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Enzymes for bacterial exopolysaccharide engineering

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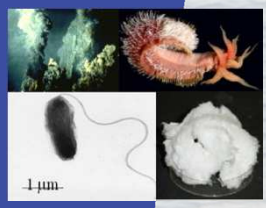
Ecosystèmes Microbiens et Molécules Marines pour les Biotechnologies

Introduction



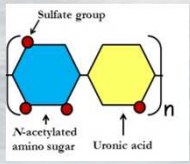
Glycosaminoglycans (GAG) found in animal tissues play a critical role in cellular processes since they bind many cellular components and interact with the extracellular matrix. Therefore, they have a great potential for the design and preparation of various therapeutic drugs. Initially extracted from animal tissues, GAG present risks of contamination, diseases and pathogen.

Natural exopolysaccharides (EPS) from marine bacteria can be alternative sources of GAG. In order to enhance and promote new biological activities, some modifications of the polysaccharide backbone have to be carried out. Until now, chemical methods for depolymerisation and sulfation are used but they lack specificity and reproducibility. In this context, new enzymes are sought to elaborate innovative bioactive derivatives.

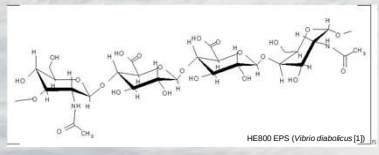
Marine-derived exopolysaccharides as glycosaminoglycan-mimetics

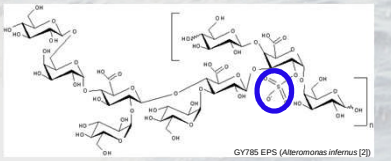
The biological activity of polysaccharides depends on the fine chemical structure, in particular the molecular weight, and the amount and position of sulfate groups.

There are very few naturally sulfated bacterial PS; most of them have been described in *Vibrio*, *Alteromonas* and *Pseudoalteromonas* strains which are consequently, very attractive bioproducts.



Schematic chemical structure of a GAG-like molecule.

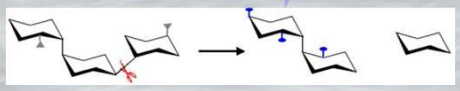




Chemical structure of two exopolysaccharides (EPS) produced by marine bacteria.
They are high-molecular-weight polyanionic (hydroxyl and carboxylic groups) heteropolysaccharides. One of them is sulfated.

Enzymes for targeted modifications on the polysaccharide backbone

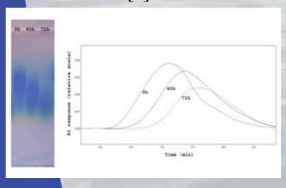
Native polysaccharide → **Biocatalyst** → **Bioactive derivatives**



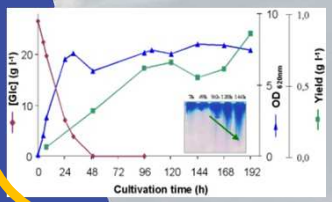
Depolymerisation	Hydrolases/Lyases
N-Deacetylation	*N*-deacetylases
Sulfation	Sulfotransferases
Biosynthesis	Glycosyltransferases

1 – Depolymerisation

The screening of various enzymes and microorganisms has been performed. Only one strain (*Enterococcus faecalis*) was shown to be able to depolymerise the polysaccharide HE800 [3].

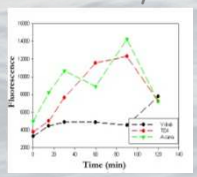


E. faecalis genome analysis and activity assays allowed the identification of the activity on the EF1800 endo-α-*N*-Acetyl-galactosaminidase (commercially available from NEB) [3].



At the end of a prolonged fermentation, MW of the polysaccharide decreases suggesting a depolymerising enzyme within the *V. diabolicus* genome.

2 – *N*-deacetylation

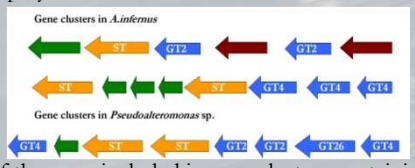


Appearing amine groups were detected using fluorescamine. These new amine groups could be further sulfated.

3 – *O*- or *N*-sulfation

	V.diabolicus	*A. infernus*	*Pseudo-alteromonas* sp. HYD721
Glycoside hydrolases (GH)	42	43	42
Polysaccharide lyases (PL)	0	1	2
Glycosyltransferases (GT)	31	54	36
Carbohydrate esterases (CE)	4	12	12
Sulfotransferases (ST)	Nd	4	5

Carbohydrate sulfotransferase genes have been identified within the genome of marine bacteria that produce a sulfated polysaccharide.



Some of them are included in gene clusters containing glycosyltransferases.

Due to the GAG-like importance and the biological activity of the marine microbial polysaccharides, new enzymes will allow new developments of the marine polysaccharides and their derivatives in the pharmaceutical field.

[1] Rougeaux H, Kervarec N, Pichon R, Guezennec J, *Carbohydr Res*, 1999. **322**(1-2): 40-45.

[2] Roger O, Kervarec N, Ratiskol J, Colliec-Jouault S, Chevolot L, *Carbohydr Res*, 2004. **339**(14): 2371-2380.

[3] Rigouin C, Delbarre-Ladrat C, Ratiskol J, Sinquin C, Colliec-Jouault S, Dion M, *Appl Microbiol Biotechnol*, 2012. **96**: 143-151.

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