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BALTIC SEA MICROBES BIOSURFACTANTS PRODUCTION QUANTIFICATION

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SCOPE

Pollution of coastal environments by petroleum hydrocarbons damages these ecosystems when lighter oil fractions spread close to the surface and heavier ones infiltrate to the sediments. Biosurfactants are amphiphilic compounds produced by diverse microorganisms to thrive in lipids or oils [1]. Their study is limited by the qualitative nature of current methods. This study aims to apply the VPBO method [2] to study the biosurfactants produced by Baltic Sea microbes with bioremediation potential.

METHODOLOGY

Yarrowia lipolytica and *Pichia anomala* isolated from Baltic Sea sediments, *Saccharomyces cerevisiae* (negative control) and *Fusarium equiseti* (positive control) were cultivated in PDA and Czapek plates for one week. Approximately 1cm^2 of biomass was harvested and suspended in water with 0.05% agar. Twelve wells plates with 2ml of Bushnell-Haas broth per well were inoculated (OD600nm ~ 0.05). 10 µl of crude oil were added to each well, and plates were incubated at room temperature. Aliquots of 400 µl of culture were taken each week and centrifuged. 10 µl of the clear supernatants were assayed in previously prepared 96 wells VPBO plates according to [2] with minor changes. After 1h incubation at room temperature **(Fig. 1)**, the absorbance at 625 nm was measured with a microplates reader to quantify the release of VPBO dye by the action of biosurfactants.

RESULTS AND DISCUSSION

After 24h, *Yarrowia lipolytica* and *Pichia anomala* produced biosurfactants to increase the availability of crude oil hydrocarbons, reaching the **highest concentration (Fig. 2)**. At this point, *Y. lipolytica* and *P. anomala* biosurfactants production was similar (**0.2 mM**).

By the first week, the biosurfactants production decreased, with *Y. lipolytica* reducing its biosurfactants to 43% of the 24h value, while *P. anomala* biosurfactant concentration decreased to 18% of the 24h value, a level similar to the negative control *S. cerevisiae*. This could indicate that *Y. lipolytica* relies mainly in the biosurfactants for the uptake of hydrocarbons while *P. anomala* uses a combination of biosurfactants and other mechanisms, such as lipase enzymes [3].

After two weeks of incubation, the biosurfactants production of both yeasts was basal, similarly to the positive and negative controls, possibly indicating that crude oil hydrocarbons had been uptaken by the fungal cells.

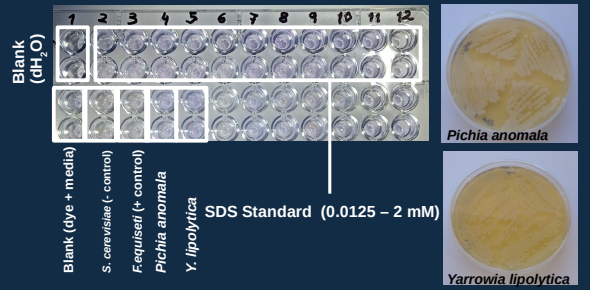

Fig. 1. Detail of biosurfactants assay plate (1 week) after incubation. *P. anomala* and *Y. lipolytica* culture plates.

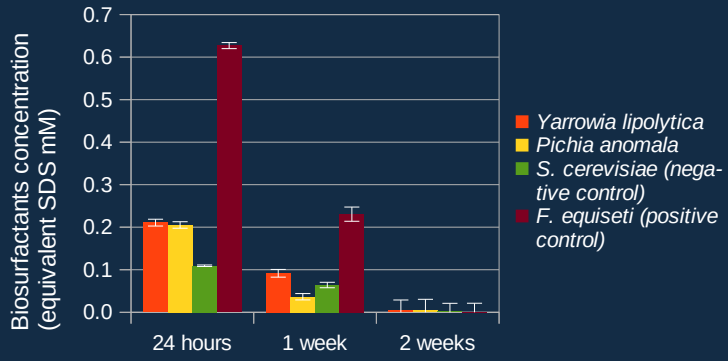

Fig. 2. Biosurfactants production vs time.

CONCLUSIONS

The tested *Y. lipolytica* and *P. anomala* yeasts produced biosurfactants up to the first week of incubation, with a peak at the first 24 hours. We intend to apply the VPBO method for the study of biosurfactants production of other oil degrading microorganisms isolated from the Baltic Sea in order to assess their bioremediation potential.

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