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Réponse de *Pseudomonas aeruginosa* au stress marin

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Pseudomonas aeruginosa exposé au stress osmotique et au jeûne en milieu marin est profondément modifié. Il donne, à côté des formes bacillaires, des formes ovoïdes dont la taille est inférieure à 0,45 µm. Son unique flagelle disparaît. Diverses sortes de colonies sont alors obtenues selon la durée d'incubation en eau de mer et le milieu de récupération :

- des colonies muqueuses : obtenues après un à cinq jours d'incubation en eau de mer sur des géloses nutritives additionnées de 15, 20 ou 25g de NaCl/litre,elles présentent les mêmes caractères biochimiques sur Api 20 NE et Api Zym que la souche parentale.

- des colonies rugueuses : obtenues sur la gélose nutritive normale (GN), sur la gélose nutritive additionnée de 23g de NaCl (GN NaCl) et sur la gélose nutritive préparée à l'eau de mer (GN EM). Elles présentent de légères modifications phénotypiques par rapport à la souche initiale. Celles qui sont obtenues après des périodes d'incubation dépassant cinq mois sont plus profondément modifiées, elles élaborent un pigment jaune localisé au niveau des colonies, donc non diffusible dans la gélose. Son intensité dépend de la durée de vieillissement et de la lumière. Ces colonies ont un % GC de 59,8, ce qui confirme qu'il s'agit de colonies de *P. aeruginosa*.

Les caractères de ces colonies varient selon la nature des milieux de récupération.

Au cours de son incubation en eau de mer, *P. aeruginosa* devient plus actif sur les galeries Api Zym : il y a apparition d'une ß-galactosidase, d'une α et d'une ß-glucosidase et d'une valine arylamidase.

Après de longues périodes d'incubation dans l'eau de mer (dépassant 8 mois), il ne disparaît pas mais donne des colonies jaune à rouge, à culture très lente nécessitant plus de deux semaines d'incubation à une température de 24°C et de très petite taille (1 mm de diamètre). Ces colonies sont inactives sur Api 20 NE. Après culture en eau de mer additionée de bouillon nutritif à 5 %, elles donnent des colonies typiques de *P. aeruginosa* avec production caractéristique de pyocyanine et de pyoverdine.

Ces modifications peuvent avoir une influence sur la spécificité des méthodes utilisées pour le dénombrement de la bactérie dans les échantillons marins.

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p-Nitrophenylphosphatase activity in Marine Microalgae *Tetraselmis suecica*

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Many species of algae are capable of obtaining phosphorus from esters in order to sustain growth in the absence of orthophosphate. Two enzymes, acid and alkaline phosphatases have been found in some species of marine microalgae. The cells contain two pools of phosphatases: 1) adaptative membrane enzymes responsible for phosphorus assimilation from the environment and 2) constitutive enzymes indispensable for intracellular formation of phosphomonoesters (Kluenzer & Perras, 1965; Lien & Knutsen,1973). However, the phosphohydrolases of algae have been rarely characterized biochemically. In this paper, the influence of pH, temperature, substrate and Pi concentration on phosphatase activity of crude cell-free extracts and whole cell of *Tetraselmis suecica* (Prasinophyceae) were analyzed.

The algae were grown in culture medium f/2 and collected at the postexponential growth phase.

Two extraction procedures for preparing crude cell-free extracts were carried out. In the first, the algae were filtered through a glass fiber filter (Whatmann GF/C), the filter was ground with glass powder in presence of buffer 100 mM Tris-HCl, pH 7.0. In the second extraction procedure, the culture was centrifuged (3.000 r.p.m for 15 minutes), the supernatant discharged and the pellet washed twice with 0.6 M NaCl. Finally 3 ml 100 mM Tris-HCl were added and sonicated for 20 minutes in two steps. The resulting extract was employed for the determination of phosphatase activities. The reaction mixture contained 1.0 ml buffer, 0.1 ml of substrate and 0.1 ml extract. When the influence of Pi was analyzed 0.1 ml of PO_4^{3-}(0-100µM) was added to the reaction mixture. The assays temperature was 40°C and the incubation time ranged from 30 to 60 minutes. The substrate concentration employed for Km and Vmax analysis ranged from 0 to 50 mM

For the whole cell phosphatase activity three series of tubes were prepared with a reaction mixture containing : a) 0.5 ml buffer + 25 µl substrate; b) 0.5 ml buffer + 0.5 ml filtrate culture + 25 µl substrate and c) 0.5 ml buffer + 0.5 ml microalgae culture diluted 1:5. The reaction mixture was incubated for 120 minutes at 20°C. Five ml of NaOH 0.1 M were added after completion of the incubation. In the case of a) 0.5 ml filtrate culture were added before the measurement. The substrate concentration for calculation of Km and Vmax ranged between 0 and 16 mM.
Results were expressed as µmol p-nitrophenol.culture $l^{-1}.min^{-1}$ for crude cell-free extracts and whole cell phosphatase activities.

In the assays carried out with crude cell-free extracts, the extraction procedure (grinding with powder glass or sonication) was very important in the preferential solubilization of enzymes. In both cases,the enzyme activity had two peaks at pH 5.5 and 8.5, but the ratio of them (pH 5.5/pH 8.5) for enzyme activity for grinding and sonication was 16.0 and 0.46 respectively.

Microscopical observations of residual pellets after two extraction procedures showed a great proportion of entire cells in the first case whereas total cell disintegration ocurred in the second one. Therefore, it should be considered that sonication results in a total cell disintegration with solubilizing of the membrane-bound enzyme, but when grindind with powder glass is employed, only a partial solubilitazion is observed.

The optimal temperature for phosphatases activities at pH 5.5 and 8.5 was found to lie within the range 40-45°C. The activation energy(Kcal.mol^{-1}) was 9.56 and 7.90 for pH 5.5 and 8.5 respectively, whereas the corresponding Km and Vmax values at pH 8.5 were 1.64 mM and 10.735 µmol.$l^{-1}.min^{-1}$. The PO_4^{3-} 100 µM inhibited competitively phosphatase activity at pH 8.5 (Ki=2.14 mM). In the range 1-10 µM inhibition was not observed for this phosphatase activity.

An exocellular phosphatase activity (pH 8.5) in incubation mixture was observed for this marine microalga, but within the range 0-100 µM PO_4^{3-} the inhibition was not clearly noticed.

The whole cell phosphatase activity showed an optimal pH 8.5, which corresponds to an alkaline phosphatase. The Km and Vmax values were 0.58 mM and 16.750 µmol $\cdot l^{-1} \cdot min^{-1}$.As in the case of the exocellular activity in the range 0-100 µM PO_4^{3-} an inhibition was not clearly observed.

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