ABSTRACT

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Exploration of the potential for biosurfactant production by fungi from oil-contaminated environments

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Abstract

Following oil spill in the environment, dispersants are used to transfer the oil slick from the surface to the water column and thus limit the oil volume that could strand on the shoreline. This transfer generates oil microdroplets in suspension, which in turn increases exchange surface between oil and water, and thus favors the degradation activity of marine bacteria (Dussauze et al., 2011). For now, these dispersants are synthetic and derived from petrochemicals. However, there are concerns about their toxicity and their possible impact on the marine environment.

Numerous studies have highlighted biosurfactant (dispersant components) production by microorganisms and more specifically by bacteria (Desai & Banat, 1997; Rizzo et al., 2018; Xia et al., 2019). The potential of fungi has been relatively less explored, yet they are known for their ability to produce metabolites of interest among which are surfactants (Bhardwaj et al., 2013; Dell'Anno et al., 2018).

In this context, the aim of the present study was to screen and select fungi able to produce surfactants. For this purpose, sampling at four sites contaminated with hydrocarbons in Brittany (France) was carried out over two periods, in winter and in summer, resulting in a total of 30 samples (12 water samples and 18 sediment samples).

Sampling was carried out following ISO 5667-19:2005-03 and ISO 19458:2006-11 standards for sediment and water sampling, respectively. The physico-chemical characteristics of these samples were recorded (pH, salinity, water temperature, conductimetry) and the sample hydrocarbon composition was determined using GC-MS (Gas Chromatography coupled to Mass Spectrometry).
Concerning sample microbiological analysis, direct plating and selective enrichments (medium enriched in diesel) were performed in order to isolate fungi and create a working collection. A total of 701 isolates were obtained and cryopreserved at -80°C before screening for surfactant production. On the other hand, total DNA of collected samples was extracted and a culture-independent approach (i.e., ITS metabarcoding) was applied to determine fungal diversity in these contaminated environments.

Isolates from the cryopreserved collection were then cultured in 24-deepwell microtiter plates on a Duetz system in two different media (i) BSF5, a medium known to enhance surfactant production containing rapeseed oil, yeast extract and glucose and (ii) Kavadia medium which is characterized by a high glucose concentration (80g/L).

Supernatants were then recovered and their surface activity evaluated by two tests, i.e., the "Parafilm M Test" (PMT), showing supernatant hydrophobicity, and the "Oil Spreading Test" (OST), which highlights repulsive activity. Among tested isolates, 25.6% were shown to possess surfactant activities and isolates positive for at least one of the two tests were identified by sequencingof a gene of taxonomic interest (ITS, β-tubulin etc.). Among them, one *Trichoderma citrinoviride* isolate showed a very promising activity. Medium composition was then further optimized to increase production of active molecules by the latter isolate. This optimization was performed using a full factorial design methodology with 4 different carbon sources at 3 different concentrations, 2 salt levels and 2 nitrogen sources. A scale up from 2.5 to 100mL was also carried out for the best identified conditions. It was found that a glycerol-based medium yielded the highest OST and PMT values and that these activities were maintained at a higher scale.

The dispersion efficiency of active molecules produced by *T. citrinoviride* is currently tested on a larger scale to determine the potential of this fungus for oil spill bioremediation comparing it with commercial surfactants used and/or dispersants currently in use. At the same time, a chemical characterization of these molecules using NMR and MALDI-TOF mass spectrometryis undergoing in order to determine the nature of these molecules. .

Keywords

Oil pollution, Dispersant, Biosurfactant, Fungi

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