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PS2: 9

Toxicity evaluation - the enhancement of the Ionic Liquids hydrophobic nature with the decrease of their toxicity

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Ionic liquids (ILs) are a novel promising class of solvents with interesting physicochemical properties. Many different applications have been described as alternatives to organic solvents in chemical processes. Despite their low vapor pressure, even the water immiscible ILs shown some solubility in water, that allows their dispersion into the aquatic systems resulting in water contamination. This work aims at enlarging the restricted knowledge about the ILs toxicity and inhibitory effects in aquatic ecosystems and to investigate the possibility of designing hydrophobic ionic liquids of low toxicity. It reports a set of toxicity results, which correspond to aromatic and non-aromatic immiscible ILs, through the use of different cations (pyridinium, piperidinium, pyrrolidinium and imidazolium) and hydrophobic anions (bis(trifluoromethylsulfonyl)imide [NTf₂]⁻ and hexafluorophosphate [PF₆]⁻). In this context, biological assays were performed using organisms of different trophic levels, such as the decomposer *Vibrio fischeri*, the producer *Pseudokirchneriella subcapitata* and the first consumer *Daphnia magna*. Contrary to the common belief that the ILs toxicity increases with their hydrophobicity it is here shown that it is possible to design ILs with an enhanced hydrophobic character, expressed as water solubility, and lower toxicity, as determined by the tests here conducted, by elimination of their aromatic nature.

PS2: 10

A new method for testing antimicrobial activity of filamentous fungi

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Fungi have been studied as a potential source of antibiotics since 1941. Some of them have a strong antimicrobial effect against other saprophytic and phytopathogenic fungi, or even against yeast and bacteria. To detect the *in vivo* production of fungal antimicrobial compounds different strategies are currently used. The most common comprise dual cultures on solid culture medium, or growth in liquid media containing the substrate previously used by the fungal antagonist. However, these strategies can be compromised by the different growth rates of both microorganisms and/or by the low stability of antimicrobial compounds. In this work, a bioassay to detect the antimicrobial activity of filamentous fungi against single-cell microorganisms is presented. The developed assay was first established using as fungal model *Hypholoma fasciculare*, a basidiomycete found in chestnut grove soils of Trás-os-Montes (Bragança), in which it displays a significant antagonistic action against different filamentous fungi. The yeast *Saccharomyces cerevisiae* was selected to be tested as the sensitive indicator strain. Parameters such as the assay temperature and the culture medium assay, fungal growth and yeast inoculum concentration were optimized during assay design. Fungal antimicrobial activity was evaluated in PDA medium after 48h at 25 °C, using a 6 days grown fungal culture and 10⁶ CFU/ml of the yeast indicator strain. The described assay can be easily used to test the *in vivo* fungal antibiotic activity against single-cell organisms such as yeasts or bacteria, allowing a faster and more reliable strategy than using, for example, fungal-grown liquid media. For confirming the method feasibility, a panel of different yeast species of industrial and clinical relevance was tested as sensitive strains. The obtained results confirm this new method as a rapid, effective and reproducible bioassay for testing antimicrobial activity of filamentous fungi against yeasts.

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PS2: 11

Antibiotic resistance in bacteria isolated from the floor of open air children playgrounds

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Antibiotic resistance is a serious problem of public health. Antibiotics are present in the environment and this leads to the emergence and dissemination of resistant genes among organisms. It is known that antibiotic resistant genes originated in the environment could be transferred to microorganisms including pathogenic bacteria. Children air playgrounds are places where children and adults go to play, but also dogs, birds and other animals share the environment. So, children could be exposed to a great number of