LIPOLYTIC ACTIVITY OF BACTERIA ASSOCIATED WITH THE ANTARCTIC MACROALGA *PALMARIA DECIPIENS*

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ABSTRACT

A total of 59 strains, isolated from surfaces of *Palmania decipiens* from Antarctic, were tested for Lipolytic activity. During the initial screening almost all strains grew in live oil and nine strains showed significant lipase activity. All lipase producer bacterial strains belong to the genus Pseudomonas. Scanning electron micrographs of the *Palmania* surfaces revealed the growth of an epiphytic diatom and bacterial community.

INTRODUCTION

The surfaces of marine eukaryotes provide relatively rich source of nutrients thus attracting numerous microorganisms. The production of enzymes by some bacteria may serve a survival tool in this highly competitive environment. Recently, it has become evident that enzymes from psychrotrophys and psychrophiles have some potential for low temperature catalysis at 0-20 °C where the activity of mesophilic enzymes tends to be reduced (Counot, 1991; Feller et al., 1992).

The diversity of bacterial isolates obtained from the surfaces of marine algae *Palmaria* was investigated in the current study. The red microalgae *Palmaria decipiens* (*Palmariales*, *Rhodophyta*) is endemic from Antarctic, and extremely abundant in terms of biomass along the western Antarctic Peninsula. The aim of this study was to investigate the lipolytic activity of bacteria isolated from the surfaces of the Antarctic algae *Palmaria*.

It is well known that bacteria are able to grow and transform vegetable oils due to the excretion of lipase, which renders the lipid substrates available to cells. Lipases acts at the interface and catalyses hydrolysis of fats and mono-and di-glycenides to free fatty acid and glycerol.

MATERIALS AND METHODS

Isolation procedure

Red algae (*Palmaria decipiens*) were collected in mid-summer (December-January 2008-2009) at Admiralty Bay, Antarctic Peninsula during the Brazilian scientific expedition.

Pieces (1 cm) of alga thalus were washed in sterilized natural sea water and placed in LB medium supplemented with 1% of olive oil. The cultures were incubated at 18 °C for five days, and the individual colonies were transferred to marine agar stored at 4 °C. The identification of bacterial strains was done through the analysis of fatty acid methyl-esters (FAMES). Examination of bacterial communities and alga surfaces was done through a field emmission scanning electron microscope Leo 982 (Zeiss + Leica).

Screening of lipase-secreting bacteria

The Rhodamine agar method was used for the presence of the extracellular lipase. The strains were inoculated in mineral medium supplemented with tween 80 and the indicator Rhodamine B (C.I. 45170) – Synth, Brazil) (1 µg/mL), to 18 °C for 24 to 48 hours. Lipase activity was identified on the plate as an orange fluorescent halo under UV Light at 350 nm after 48 hours of incubation at 20 °C. Lipase activity was quantitatively assayed from supernatant of the liquid culture.

RESULTS AND DISCUSSION

A total of 59 isolates were obtained from the alga surface, and were deposited in the Culture Collection of Environmental Microbiology Laboratory of Embrapa Environment, in Jaguariúna, São Paulo State, Brazil. Each isolate was tested for its lipolytic activity at 18 °C. Nine isolates screened showed significant lipase activity as indicated by the comparison of degradation halos in agar plates. The halos ranging from 23,8 mm to 42,5 mm diameter (Table 1) . .

Analysis of identification revealed that the all bacterial isolates able in producing lipase belonged to the genus *Pseudomonas*, with two species *P. putida* and *P. savastanoi fraxinus*. One high producer lipase strain of *P. savastanoi fraxinus* (Antar10) was assayed for its potential in producing lipase.

Table 1. Identification of lipase – producer bacterial strains in agar plate. Diameter of the degradation halos (mm ± SD) by nine bacterial strains isolated from *Palmaria*.

Strain	Similarity	Identification	Halos (mm ± SD)
Antar2	0,856	*Pseudomonas putida biotipo B/vancouverensis*	28,8 ± 4,8
Antar3	0,912	*Pseudomonas putida biotipo B/vancouverensis*	24,5 ± 3,1
Antar4B	0,436	*Pseudomonas putida biotipo B/vancouverensis*	34,8 ± 0,5
Antar5	0,925	*Pseudomonas putida biotipo B/vancouverensis*	27,5 ± 2,9
Antar6	0,384	*Pseudomonas putida biotipo B/vancouverensis*	26,0 ± 1,2
Antar7	0,861	*Pseudomonas putida biotipo B/vancouverensis*	23,8 ± 2,5
Antar8	0,900	*Pseudomonas putida biotipo B/vancouverensis*	37,5 ± 2,9
Antar9	0,895	*Pseudomonas savastanoi fraxinus*	27,0 ± 2,4
Antar10	0,822	*Pseudomonas savastanoi fraxinus*	42,5 ± 2,9

Scanning electron micrographs (SEM) demonstrate the growth of an epiphytic bacterial community on the surface of *Palmaria in situ(Figure 1)*. It is shown a complete lawn of one similar species covering the surfaces. It is also demonstrated the presence of different species of diatoms. The distribution patterns suggest strongly that bacteria utilize compounds derived from the alga.

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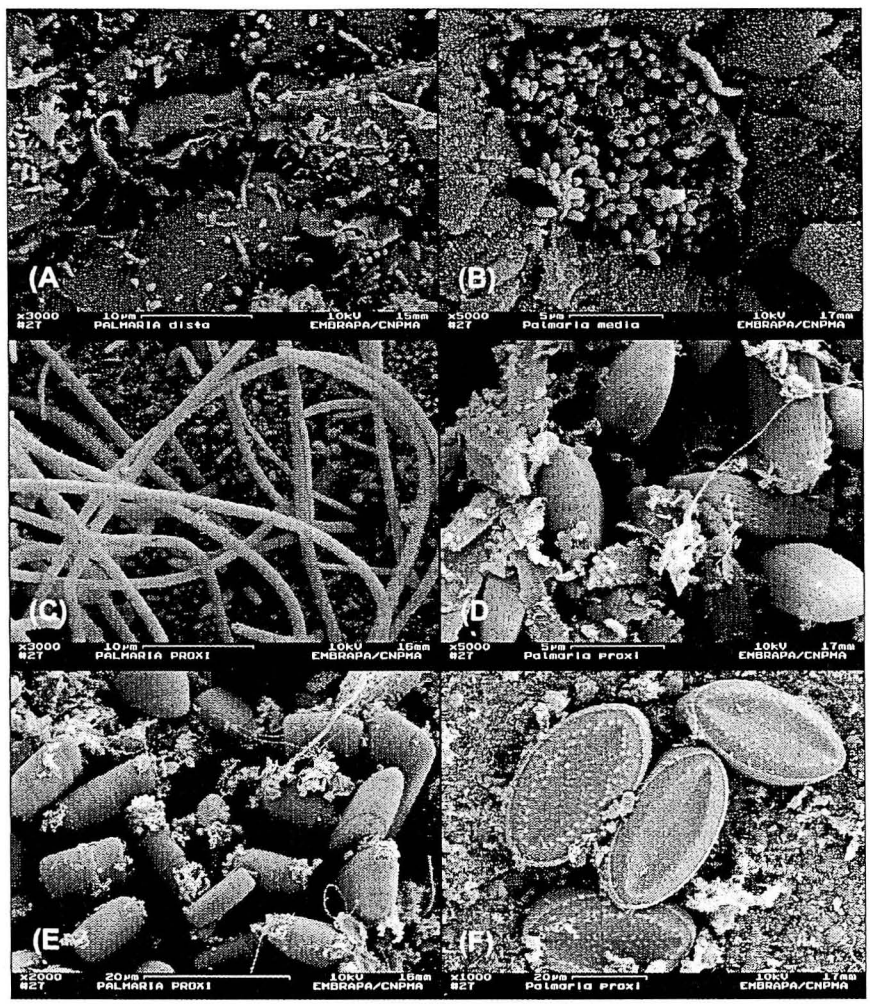

Figure 1. Scanning electron micrographs of the surface of *Palmaria*. The surface is colonized by rod-shaped (A) and phylamentous (C) (like actinobacteria) bacteria and by different diatoms(D,E and F). Diatoms were frequently the microbial dominants in the surfaces of the alga studied. Bacteria often grew from within cavities of cells(B).