Oceanobacillus oncorhynchi subsp. *incaldanensis* subsp. nov., an alkalitolerant halophile isolated from an algal mat collected from a sulfurous spring in Campania (Italy), and emended description of *O. oncorhynchi*. It is capable of growing under alkaline and high salinity conditions. The purification and characterization of its catalase-peroxidase are described.

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51.
Molecular characterization and recuperation of bacteriocins produced by lactic acid bacteria isolated from dry cured-meat products

Rebeca Miñambres*, Alejandro Rodrigo, Mercedes Villa-Carvajal, José García-Reverter, David Tomás

Ainia Centro Tecnológico, C/Benjamin Franklin 5-11, Parque Tecnológico, 46980 Paterna, Valencia, Spain

Lactic acid bacteria (LAB) are generally recognized for their fermentative ability, contributing to the organoleptic profile, as well as for providing health and nutritional benefits. Some of the lactobacilli strains produce bacteriocins which are ribosomally synthesized peptides that exhibit bactericidal activity against certain microorganisms species. The application of bacteriocins as food preservatives has a promising biotechnological approach in food safety and in the inhibition of spoilage and pathogenic microorganisms. The prevalence of food toxiinfections caused by emergent pathogen microorganisms such as toxigenic *Escherichia coli O157* and *Listeria monocytogenes* has revealed the importance of the development of new bacteriocins active in foods.

In this sense, the aim of our work has been the isolation and molecular characterization of LAB from traditional cured-meat products and the analysis of the bacteriocin expression with inhibitory activity against pathogen.

Eighty five LAB strains were isolated from different Spanish traditional dry cured-meat products using MRS agar at 30 °C for 72 h and stored at −20 °C. Bacteriocin production was assayed by agar diffusion test and the isolated strains showing activity were identified by the amplification of 16S gene fragment by PCR methods. The bacteriocin producer strain was cultured in a bioreactor and the production of the secreted bacteriocin was analyzed by the diffusing agar assay, using the strain *Lactobacillus curvatus LTH1174* as positive control of the production of curvacin A. After the characterization of the bacteriocin expression, the isolated strain was cultured in MRS broth and the secreted proteins were precipitated using different precipitation methodologies and agents to finally test the inhibitory properties of the precipitated bacteriocin against both pathogens. Finally, molecular bacteriocin characterization was developed using specific primers in a high-throughput analysis to detect gene fragments of Curvacin A, Plantaricin A, Nisin and Sakacin P. DNA was isolated from LAB according to a thermal protocol with lysis buffer, amplificated by PCR and analysed by electrophoresis.

Our results demonstrate that one of the evaluated strains, strain number 11, showed a good inhibitory activity against both pathogens tested and two of them had a partial biocide activity in agar diffusion test studies. Under optimized PCR conditions with all primers pairs, we found that nine strains were positive for the expression of bacteriocin genes. The dry cured-meat isolated strain number 11 secretes a bacteriocin which shows an inhibitory effect against *Escherichia coli O157* and *Listeria monocytogenes,* according to our positive control *L. curvatus LTH1174*. This protein is effectively precipitated and renaturalized in phosphate buffer showing an inhibitory effect on the pathogens growth. In our screening analysis the strain number 11 was the only that exhibited inhibitory effect on pathogens growth and which bacteriocin gene was detected by PCR methods. The sequence of the PCR amplified fragment showed a homology of 93% with a known bacteriocin previously described.

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52.
Marine natural product bioprospecting: Screening and production bioprocess development of novel bioactive compounds

Elvira Casas[a], Javier Martín[b], Lidia Tomás-Cobos[a], José García-Reverter[a], Mercedes Villa-Carvajal[a,*]

[a] *Ainia Centro Tecnologico, Parque Tecnológico C/Benjamin Franklin 5-11 E46980, E46980 Paterna Valencia, Spain*
[b] *Mediterráneo Servicios Marinos S.L., Alicante, Spain*

The latest tendencies in biotechnology research point to marine ecosystems as a source of immeasurable value for obtaining molecules with extraordinary properties that are used in the development of drugs, cosmetics and other products of high added value for specific purposes. It is due to the extensive biodiversity of submarine environments and to the secondary metabolites produced by the marine organisms as a result of the environmental conditions in which they are found, these being completely different to terrestrial ones. With this aim, Mediterráneo Servicios Marinos, S.L (MSM), a company dedicated mainly to the marine prospections and ainia Technological Center as a services and R&D organization have developed a research project to obtaining bioactive compounds from marine organisms with a potential application in the development of new natural products. Concretely, various hundreds of samples from marine sources collected in different bioprospections have been studied by different screening assays obtaining marine extracts

and isolated microorganisms which have been tested on the basis of their potential biocide activity against different kinds of pathogenic microorganisms.

In this project, our attention has been focused to obtain substances with antimicrobial activity from marine samples of three differentiated ecosystems. Samples of macroorganisms such invertebrates, algae and marine sediments, as well as, the microorganisms included in the same ones (mainly, bacteria, fungi and microalgae) have been recollected. First, the precise locations of the studied ecosystems were selected (Mediterranean and South America marine environments) and samples were compiled and preserved for the next step. Then, samples, previously prepared (*in situ* frozen) and codified, were taxonomically classified and transported to the laboratory facilities. Later, we designed two specific screening protocols which were developed in parallel in order to extract potential bioactive compounds directly of marine samples using extraction and microbiology techniques. The extracts and the supernatants of cultured marine isolates were tested against three specific pathogens (*Escherichia coli*, *Candida albicans* and *Aspergillus niger*).

In these screening tests, we have obtained 120 extracts and 70 isolated microorganisms of which 17 extracts and 42 microorganisms showed to inhibitory activity against pathogens tested. It is necessary to emphasize that in most of the cases (extracts and microorganisms) the biocide activity (antibacterial and antifungic inhibition) was simultaneously observed against studied pathogens. Moreover, it is very relevant the high percentage of isolated microorganisms with potential biocide activity that were detected in this study (60% of isolated microorganisms). These results reveal the great potentiality of the marine microorganisms as a source of new biocide compounds for the industry. These activities have been carried out during 3 years, and now, the project is being continued. In that sense, we are developing the bioactive compounds production bioprocesses at pilot scale and we are testing another interesting activities (anti-inflammatory, antitumoral activities and enzymatic profiles) with the extracts and isolated microorganisms obtained.

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53.
Isolation and characterization of antimicrobial peptides from larvae of flesh fly *Neobellieria bullata*

Tereza Neubauerová*, Alice Ciencialová, Martina Macková

Department of Biochemistry and Microbiology, Institute of Chemical Technology Prague, Technická 5, 16628 Praha 6, Czech Republic

In last few decades number of resistant bacterial pathogens is increasing and thus finding of new antibiotics is in front of the microbiological research. A new potential seems to be short cationic peptides produced as part of innate immunity. Like a source of these peptides was chosen larvae of the fleshly *Neobellieria bullata*.

Production of these peptides was induced by injection of *Escherichia coli* suspension into the larvae. After few hours of incubation the haemolymph was picked up, gradually centrifuged and precipitated. Subsequently these fractions were separated by chromatographic methods (adsorption and ionic chromatography, RP-HPLC) to gain fractions of short peptides. Identification and characterization of these fractions were made by SDS-PAGE, mass spectrometry MALDI-TOF and N-terminal sequencing. Results were compared with databases of peptides.

Antimicrobial activity of fractions was tested by two methods. Diffusion method was used for first screening; diameter of inhibition zones of fractions was compared with diameters made by commercial antibiotics. The growth of pathogen with peptide fractions at defined concentration and potential inhibition was monitored continually by turbidimeter BIOSCREEN C. We can measure and calculate maximal growth speed and express the inhibition. Tested organisms were gram-negative bacteria – *Escherichia coli*, *Pseudomonas aeruginosa*, and gram-positive bacteria – *Staphylococcus aureus*.

We found few active fractions. These fractions were precipitated by methanol and not observed in haemolymph of noninduced larvae. One fraction (called M13) inhibited completely pathogen *Pseudomonas aeruginosa* at concentration 1000 μg/ml. Next two fractions were active against *Staphylococcus aureus* and less active against *Escherichia coli*. In both fractions sapecin A and C were characterized. These peptide fractions were active in the same range like commercial antibiotics (vankomycin, kanamycin).

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54.
Rhizosphere biodegradation studies on long-term PCB contaminated soil; isolation and characterization of different rhizosphere microbial communities from PCBs soil

Marieta Ionescu*, Katarina Beranova, Lucia Kochankova, Katerina Demnerova, Tomas Macek, Martina Mackova

Institute of chemical Technology, Rague, 14800 Prague, Czech Republic

Bacteria generally can contribute to the health, growth and development of plants. Plant's growth promotion by rhizosphere bacteria may result either from indirect effects such as the biocontrol of soil-borne diseases or from direct effects such as the production of phytohormones, solubilization of minerals, synthesis of vitamins, siderophores and other growth stimulating compounds. Similarly plant root exudates (sugars, alcohols, organic acids) act as carbohydrate sources for the soil microflora and enhance microbial growth and activity of indige-