SEAWATER-BASED BIOCATALYTIC STRATEGIES USING MARINE MICROORGANISMS

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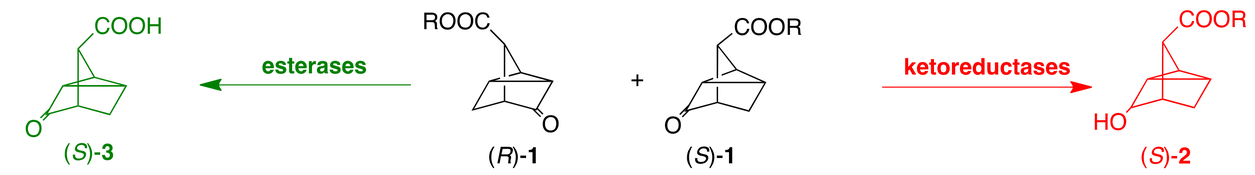
OBJECTIVES Bioprospecting of enzymes from marine bacteria and fungi from untapped deep-sea extreme habitats is the aim of this work, with reference to ketoreductases, esterases, fructofuranosidases, and transaminases. Free enzymes or whole cells were used for biotransformations in fresh- or seawater.

STEREOSELECTIVE ENZYMES FROM MARINE BACTERIA

Halotolerant bacterial strains have been screened with colorimetric screening from a wide collection of isolates obtained from the seawater-brine interface of different deep hypersaline anoxic basins (DHABs) of the Eastern Mediterranean Sea, namely, Urania, Bannock, Discovery and Atalante basins.

KETOREDUCTASES (KRED) AND ESTERASES (EST)

33 Strains were selected and used for the resolution of racemic propyl ester of anti-2-oxotricyclo[2.2.1.0]heptan-7-carboxylic acid, a key intermediate for the synthesis of D-cloprostenol.



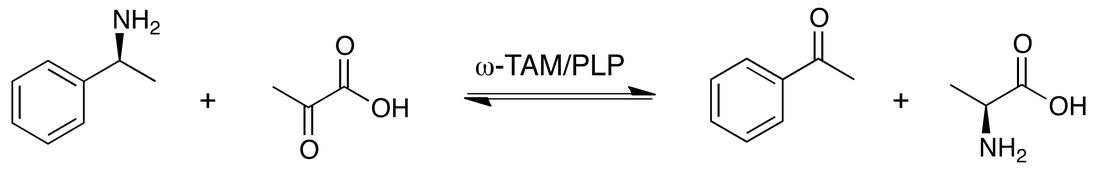
Strain	Growth optimum (NaCl %)	3 (%)	3 ee (%)	2 (%)	2 ee (%)	Time (h)
Bacillus horneckiae 15A[a]	0-3	<5	n.d.	58	100	3
Halomonas aquamarina 9B[a]	3	57	100	<5	n.d.	5
Virgibacillus pantotheticus 21D[a]	6-9	<5	n.d.	58	100	6
Virgibacillus pantotheticus 21D[b]	6-9	46	88	7	n.d.	6

Activity on racemic propyl ester **1** of whole cells of marine bacteria. [a] Biotransformation in the presence of glucose (5%); [b] Biotransformation in the absence of glucose.

Virgibacillus pantotheticus 21D showed high halotolerance, enantioselective KRED activity in the presence of glucose, and Est activity was prevalent in the absence of glucose.[1]

TRANSAMINASES (TAM)

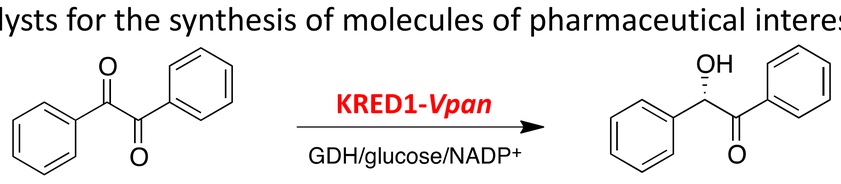
A screening was also carried out for finding transaminase (ω-TAM) activities.



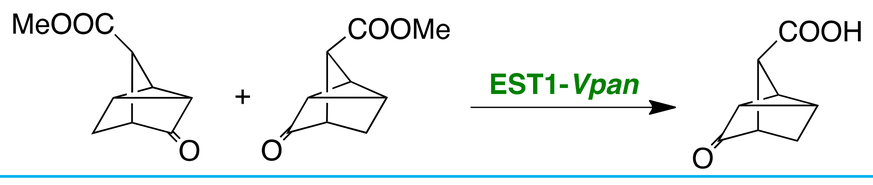
V. pantotheticus 21D showed high activity. Since its potential (different enantioselective activities, significant halotolerance), **the genome of *Virgibacillus pantotheticus* 21D was sequenced**.

Three genes (KRED1, EST1, TAM1) were identified by homology sequence and produced as recombinant proteins in *E. coli* and are currently used for evaluating their potential as enantioselective biocatalysts for the synthesis of molecules of pharmaceutical interest.

KRED1-*Vpan*:



EST1-*Vpan*:



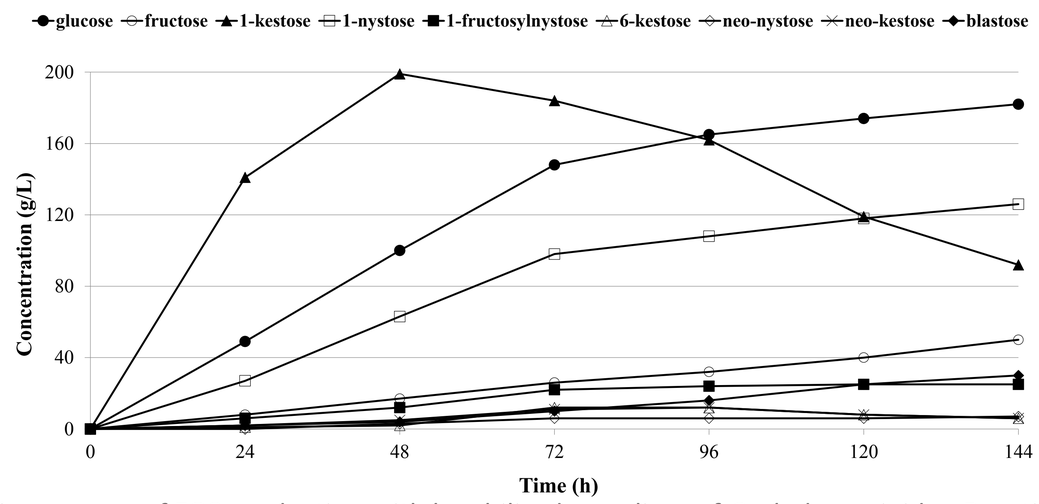
FUNGAL STRAINS FOR SEAWATER-BASED BIOCATALYTIC STRATEGIES

The large consumption of freshwater in bioprocesses (fermentations and biotransformations) is a matter of concern about the sustainability of many bioprocesses. The use of seawater for carrying out bioprocesses seems a sustainable alternative. Halotolerant marine microorganisms (and more generally halophilic and/or halotolerant microorganisms) could be a plentiful source of enzymes for accomplishing biocatalysis in seawater.

SWEET-AND-SALTY BIOCATALYSIS: FRUCTOOLIGOSACCHARIDES (FOS) PRODUCTION USING MARINE FUNGI IN SEAWATER

Marine yeasts and moulds were screened for fructooligosaccharides (FOS) production from sucrose (fructofuranosidases activity).

A halotolerant strain of *Cladosporium cladosporioides* (grown and used in seawater) resulted an efficient producer of FOS from sucrose (600 g/L).[2]

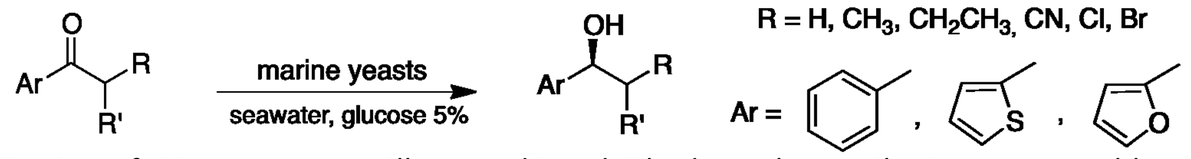


Time-course of FOS production with lyophilized mycelium of *C. cladosporioides*. Reaction conditions: 600 g/L sucrose, 40 g/L lyophilized mycelium, seawater (pH 7.5), 50 °C

The activity of the lyophilised mycelium showed to be stable for at least 30 cycles and was used as immobilized system for a continuous process in flow reactors.[3]

STEREOSELECTIVE REDUCTIONS OF KETONES WITH MARINE YEASTS

Marine yeasts from deep-subseafloor sediments (isolated by University of Brest) were grown and used in seawater for the stereoselective reduction of different ketones.



Strains of *Meyerozyma guilliermondii* and *Rhodotorula mucilaginosa* were able to enantioselectively reduce various aromatic ketones with high yields and moderate-to-high enantioselectivity, with no major differences between biocatalysis carried out in seawater or freshwater. Finally, the selected marine yeasts were used for the reduction in seawater of key-intermediates for the synthesis of molecules of pharmaceutical interest (desogestrel, norgestrel, gestodene, pramipexole).[4]

REFERENCES **1.** De Vitis *et al.*, *Mar. Biotechnol.* **2015**, *17*, 144. **2.** Zambelli *et al.*, *Process Biochem.* **2015**, *50*, 1086. **3.** Zambelli *et al.*, *Food Chem.* **2016**, *190*, 607. **4.** Serra I. *et al.; CHEMSUSCHEM submitted*

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