information to manage the environmental impact of visitor activities on the main conservation purpose. In this context, we studied Fecal Bacteria Indicators (FIB) in three points of the main river crossing the park (River Manzanares). The objective was to assess the influence of visitors' affluence to the park on FIB counts in different seasons of a year as well as to evaluate the short-term effects on the river.

Methodology & Theoretical Orientation: Three points were selected in the river based on differential accessibility to visitors during four sampling dates in a year. Samples were taken in two dates during the same week to count total bacteria, total coliforms, fecal coliforms, enterococci and sulfite-reducing Clostridium. Sampling procedures were repeated four times in a year during different seasons. The main hypothesis was that visitors would influence in the FIB populations.

Findings: The bacteria highest abundances were found after a weekend in the summer season when visitors' affluence was the highest. Total aerobic counts (22 °C and 37 °C) and total coliforms remained stable or increased from Monday to Thursday sampling dates of each campaign. However, fecal coliforms and enterococci always decreased their abundance from Monday through Thursday which showed that these were preferentially eliminated or had a low short-term survival in the river compared to other bacterial groups. No significant differences were found for enterococci when sampling points were compared.

Conclusion & Significance: Recreational activities in the park have indeed an effect increasing bacterial populations in the river. These bacteria were even raised in the short-term period of study after a weekend; however, main FIBs were significantly reduced during this term.

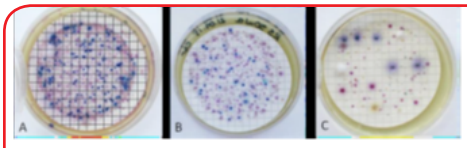

Figure 1. Filters on Chromocult® agar to assess total and faecal coliforms on the first day after a weekend in three of the sampling campaigns: July (A), September (B) and February (C). 50 ml volumes were filtered from the last sampling point (R3) where the highest bacterial concentrations were found. Pink colonies are total coliforms and blue are *E. coli* (faecal coliforms).

Biography

Blanca Perez-Uz has her expertise in microbial ecology and the use of microbial bioindicators both prokaryotic and eukaryotic in different natural and artificial environments. Her main interest has been the adaptations and effects of predatory activities of protist on bacterial populations and the possibility to use these activities to test the effects on wastewater treatment plants and natural environmental setups.

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Predatory activities of ciliates and their potential capacity to eliminate fecal indicator bacteria in natural environments

Blanca Perez-Uz, Elena Alonso Fernandes and Mercedes Martin-Cereceda
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Statement of the Problem: The increase of bacterial populations in natural environments in response to impacts caused by human activities is also associated with the increase of bacterial predators such as ciliates. These protists are able to eliminate bacteria, as part of their activities in the microbial loop, channeling this matter and energy to other links in the food web. Therefore, the bacterial production could be potentially eliminated through the activities of other microbial components of the ecosystem. We worked during a year assessing the environmental impact of visitor activities through the study of Fecal Indicator Bacteria (FIB) in three points of the main river crossing a National Park. During this time, we isolated bacterivorous ciliates and bacteria, both coliform and enterococci from some of the samples and developed experiments to assess the capability of ciliates predatory activities on these bacteria.

Methodology & Theoretical Orientation: Ciliates (*Cyclidium sp*. and *Colpidium colpoda*) were isolated from samples retrieved to enumerate FIB. These ciliates were kept in cultures with bacterial suspensions promoted with wheat grass. FIB bacteria isolated were fecal Enterococci (EC), selected on Slanetz-Bartley and Bile Sculin Agars and fecal coliforms Enterobacteria (EB) on EMB, Chromocult and EC. Short term experiments with Fluorescently Labeled Enterococci (FLE) and Coliforms (FLC) were used to evaluate the capacity of ciliates to capture them at concentrations between105-106 bacteria ml-1. Long term experiments were carried out to assess the ability of ciliates to grow in the presence of both bacteria. This would be an indication that those bacteria could be used as a food source and therefore eliminated effectively.

Findings: Both ciliates *Cyclidium sp*. and *Colpidium colpoda* were able to ingest the EC and the EB, but they only grew on EB. Cyclidium ingestion was much less effective than Colpidium colpoda and had as well a preference to ingest EC over the EB. Colpidium colpoda had the opposite preference, ingesting more EB (9 times more than Cyclidium) than EC and these were more than twice those ingested by Cyclidium.

Conclusion & Significance: These results show that capture of preys by ciliates did not predicted their capability to eliminate them from the environment. Growth experiments would be important to confirm their possible use in the elimination of FIB.

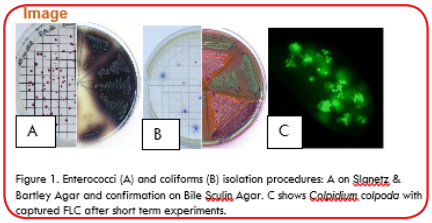

Figure 1. Enterococci (A) and coliforms (B) isolation procedures: A on Slanetz & Bartley Agar and confirmation on Bile Sculin Agar. C shows *Colpidium colpoda* with captured FLC after short term experiments.

Biography

Blanca Perez-Uz has her expertise in microbial ecology and the use of microbial bioindicators both prokaryotic and eukaryotic in different natural and artificial environments. Her main interest has been the adaptations and effects of predatory activities of protist on bacterial populations and the possibility to use these activities to test the effects on wastewater treatment plants and natural environmental setups.

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Optimization of confocal microscopy for visualization and quantification of yeast biofilm

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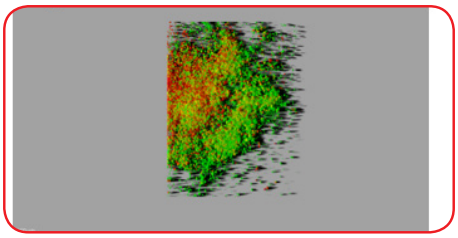
[2]University of Chemistry and Technology, Czech Republic

Introduction & Aim: Due to the resistance of biofilm to conventional cleaning processes, an industrial biofilm, especially in dairy industry, could cause extensive problems in final products in case of insufficient sanitation process. Our work focuses on visualization and quantification of yeasts biofilm using confocal microscopy. Commercial dying kits are optimized for pathogenic bacteria; therefore, the dying protocol for yeasts must have been created. The yeasts were isolated as industrial contaminants from cheese and saline solution and were identified as Yarrowia lipolytica and Debaryomyces hansenii using MALDI-TOF.

Material & Methods: µCLEAR® Chimney well plate was used for yeasts cultivation and the cultivation time, washing, dying and visualization of yeasts biofilm was optimized. LIVE/DEAD® BacLight™ Bacterial Viability and Counting Kit was used for dying and the biofilm visualization was performed by a confocal laser scanning microscopy with a rotating disc, a ten-fold magnification and a 1 µm z-step. For the living, dead and damaged cells, biomass volume and total biofilm structure were determined using Imaris software.

Results: The cultivation time was set to 24 hours with fresh broth well washing after 2 hours which better corresponds with real conditions. While living cells predominated in D. hansenii biofilm, the damaged cells prevailed in the *Y. lipolytica* biofilm, which may have been related to the different aging rates of the biofilm of both strains. The dying time was shortened to 60 minutes to reduce the toxic effect of propidium iodide.

Conclusion: New approaches for visualization and quantification of biofilm are needed for better understanding of the biofilm forming and persistence. We have shown that visualization of yeast biofilm is one of the ways to study these biofilms. These findings can lead to monitoring the effectiveness of sanitation solutions and to designing better cleaning processes.



Biography

Jana Smolova is a PhD candidate working under many research works related to microbiology and biotechnology. She has been working on biofilms with different methods and techniques to get the best results with the latest development of biofilms. She has interest in biotechnological experiments based on microbes.

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Fortification of dairy products with vitamin D3 and calcium and its influence on dairy microorganisms

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Purpose: The aim of our work was to test the effect of vitamin D and calcium supplementation on dairy microorganisms and subsequently to design the technological processes of dairy products with an increased nutritional value. It is important to supply the diet of temperate climate inhabitants with these nutrients, especially in winter period when the exposure to UV light is reduced. The lack of these nutrients contributes many civilization chronic diseases (diabetes, osteoporosis, cancer, immunity disorders).

Methods: In the first stage, the growth curves of yoghurt, acidophilic, bifidogenic and mesophilic cultures in UHT milk with the addition of vitamin D3, Aquamine F or Lactoval were studied. The supplements were added to cover 30% of the recommended daily allowance (DDD) in a 100 ml portion. Afterwards, samples of yoghurt drinks and quark desserts were prepared with an emphasis on studying the influence of fortification and flavoring components on dairy microorganisms, selected physicochemical parameters and sensory evaluation of the products.

Results: The addition of vitamin D and Aquamine F did not significantly affect the growth curves of tested cultures. The addition of Aquamine F even had a positive effect on the density of bifidobacteria. On the other hand, the addition of Lactoval slowed down the growth of acidophilic culture but the density of microorganisms was comparable across all samples. Due to the buffering capacity of the calcium preparations, there has been a low pH drop in the developed dairy products.

Conclusion: Our study suggests possibilities to fortify dairy products fulfilling nutritional, microbiological and sensory criteria.

Biography

Sarka Havlikova is working as an engineer in the Department of Cheese Technology at the Research Institute of Dairy. She has experience in the field of microbiology and biotechnological related research work. She has interest in dairy products and dairy microorganisms related projects and technical challenges.

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Production of poly-3-hydroxybutyrate and oxygen transfer characterization by *Azotobacter vinelandii* in a 30L bioreactor

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Polyhydroxybutyrate (PHB) is a biopolymer produced by *Azotobacter vinelandii*. The PHB is a biodegradable thermoplastic material, used in packaging production, drug encapsulation and medical implants. The objective of this work was to develop a PHB production process in 30L bioreactor. Cultures of *A. vinelandii* OP were performed in batches in an Infors HT stirred tank reactor model Techfors-S with 20L of culture medium composed of sucrose as a carbon source and yeast extract as nitrogen source. The culture was developed at 100 rpm, 1 vvm, 30 °C and pH 7.1 controlled with 2N NaOH. The Dissolved Oxygen Tension (DOT) was characterized with a polarographic sensor and the Oxygen Transfer Rate (OTR) was estimated by analyzing O2 and CO2 in the gas phase. The results show that the biomass reached a maximum value of 7.5g L-1 after 70 hours of cultivation. Sucrose was completely consumed, indicating that it was the nutrient limiting growth. The DOT remained at values close to zero during the cell growth phase and the OTR reached a constant maximum value for 30 hours, reaching 10 mmol L-1h-1. This OTR behavior is typical of oxygen-limited cultures. The concentration of PHB was increased during cell growth, until reaching a maximum concentration of 6.0 g L-1. Likewise, the percentage of intracellular accumulation of PHB varied between 65% and 82% between 35 and 50 hours of culture. Results of PHB are like that reported by Millán et al., in 2017 with percentages of accumulation of constant PHB over 70%. YPHB/S of 0.24 gg-1 with qPHB of 0.01 gg-1h-1. Our results have demonstrated it was possible to develop a PHB production process on a 30L scale getting PHB concentrations of 6 g L-1. Furthermore, by characterizing the oxygen transfer it is possible to explain the high percentage of PHB accumulated by *A. vinelandii*.

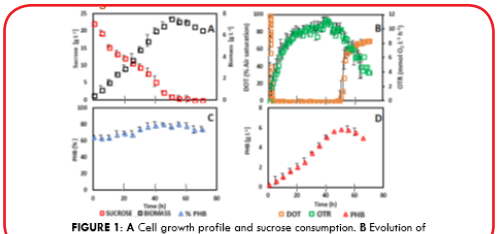

FIGURE 1: **A** Cell growth profile and sucrose consumption. **B** Evolution of DOT and OTR. **C** Profile of intracellular PHB percentage. **D** Evolution of

Biography

Andres Perez is a student of Master of Science of Engineering in Biochemical Engineering at Pontificia Universidad Católica de Valparaíso, Chile. His profession is a Biotechnologist Engineer of Concepcion University of Chile.

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Bacteriophages for prevention and treatment of Salmonella infection in poultry

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G. Eliava Institute of Bacteriophage, Microbiology and Virology, Georgia

Enteric Salmonella infection is a global problem both in human and animals and has been attributed to be the most important bacterial etiology for enteric infections worldwide with massive outbreaks occurring in recent years. Food animals are the primary reservoir for human non-typhoid Salmonella infections. Poultry products are considered one of the major sources of Salmonella infections. In many cases multi-resistant bacteria infecting humans have been directly linked to resistant organisms in animals. Existence of such pathogens is problematic because of possible transmission of antibiotic resistant bacteria from animals to humans through the food supply. The development of alternative anti-microbial remedies has become one of the highest priorities of modern medicine and biotechnology. One of such alternatives might be bacteriophages as a prospective biocontrol method against contaminations caused by antimicrobial resistant pathogens. Main goal of this work is development of bacteriophage-based product that can be used to control Salmonella contamination on farm level. For formulating polyvalent phage preparation 3 phages with wide, complementary, not-fully-overlapping host ranges were selected. Salmonella phages Sal.phi13, Sal.phi18 and vB_Stm 21 were mixed in the proportion 1:1:1 and lytic activity and host specificity of each individual phage was compared with that of the phages cocktail. It was observed that the phage cocktail possessed broader host specificity within *S. typhimurium* serotype than each of three phages alone. It was found that the host specificity of Salmonella cocktail was noticeably wider than that of the individual Salmonella phages. Salmonella phage cocktail was effective against 65 out of 66 (98%) tested Salmonella strains in *in vitro* experiments.

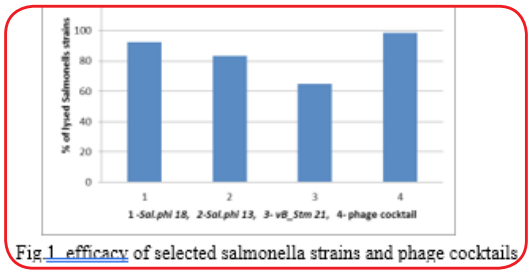

Fig 1. efficacy of selected salmonella strains and phage cocktails

Biography

T Gabisonia is the Head of the Laboratory of Applied Microbiology at the G. Eliava Institute of Bacteriophage, Microbiology and Virology, Georgia. He is author of more than 30 scientific articles and has participated in many local and international scientific projects.

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Bacteriophages for decontamination of artificially contaminated chicken meat

M Loladze, T Gabisonia, N Chakhunashvili, M Nadiradze, M Alibegashvili, N Tamarashvili, T Katamadze, T Kalandarishvili, T Eliava and K Samkurashvili
G. Eliava Institute of Bacteriophage, Microbiology and Virology, Georgia

Introduction: Bacterial food poisoning remains a major worldwide health problem. The increased incidence of foodborne illness has caused substantial morbidity and mortality worldwide annually. A substantial number of works have described the use of bacteriophage biocontrol to target a variety of bacterial pathogens in food and bacteriophage biocontrol is increasingly recognized as an important tool for elimination of pathogenic bacteria from food.

Aim: This work was conducted to study the efficacy of bacteriophage cocktail for reduction of contamination in chicken meat, on the model of artificial contamination with *E. coli*.

Method: A total of 30 random samples of raw chicken were enrolled in our work. The samples were divided into three equal groups (10 samples each). A 0.5 ml of the diluted (105 CFU/ml) *E. coli* culture was distributed over the surface of the chicken meat. The first and second groups were inoculated in such method. Samples from third group were left untreated. All samples were left for 30 minutes at room temperature to allow attachment and adsorption of the inoculated bacteria. After 30 minutes samples from the contaminated first group were treated with 50 ml phage cocktail (105 PFU/ml), samples from the second contaminated and third non-contaminated groups were treated with 50 ml of sterile saline. After 1 hour at room temperature the tested microorganism *(E. coli)* was enumerated from all samples.

Results & Conclusion: After bacteriophage cocktail treatment of the chicken meat, experimentally contaminated with *E. coli*, a significant reduction of contamination was observed. These data suggest that bacteriophages can be successfully used to reduce food-borne pathogen contamination in food chain.

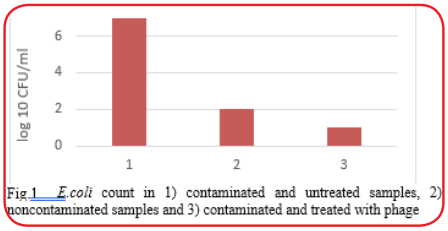

Fig 1 *E.coli* count in 1) contaminated and untreated samples, 2) noncontaminated samples and 3) contaminated and treated with phage

Biography

M Loladze is a leading Scientist of the Laboratory of Applied Microbiology at the G. Eliava Institute of Bacteriophage, Microbiology and Virology, Georgia. She is author of more than 30 scientific articles and has participated in many local and international scientific projects.

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Escape of genetically modified microorganisms in the food and feed chain: Strategy to support enforcement laboratories

Marie-Alice Fraiture, Marie Deckers, Sigrid C J De Keersmaecker, Bas Berbers, Kevin Vanneste
Nina Papazova and Nancy H C Roosens Sciensano, Belgium

In the food/feed industry, Genetically Modified Microorganisms (GMM) are commonly used to produce food enzymes, food additives, feed additives and food flavorings. For instance, around 50% of food enzyme producing microorganisms from the 304 submitted dossiers to the European Food Safety Authority (EFSA) for authorization are GMM. The use of GMM presents several advantages such as an increase of the yield, an external secretion of the product using signal peptides, a production not constitutive of products and a safer strain in inhibiting the possible toxin production. In the European Union, the use of GMM has to respect the following regulations: EC/1332/2008 (food enzymes), EC/1333/2008 (food additives), EC/1831/2003 (feed additives) and EC/1334/2008 (food flavorings). These regulations required the absence in the final product of viable production GMM and associated recombinant DNA. Given that manufacturers are responsible for the quality control of their products, it is not mandatory to provide GMM-specific detection methods to enforcement laboratories. However, accidental escapes of unauthorized GMM have already been reported in 2014 (RASFF 2014.1249) and 2017 (RASFF 2017.1544). Therefore, there is a crucial need to develop a strategy for enforcement laboratories to control the food/feed chain. This strategy includes the DNA extraction from GMM earlier isolated from the food/feed matrix to subsequently perform a whole-genome-sequencing. Based on bioinformatics analysis, sequences of interest are selected to develop GMM-specific detection methods that could be then directly applied on the food/feed matrices. However, the implementation of this strategy could be challenging, especially with the sequencing of plasmids. This strategy will be tested on food enzyme preparations in the frame of the SPECENZYM project.

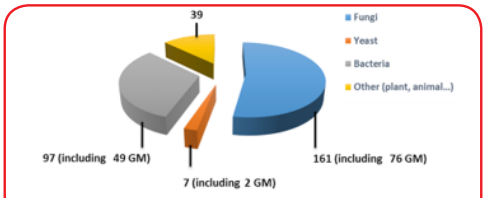

Figure 1 : Food enzyme production sources from the 304 submitted dossiers to EFSA for authorization.

Biography

Marie-Alice Fraiture is currently working as a scientific collaborator in transversal and applied genomics in Sciensano. She completed her PhD from Ghent University, Belgium and have a work experience in the Plant Sciences Division at the University of Nottingham (Great-Britain) in the laboratory of Prof. Malcolm Bennett. She has an expertise is in management and in collaboration of national and international projects, in detection of GMO in the food and feed chain, in detection of arboviruses in human samples, in detection of GMO and pathogens in case of accidental/deliberate escapes and in development of tools (such as high-throughput technologies) for applied genomics.

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Quantification of functional genes associated with microbial transformations of mercury in Colombia Amazon ecosystems

Maria Camila Escobar[1], Gladys Cardona[1], Silvia Marques[2] and Alejandro Acosta-Gonzalez[3]
[1]Sinchi Amazonic Institute of Scientific Research, Colombia
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Methylmercury (CH3Hg) is a potent neurotoxin produced by methylation of mercury (Hg) by anaerobic bacteria, especially sulfate-reducing bacteria (SRB) that carriers the hgcA gene, and which is biomagnified through the trophic chain. Many aerobic bacteria resistant to Hg possess mer operons that allow the reduction of Hg2+ to Hg0, a less toxic form of the metal. The objective of this study was to evaluate the abundance of genes involved in the reduction and methylation of Hg in the bacterial communities of Amazonian ecosystems. To do this, DNA was extracted from sediments, forest soils and waters from two locations with different degrees of intervention of gold mining: Tarapaca-Amazonas (low intervention) and Tarair- Vaupés (high intervention). The genes were quantified by real-time PCR (qPCR) in standardized curves. Clone libraries were generated and sequenced for dsrA gene, which detects SRB, hgcA and merA. The primers for dsrA amplified Deltaproteobacteria; two sets of primers of the hgcA gene were used to quantify Deltaproteobacteria and Firmicutes, the primers of the merA gene detected Beta and Gammaproteobacteria. In general, the dsrA gene was the most abundant in all the samples of both localities, especially in samples of superficial and deep sediment, being in the order of 103-104 copies of the gene/ng of DNA. Only a proportion of the SRBs carried the hgcA gene, confirming that only a part of the population of these bacteria is capable of methylation. In Tarapaca, the highest number of copies of the hgcA gene was found in sediments (102 copies/ng) and in Taraira in forest soils (103 copies/ng), which in turn detected some of the highest concentrations of Hg (13-43 ppm) and CH3Hg (0.02-0.05 ppm). Regarding the merA gene, it was more abundant in waters (104) and sediments (103) of Taraira.

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September 17-18, 2018 Lisbon, Portugal

Reduction of selenite to selenium using bacteria isolated from polluted areas

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In this work toxic selenite which is also highly soluble is transformed to selenium a less toxic element. Selenium is transformed through biotic transformation using different strains of bacterial like *Pseudomonas, Exiguobacterium sp*., *Bacillus subtilis* and *licheniformis*. Selenium can exist in different forms like reduced form (Selenide, Se2-), water dissolved form (Selenite, So3-2/Selenate SeO4-2) and in the form of element (Seo). Different physical parameters were changed for optimizing conditions like different concentrations of Selenium (Se) varying from 200 to 400 and finally to 600 µg ml-1, temperature, pH, aeration along with incubation time for high reduction of selenite. It was found that selenite reduction rate was increased by increasing pH. It was found that at pH 3 around 15-33% Selenite was reduced and this trend kept on increasing to 28-90% at pH 9. For evaluating optimum temperature for Selenite reduction three levels of temperature were selected (32 °C, 37 °C and 42 °C) were selected. The selenite reduction was found at different temperatures and the results showed that for optimum reduction of selenite all strains possess varying preferences. The reduction in Selenite was also checked at different concentrations of selenite and it was found that maximum reduction of selenite was observed at lower concentration. This study concluded that in aerobic and anaerobic environment Se can be remediated by using selenite reducing bacteria.

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Prevalence and control of *Cronobacter sakazakii* containing putative virulence genes

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Cronobacter sakazakii is an emerging food-borne pathogen that is associated with a number of infections in infants, neonates and immune-compromised individuals. It is associated with a number of life-threatening diseases like necrotizing enterocolitis, sepsis, meningitis, bacteremia, CSF infection, brain abscess and cyst formation. In the present study prevalence of C. sakazakii was investigated in dairy products of Agra city, India. Total 243 dairy product samples were analyzed in different seasons and a total of 480 isolates were obtained. These isolates were biochemically characterized and were further confirmed using 16S rRNA gene sequencing. The isolates were subjected to *in vitro* and *in vivo* pathogenicity testing. The presence of virulence associated genes was checked in these isolates and the effect of various stresses like acid, alkaline, heat, cold and desiccation was also studied on these isolates. qPCR based studies were conducted to check the expression of putative virulence gene under stressed and unstressed cells. A number of antibiotics and probiotics were tested to control these *C. sakazakii* isolates. Amongst antibiotics Ofloxacin, Piperacillin, Cefotaxime and Chloramphenicol were the most effective antibiotics and amongst probiotics *L. fermentum* and *Pediococcus acidilactici* were the most effective in controlling the *C. sakazakii* isolates.

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Salt tolerance ability of coliform bacteria in Hooghly estuary, West Bengal, India

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Population of total coliform and fecal coliform bacteria was monitored both spatially and seasonally from the Hooghly estuarine water for assessing their salt tolerance ability. The coliform bacteria isolated were allowed to grow in different saline medium and our study revealed that both total and fecal coliform bacteria could tolerate maximum salinity of 30 psu. Physicochemical parameters of estuarine water showed a significant control over the population of coliform bacteria. Human activity, rather than monsoonal cycle, was found to be more effective parameter to control population of coliform bacteria in the Hooghly estuarine water of West Bengal, India.

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Antibacterial activity of methanol extract of Mazouj and Ghalghaf galls extracts of Oak against *Pseudomonas aeruginosa*

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Background & Aim: Most of the infections caused by *Pseudomonas aeruginosa* resistant strains are originated from hospitals and its prevalence is increasing worldwide. Therefore, many efforts have been made to find new effective plant compounds as a substitute for antibiotics. The aim of this study is to evaluate the active constituents, antibacterial and anti-biofilm activity of aqueous and methanol extracts of Mazuj and Ghalghaf galls against *Pseudomonas aeruginosa*.

Method: This study was performed by standard strains of bacteria. The methanol extract of Mazuj and Ghalghaf galls were prepared by Soxhlet apparatus. Antibacterial activity of extracts was evaluated by the diffusion method. Moreover, Minimum Inhibitory Concentration (MIC) was assessed by micro dilution method. In order to evaluate anti-quorum sensing activity of methanol and aqueous extracts of Mazuj and Ghalghaf galls, effects of the extract on anti-biofilm activity, production of elastase, proteases and pyocyanin were examined. Active compounds Mazuj and Ghalghaf galls were identified by gas chromatography-mass.

Results: The methanol and aqueous extracts of Mazuj and Ghalghaf galls exhibited strong inhibitory effects against *Pseudomonas aeruginosa*. The MIC values of extracts were similar and ranged from 6.25 mg/ml to 25 mg/ml. The extracts of Mazuj and Ghalghaf galls strongly inhibited the formation of *Pseudomonas aeruginosa* biofilms, production of elastase, proteases and pyocyanin at concentrations higher than 6.25 mg/ml. The highest compound isolated from the extract of Mazuj galls 9-Octadecenoic acid and the extract of Ghalghaf galls 9-12 octadecenoic acid and oleic acid.

Conclusion: According to the results of this study, these extracts demonstrate favorable antibacterial activity against tested bacteria. The methanol extract proved to have more antibacterial activity than the aqueous extract. However, a profound understanding of the field identification of antimicrobial agents in diseases treatment as the alternative to antibiotics requires more experimental research.

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Successfully activating positive behaviors of the stakeholders involved in vaccine purchasing and usage through technological advances

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The vaccine segment is anticipated to be one of the fastest growing one of the healthcare industry and several leading firms have stepped up vaccine investments in recent years. Unlike therapeutic agents, vaccines are administered to healthy individuals only once or very infrequently during a life time. Vaccines generate well-documented positive externalities, yet their poor awareness and acceptability among vaccine end-users may contribute to resurgence of transmissible diseases and consequently trigger governmental interventions such as mandating vaccination. In addition to technical and clinical development per the highest quality standards, bringing new vaccines to market requires carefully orchestrated programs targeting the multiple types of stakeholders along the entire value chain and addressing their respective purchasing behavioral drivers. Against a backdrop of anti-vaccination buzz and vaccine fatigue, successful global launch and sustainable usage of a vaccine requires the development of a multi-pronged strategy addressing all aspects in relation to acceptability (e.g. the motivation to immunize despite the quasi-disappearance of the disease), accessibility (e.g. supply chain services), availability (e.g. mechanisms ensuring reliability of supply) and affordability (e.g. tiered pricing policy taking country differences in per capita income into account). Leveraging novel technological advances can positively influence the ability to activate these levers successfully.

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Vaccines against bacterial diseases: Immunological requirements for protection and safety concerns in developing countries

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Bacterial diseases cause approximately six million deaths per year. Antimicrobial resistance is increasing and better vaccines are needed. Vaccine design against many bacteria is still empirical and not always based on the knowledge of the immunological requirements for protection. We are currently studying immune responses and vaccines against invasive Salmonella diseases. Invasive Non-Typhoidal Salmonella (iNTS) disease is a leading cause of morbidity and mortality especially in Sub-Saharan Africa (SSA) and is estimated to cause over 1.9 M illnesses and 400,000 deaths annually in SSA, which carries 57% of the global burden. These infections affect in particular young children and individuals with immunological defects or co-morbidities such as malaria, HIV and malnutrition. Using interdisciplinary approaches that combine preclinical models, epidemiological observations and *in vitro* studies in humans we have identified several of the immunological mechanisms that are required for host resistance to iNTS. This knowledge needs translating in the generation of vaccines that are able to confer protection also in the presence of underlying immune-compromise. We are also evaluating new classes of non-living vaccines and we are searching for gene-mutations that will make live attenuated vaccines safer to use in areas of the world where underlying pathologies would make current live vaccine candidates excessively dangerous.

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Eliminating the vaccine cold-chain distribution hurdle

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Statement of the Problem: The requirement for vaccines to be kept sufficiently cold (unbroken cold chain) during transport makes vaccine distribution to some populations (where the vaccines are greatly needed) extremely challenging, if not impossible. Thus, various strategies are being explored to eliminate the need for the expensive cold chain. We have designed a versatile Vibrio Cholerae Ghost (VCG) vaccine delivery platform that eliminates the need for the expensive cold chain and thus alleviate cold chain-associated constraints on global vaccine access. Moreover, this platform is self-adjuvanting and capable of simultaneously delivering multiple vaccine antigens to the immune system. It also offers an attractive approach for developing combination vaccines, especially against diseases with epidemiological overlap. Here, we present data showing a VCG-based chlamydial vaccine protects against infertility in mice.

Method: Groups of mice were Immunized Rectally (IR) with VCG expressing the *Chlamydia trachomatis* porin B and polymorphic membrane protein D proteins (rVCG-PmpD/PorB) or glycoprotein D from HSV-2 (rVCG-gD2 or gD2) as antigen control. Vaccine efficacy was assessed by evaluating the intensity and duration of genital chlamydial shedding following intravaginal challenge with live chlamydiae. Protection against upper genital tract pathology was determined by assessing infertility and tubal inflammation. Analysis of Variance (ANOVA) was used to compare differences between groups.

Finding: We demonstrated that elicited immune effectors following immunization cross-reacted with the serovar E chlamydial antigen and reduced the length and intensity of genital chlamydial shedding. Moreover, immunization with the VCG-vaccine reduced the incidence of tubal inflammation and protected mice against Chlamydia-induced infertility.

Conclusion: These results highlight the potential of the VCG platform for eliciting immunity in the female genital tract and preventing the sequelae of chlamydial infection such as infertility and upper genital tract inflammation.

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Colon cancer stem cell-based vaccine reduces efficiently both tumor growth and cancer stem cell subpopulation in a mouse colon carcinoma mouse model

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Statement of the Problem: Colon cancer is the most common malignant gastrointestinal cancers that are still the most frequent cause of cancer-related mortality in China. Colon Cancer Stem Cells (CCSCs) are the main reasons that result in the drug and radiation resistance, invasive growth, metastasis and cancer relapse. Though many factors involving immuno surveillance and immunosuppression were recently validated as important for patient prognosis, a lot of experimental immunotherapies to fight unresectable metastatic colorectal cancer, only few cases have successfully induced antitumor immune response against malignancies. The aim of this work was to investigate the effects on the inhibition of colon cancer growth by vaccination of CCSC vaccines.

Method: The $CD133^+$CSCs were isolated from human LOVO and mouse CT26 cell lines by using a magnetic-activated cell sorting system, respectively. The xenograft or syngeneic mice were subcutaneously inoculated with the LOVO or CT26 $CD133^+$CSC vaccine inactivated with again and again freeze thawing three times before the mice were challenged subcutaneously with LOVO or CT26 cells. The inhibition tumor efficacy was assessed by the tumorigenicity, immune efficient analysis by flow cytometer and enzyme-linked immunosorbent assays, respectively.

Result: The results showed that, compared with the non-CSC vaccine, the inhibition tumor growth efficacy of LOVO or CT26 CSC vaccine was significantly increased in the xenograft or syngeneic mice. Vaccination of LOVO or CT26 $CD133^+$CSC vaccine resulted in increasing cytotoxic activity of natural killer cells, enhancing serum IFN-γ and decreasing TGF- β levels in the mice. The LOVO and CT26 $CD133^+$CSC vaccines significantly reduced the CSC subpopulations in the colon cancer tissues.

Conclusion: The data provided the first evidence that the human LOVO or mouse CT26 $CD133^+$CSC-based vaccine may be an attractive therapeutic approach to excitation of anti-tumor immunity for treatment of colon cancer.

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5th World Congress on
Microbial Biotechnology & Vaccine Design
September 17-18, 2018 Lisbon, Portugal

Regulation of TCR-coupled signaling pathways by Crk adaptor proteins

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Cellular responses to environmental cues are controlled by cell surface receptors that are functionally linked to intracellular networks of signal transduction pathways. A hallmark of these signaling pathways is the temporal and spatial assembly of multi-molecular complexes at the site of the engaged receptors. Formation of these complexes is regulated by conformational changes and posttranslational modification of the activated receptors, as well as scaffold and adaptor proteins, which create docking sites for effector molecules, predominantly enzymes and their substrate proteins. The Crk adaptor proteins constitute an integral part of many receptor-coupled signaling networks, thanks to their Src homology 2 (SH2) and SH3 protein-protein binding domains, which enables the interaction with activated receptors and with effector molecules that recruit to the receptor site. A viral form of Crk (v-Crk) is also involved in oncogenesis, while cellular Crk can serve as preferred targets for a number of cell-invading pathogens. Thus, the Crk proteins contribute to bacterial pathogenesis by promoting their entry into cells, and serve as targets for virulence factors that divert host cell signaling pathways to the benefit of the pathogen. We found that TCR/CD3 crosslinking in Jurkat T cells promotes the association of Crk adaptor proteins with the transiently phosphorylated CD3ζ chain. Binding studies and pull down assays revealed that the Crk-SH2 domain mediates binding of phospho-CD3ζ. Crk-mediated binding of phospho-CD3ζ is selective and is not mediated by other SH2 domain-containing adaptor proteins, including Grb2, GRAP and Nck. Our results support the involvement of Crk adaptor proteins in the early steps of T cell activation and suggest a role for Crk in the recruitment of signaling proteins to the activated TCR where Crk might contribute to the fine-tuning of the TCR/CD3-coupled signal transduction pathways.

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