

MICRO BIOTEC'11

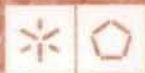
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investigate the antibacterial activity of *N. officinale* extracts and evaluate the effect of 2-Phenylethyl isothiocyanate (PEITC), a predominant isothiocyanate derived from the sulphur compound gluconasturtiin, particularly important in *N. officinale*, against Gram-negative and Gram-positive bacteria, isolated from Human and animal gastrointestinal segments. Using a disc diffusion method and different doses of hydrophilic *N. officinale* extracts and PEITC prepared in dimethyl sulfoxide (DMSO) were tested. Positive (commercial standards antibiotic) and negative control were used. All experiments were conducted in triplicate. The antibacterial activity was also assessed by the application of relative inhibition zone (% RIZD) in relation to the antimicrobial effect of commercial standards antibiotics. Following, total phenolics with Folin-Ciocalteu method, total flavonoids with aluminum-chloride method and the antiradical activity of *N. officinale* extracts was evaluated using the methods of the free radical scavenging activity (the 2,2-diphenyl-1-picrylhydrazyl-DPPH) and the Ferric reducing antioxidant power (FRAP). The results showed that both methanolic extract of *N. officinale* and PEITC was more effective in Gram-positive bacteria. The results indicate that *N. officinale* extracts could be useful as antimicrobial agent and might be used as alternative to, or in combination with current antibiotic-based controls used for treating bacteria. Also, the natural compound PEITC possesses interesting antimicrobial activity and enormous antiradical potential activity probably mediated through direct trapping free radicals, reducing power, and also through metal chelating. Based on its antiradical potential, *N. officinale* could be used in the prevention of free radical diseases.

PS3: 4

Characterization of antibiotic susceptibility profile and of β -lactamase genes content in *Bacillus pumilus* from different sources

Raquel Branquinho¹, Maria Manuela Amorim², Ângela Novais¹, Maria Estevez Pintado², Luísa Peixe¹

¹REQUIMTE. Serviço de Microbiologia. Faculdade de Farmácia. Universidade do Porto, Portugal; ²CBQF – Escola Superior de Biotecnologia – Universidade Católica Portuguesa. Porto, Portugal

Background: *Bacillus pumilus* is associated with a wide range of biotechnological activities, being used as probiotic in humans, animal feed supplement and growth-plant promoter. Concerns about the possibility of dissemination of resistance genes by microorganisms used as human nutrition supplements or in food production for human consumption have led to the need of demonstration of absence of antimicrobial resistance as a universal qualification of microorganisms' safety (EFSA, 2010). Nevertheless, data on antimicrobial susceptibility using standardized methodology and β -lactamase genes content in isolates of *B. pumilus* is lacking. Four putative β -lactamase genes were found in *B. pumilus* SAFR-032 genome, although their contribution to β -lactams phenotype is unknown. Objective: To determine β -lactam antibiotic susceptibility profile of *B. pumilus* isolates from different origins, and establish its association with putative β -lactamase genes. Methods: Thirty *B. pumilus* isolates, recovered from a variety of sources, including food (n=6), medicines (n=12) and cosmetics (n=4) contaminants; plants (n=4), gastropods' normal flora (n=3), and *B. pumilus* ATCC14884, were assayed. Identification was conducted by biochemical tests, 16S rDNA-PCR and sequencing. Clonal relationship was evaluated by *Apal*-PFGE. Presence of putative narrow-spectrum- β -lactamases (B1, B2) and metallo- β -lactamases (M1, M2), described in *B. pumilus* SARF-032, was verified by IEF, PCR and sequencing. Susceptibility patterns to 8 β -lactams were evaluated by broth microdilution (CLSI/NCCLS/M45-A). Results: All isolates revealed β -lactamase production at a later sporulation phase. They harbored 3 (50%), 4 (23.3%), 1 (16.7%) and 2 (10%) β -lactamase genes, but were susceptible to β -lactams, although isoelectric focusing revealed different bands. Moreover, no correlation was found among MIC values and β -lactamase content. None of β -lactamase gene was consistently found, and the most frequent one (M1) was identified in 80% of the isolates. Twenty-two PFGE-types were defined and associated with high variability of β -lactamases (number/type), being particular pulsotypes associated with isolates' origin. Conclusion: Variable β -lactamase gene content was observed in *B. pumilus* suggesting their mobilization throughout horizontal transfer. Nevertheless, current recommended methodology for testing β -lactams susceptibility tests does not reflect the presence of these putatively transmissible enzymes.

PS3: 5

Screening of the antimicrobial activity of wild mushrooms phenolic extracts against clinical isolates

Maria Alves^{1,2,3}, Isabel Ferreira³, Anabela Martins³, Manuela Pintado¹

¹Universidade Católica Portuguesa Porto, Portugal; ²Hospital Trás-os-Montes e Alto Douro; ³Instituto Politécnico de Bragança, Portugal

Antimicrobial drugs have long been used for prophylactic and therapeutic purposes; however the drug-resistant bacterial strains have been creating serious treatment problems. This situation has forced the

search of new antimicrobial substances effective against pathogenic microorganisms resistant to conventional treatments. Natural resources have been tested and among them mushrooms could be an alternative. This work aimed to screen the antimicrobial activity of phenolic extracts of 13 edible mushroom species, collected in Bragança, against several clinical isolates obtained in Hospital Center of Trás-os-Montes and Alto Douro, Portugal. Microdilution method was used to determine the Minimum Inhibitory Concentration (MIC) and the Minimum Bactericidal Concentration (MBC). MIC results showed that *Russula delica* and *Fistula hepática* extracts inhibited the growth of Gram negative (*Escherichia coli*, *Pasteurella multocida*, *Morganella morganni*, isolated from urine) and Gram positive (*Staphylococcus aureus*, MRSA- isolated from wound exudates, *Enterococcus faecalis*, *Listeria monocytogenes*- isolated from blood culture, *Streptococcus agalactiae* and *Streptococcus pyogenes*- isolated from vaginal swab) bacteria. A bactericide effect of both extracts was observed in Gram positive bacteria (*L. monocytogenes*, *S. agalactiae* and *S. pyogenes*); regarding Gram negative bacteria, a total inhibition effect was observed for *P. multocida* at the highest concentration tested (20 mg/ml). *Lepista nuda* extract exhibited a bactericide effect upon *P. multocida* at 5 mg/ml, and inhibited *Proteus mirabilis* (isolated from urine) at 20 mg/ml. *Ramaria botrytus* extract did not present activity against Gram-negative bacteria; nevertheless, it showed activity against *E. faecalis*, being bactericide for *S. agalactiae* and *S. pyogenes*. Moreover, this was the extract with the highest activity against *L. monocytogenes* with MBC of 10 mg/ml. *Leucopaxillus giganteus* extract inhibited the growth of *E. coli* and *P. mirabilis*, being bactericide for *P. multocida*, *L. monocytogenes*, *S. pyogenes* and *S. agalactiae*. Among all the studied mushrooms, *Agaricus arvensis* seemed to present the lowest antimicrobial activity against all the tested bacteria. The extracts are chemically characterized and the individual/combined phenolic compounds will be submitted to antimicrobial assays in order to identify compounds responsible for the mushrooms bioactivity.

PS3: 6

Production of extracellular melanin from the fungus *Gliocephalotrichum simplex*

Pratibha Jalmi¹, Pranali Bodke¹, Solimabi Wahidullah², Raghukumar S.¹

¹Mykotech Private Limited, India; ²National Institute of Oceanography, Dona Paula, Goa, India

Melanin pigments perform many biological functions in bacteria, fungi, plants and animals. They protect cells against ultraviolet radiation and there are indications that they also have excellent radioprotective properties. Despite their potential use in cosmetics and various other industries, their use has been limited because melanins are generally not produced extracellularly and are expensive to prepare. We report here the copious production of extracellular melanin by three fungi: *Gliocephalotrichum simplex*, *Gliocladiopsis* sp. and an unidentified fungus. Qualitative tests confirmed these pigments to be melanins. Among these, melanin produced by the fungus *Gliocephalotrichum simplex* was studied in detail. The pigment was induced by tyrosine and was inhibited in the presence of kojic acid, suggesting that it was a eumelanin. Typical UV-absorbance, as well as IR, ¹³C solid-state and ¹H NMR spectra confirmed this. The melanin appears to be a copolymer of dihydroxyindole carboxylic acid, and dihydroxy indole, and associated with some carbohydrates and proteinaceous matter. Preliminary media optimization studies were carried out. A maximum of up to 6.6 g L⁻¹ melanin was produced in culture under optimal conditions. Such a high yield suggests that *Gliocephalotrichum simplex* is a potential source of melanin for biotechnological applications by virtue of high yields of extracellular melanin.

PS3: 8

Ocurrence of environmental *Pseudomonas aeruginosa* and other associated species in Hospital de Faro, Portugal

Pedro M de Abreu^{1,2}, Susana Santos¹, Gabriel Paiva¹, Ana Almeida², Paula V. Morais¹

¹IMAR-CMA, 3004-517 Coimbra, Portugal; Department of Life Sciences, FCTUC, University of Coimbra, Coimbra, Portugal; ²Unidade de Investigação em Ciências da Saúde, CIIERT, Instituto Piaget, Portugal

Nosocomial infections have been recognized for over a century as a critical problem affecting the quality of health care. *Pseudomonas aeruginosa* has been one of the most common pathogens isolated from hospitalized patients and is responsible for many nosocomial diseases. The aim of this study was to assess the prevalence and the genetic variability of *P. aeruginosa* strains present in four hospital units of the Hospital de Faro – Portugal, and identify strains associated to *P. aeruginosa* positive samples. *P. aeruginosa* from environmental and clinical samples were investigated. During 7 months, 290 environmental samples were obtained from mainly humid and moist areas (sink, shower and tap) as well as medical equipment, bed and floor. Additionally 30 clinical samples, from the same clinical units were analyzed. Samples presenting fluorescence on recovering medium (PIA) were then assessed for *P. aeruginosa* by isolation of fluorescent colonies, fluorescence confirmation, DNA extraction, 16S rRNA gene sequencing. Finally, random amplification of polymorphic DNA (RAPD) analysis was performed, in order to