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Chromogenic bacterioneuston from littoral pools and its role in mineralization process

Bacterioneuston cromógeno de pozas litorales y su participación en el proceso de mineralización

Pablo Conejeros [1], Patricio García-Tello [2] and Jorge Q León [3]

[1] *Instituto de Oceanología, Universidad de Valparaíso , Chile, Casilla 13-D , Viña del Mar, Chile.*
[2] *Instituto de Biología, Laboratorio de Microbiología Marina, Universidad Católica de Valparaíso, Casilla 4059, Valparaíso, Chile.*
pgarciat@ucv.cl
[3] *Laboratorio de Microbiología Ambiental y Biotecnología, Universidad Nacional Mayor de San Marcos, Perú.*

RESUMEN

Bacterioneuston cromógeno fue aislado de pozas litorales y sometido a pruebas de tinción de Gram, morfología y motilidad, bioluminiscencia, presencia de citocromo oxidasa, catalasa, nitrato reductasa y presencia de enzimas hidrolíticas. Los resultados sugieren que las bacterias cromógenas psicrófilas aisladas constituyen básicamente una contribución del sedimento a la película superficial cuya participación en la mineralización del nitrógeno y carbono puede ser importante.

Palabras clave: propiedades hidrolíticas, nitrato reductasa, tinción Gram, sedimentos, mineralización.

Key words: hydrolytic properties, nitrate reductasa, Gram-stain, sediments, mineralization.

The sea water surface film has a microflora which includes microalgae, varied fungi and algae, spores, protozoa, phytoflagellates and bacteria, being the latest one an important component in the mineralization processes. This film and its microflora receive the influence of important environmental factors such as temperature, salinity, ultraviolet radiation (Fehon & Oliver 1979), and also organic nutrients with carbon concentrations that reach 1.350 mg of carbon per liter (Sieburth 1979). Extreme fluctuations of these physicochemical parameters could be a negative influence on bacterial numbers, nevertheless the great quantity of organic matter and carbon in the surface film (Sieburth 1979) play an important role producing, most probably, a notorious enrichment of psichrophylic saprophytic bacteria *in situ*. Sieburth (1971) counted 2398 colony forming units /ml (CFU/ml). Higher numbers can also be found in literature (De Souza-Lima & Chretiennot-Dinet 1984, Sieburth *et al.* 1976). In the water column heterotrophic viable numbers being much lower. An important part of bacteria from the sea-water surface film growing on a solid media produce pigmented colonies. Pigmented strains in the atmosphere have been shown to be protected against induced toxic forms of oxygen produced during aerobic respiration (Shapiro *et al.* 1977).Carotenoid pigmented organisms isolated from sea-water are also more resistant to ultraviolet radiation than non pigmented bacteria (Sieburth 1968), and in *Serratia marcescens* prodigiosin is related to hydrophobic adhesion mechanisms (Rosenberg 1984). In general, pigments play a protective role. Considering that chromogenic bacteria could represent a group fitted to the surface film conditions, we have investigated some features of their metabolism and morphology that we consider importants.

MATERIAL AND METHODS

Samples were taken from the sea-water surface film at the rocky littoral pools of the Marine Biological Station at Montemar, Universidad de Valparaíso, Valparaíso. Ninety-eight

samples were taken between may and july 1995 with a Garrett (1965) sampling net for bacterioneuston. All samples were taken to the laboratory in a refrigerated box, at aproximately 7° C and processed during the three next hours. Each sample (0.1 ml) was surface plated on marine agar 2216 (Difco) and incubated at 20° C in a low temperature controlled incubator. For counts and isolation of pure cultures, plates with 30 to 300 colonies were used. Fifty pigmented colonies were picked at random and further purified. Marine agar (MA) and marine broth (MB) were used for these procedures.

Media were prepared with or without agar (Difco) for MA and MB respectively according to the following formula: Bacto peptone 0.4% (w/v), Bacto yeast extract 0.1% (w/v), Bacto agar 1.5% (w/v), aged sea water 75%, (v/v) and distilled water 25% (v/v), pH 7.6. The Gram identification technique was done according to Buck (1982), morphology was determined by staining cells with crystal violet. Motility was determined by the hanging drop method using a 24h MB culture. Observation was done with a Nikon phase contrast microscope. Chromogenesis was determined by observation of colonies grown in 2216 after 8 days of incubation at 20° C. Bioluminiscence was done after the technique of Nealson & Hasting (1992) and cytochromo oxidase after the method of Kovacs (1956). Catalase was determined in MA cultures after 8 days incubation at 20° C with 3% H_2O_2. Nitrate reductase was done with the method described by MacFaddin (1980).

Determination of extracellular enzymatic activity was done by diferent methods. Caseinase was determined in MA with 1% casein (Merck). Incubation was done at 20° C for 4 days. Casein positive strains formed a clear zone around the colony. Gelatinase in MA was done with 5% gelatin (Merck). Incubation was done at 20° C for 4 days, and a positive reaction of proteolysis was indicated by a clear zone around the colony. Amylase was done with the method recommended by MacFaddin (1980), using starch (Merck). Esterase was done using 1% Tween 80 (Sigma) (w/v) in MA. Incubation was done at 20° C for 10 days. A positive reaction is indicated by an opaque zone around the colony. Dnase cultures were done under MacFaddin (1980) recommendations in DNase media (Difco) prepared with 75% of sea-water. Determination for glucose metabolism was done in Hugh-Leifson's media under the recommendations of Gerhard *et al.* (1994). Resistance to 0/129 compound was done.

RESULTS AND DISCUSSION

Most part of results are summarized in Table 1 and Figure 1. Counts of viable saprophytes from the surface film in MA 2216 yielded 10^3 -10^4 CFU/ml. Chromogenic strains represent *circa* 1/3 of the total viable counts. Forty-five strains survived after purification procedures in MA and MB. The colors of isolated chromogenic colonies were orange, pale rose, red, and different intensities of yellow. Facultative anaerobes represented 82% of the isolates, the diference being strict aerobes (18%). Obviously this condition is strongly related with the presence of oxidase (71 %), and catalase (96%) positive strains. The high number of facultative anaerobic, chromogenic strains, was not found either by Fehon & Oliver (1979) who report only 2% of chromogenic facultative anaerobes, and Gauthier (1975) who found all his isolates to be aerobes and catalase-negative.

Gram positive bacteria represented 44% of the chromogenic strains of which 69% had streptococcus, staphylococcus or diplococcus morphology. One Gram-negative isolate was a filamentous form. Gram-negative strains comprised 55% of the isolates and the majority of these were non-motile strains. Crow *et al.* (1975), Fehon & Oliver (1979),

Table 1. Characteristics of chromogenic bacteria. (+) Positive reaction; (-) Negative reaction.

Tabla 1. Características de bacterias cromogénicas. (+) Reacción positiva; (-) Reacción negativa.

Test / Strain	1p	2p	3p	4p	5p	6p	7p	8p	9p	10p	11p	12p	13p	14p	15p	16p	17p	18p	19p	20p	21p	22p	23p
Motility	+	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	+	-
Gram	-	-	+	-	-	+	-	-	-	+	-	+	+	-	+	-	+	+	+	+	-	-	+
Oxidase	+	+	+	+	+	-	+	-	-	+	-	+	+	+	+	+	-	+	+	+	+	+	+
Catalase	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Amylase	-	+	-	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	+
Caseinase	+	+	+	+	+	+	+	+	+	-	-	-	+	-	+	+	+	+	-	+	+	+	-
Dnase	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Gelatinase	+	+	-	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	-
Tween 80	+	-	+	-	-	+	+	+	+	-	+	-	-	+	-	+	+	+	-	-	-	+	+
Nitrate reductase	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	-	-	+
Antibiotic reaction	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Aerobic growing	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Anaerobic growing	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	-	+

Test / Strain	24p	25p	26p	27p	28p	29p	30p	31p	32p	33p	34p	35p	36p	37p	38p	39p	40p	41p	42p	43p	44p	45p
Motility	+	+	-	+	-	-	-	-	-	-	-	+	-	-	-	-	-	-	+	+	-	-
Gram	-	+	-	-	-	+	+	-	-	-	-	-	+	-	+	+	-	-	+	+	-	+
Oxidase	+	-	-	+	-	+	+	+	+	+	+	+	-	+	-	-	-	+	+	+	+	-
Catalase	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+
Amilase	-	+	+	+	-	+	-	-	+	+	-	+	+	-	+	+	+	-	+	+	+	+
Caseinase	+	-	+	+	+	-	-	-	-	-	-	+	-	-	+	+	+	+	+	+	+	+
Dnase	+	+	+	+	+	+	-	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+
Gelatinase	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+
Tween 80	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	-
Nitrate reductase	+	+	+	-	-	-	+	+	+	+	+	+	-	+	+	+	+	-	-	-	+	+
Antibiotic reaction	-	-	-	+	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-
Aerobic growing	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Anaerobic growing	+	+	+	+	+	+	+	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+

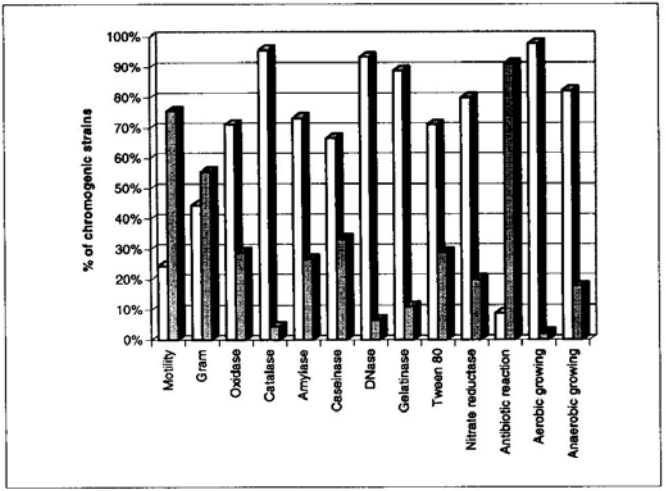

Figure 1. Characteristics of chromogenic bacteria.

Figura. Características de las bacterias cromogénicas.

□ Positive reaction.

□ Reaction positiva

■ Negative reaction.

■ Reacción negativa.

Gauthier (1975), and León (1996) found that Gram negative rods predominate in the surface film. Also Nair *et al.* (1987) isolated chromogenic bacteria from surface waters, all of them Gram-negative strains.

Depending on the substrate, extracelular enzymatic activity from pigmented strains fluctuated between 67% and 96%. García-Tello *et al.* (1994) also found high values for enzymatic activity of hydrophobic non-pigmented neustonic strains. The high percentage of DNase and gelatinase positive strains suggest an important role in mineralization of DNA and proteinaceous compounds; also amylolitic, and proteolytic activity was important in our strains. Amylolitic activity of strains from the sea water surface was found not to be important by Fehon & Oliver (1979) and León (1996), but Gauthier (1975), Soto *et al.* (1984) and García-Tello *et al.* (1994) find excellent amylolitic activity. We suggest that the abundance of these types of bacteria depends on enrichment by starch producing Phaeophyceae in the nearby seawater, particularly *Lessonia*, that grows abundantly in the exposed rocks in Montemar.

It is noteworthy that 80% of the pigmented strains performed nitrate reduction. Schropp & Schwarz (1983) have

shown that nitrate reduction operates in sea water. This observation raises a question about the fate of nitrogen from the surface film of littoral pools. Our results point out at a rather active chromogenic, mineralizing microflora; nevertheless, De Souza-Lima & Romano (1983) and Williams *et al.* (1986) consider heterotrophic activity as weak, or variable due to the extreme conditions that affect the surface film. The considerable differences of our results compared to those in the literature about bacteria from the surface film suggest that we are not in the presence of typical marine neustonic strains. Chromogenic bacterial isolates could be strains adapted to the surface film in litoral pools, and its particular tidal rithm and physico-chemical condition.

LITERATURE CITED

Buck JD. 1982. Nonstaining (KOH) method for determination of Gram reaction of marine bacteria. Applied and Environmental Microbiology 44: 992-993.

Crow S, D Ahearn, W Cook & A Bourquin. 1975. Densities of bacteria and fungi in coastal surface films as determined by a membrane-absorption procedure. Limnology and Oceanography 20: 6-8.

De Souza-Lima Y & J-C Romano. 1983. Ecological aspects of the surface microlayer. I. ATP, ADP, AMP contents, and energy charge ratios of microplanktonic communities. Journal of Experimental Marine Biology and Ecology 70: 107-122.

De Souza-Lima Y & MJ Chretiennot-Dinet. 1984. Measurements of biomass and activity of neustonic microorganism. Estuarine Coastal Shelf Science 19: 167-180.

Fehon WC & JD Oliver. 1979. Taxonomy and distribution of surface microlayer bacteria from two estuarine sites. Estuaries 2 : 194-197.

García-Tello P, J Llanos, M Cid, B Prado y S Navarro. 1994. Actividad metabólica de bacterioneuston hidrofóbico. Anales del Museo de Historia Natural de Valparaíso 22: 27-32.

Garrett W. 1965. Collection of slick-forming materials from the sea surface. Limnology and Oceanography 10: 602-605.

Gauthier MJ. 1975. Morphological, physiological and biochemical characteristic of some violet-pigmented bacteria isolated from seawater. Canadian Journal of Microbiology 22: 138-149.

Gerhard P, R Murray, W Wood, N Krieg. 1994. Methods for general and molecular bacteriology. Eds. American Society for Microbiology. Washington DC. 253-257.

Kovacs N. 1956. Identification of *Pseudomonas pyogenes* by the oxidasa reactions. Nature 178: 703

León J. 1996. Cepas nativas del bacterioneuston marino con actividad antagónica frente a bacterias ictiopatógenas. Caracterización preliminar de substancias inhibitorias. Tesis para optar al grado de Magister en Ciencias Microbiológicas. Universidad Católica de Valparaíso. Chile : 1-114.

MacFaddin J. 1980. Pruebas bioquímicas para la identificación de bacterias de importancia médica. Editorial Médica Panamericana, S.A. Buenos Aires : 1-198.

Nair S & U Simidu. 1987. Distribution and significance of heterotrophic marine bacteria with antibacterial activity. Applied Environmental Microbiology 53: 2957-2962.

Nair S D Chandramohan & P A Loka-Bharathi. 1992. Differential sensitivity of pigmented and non-pigmented marine bacteria to metals and antibiotics. Water Research 26: 431-434.

Nealson K & W Hasting. 1992. The luminous bacteria, p. 625, In: Balows A, HG Truper, M Dworkin, W Harder. K Schleifer, (eds.)The prokaryotes, a handbook on the biology of bacteria: ecophysiology, isolation, identification, application. vol 1. Springer Verlag.

Rosenberg M. 1984. Isolation of pigmented and nonpigmented mutants of *Serratia marcescens* with reduced cell surface hydrophobicity. Journal of Bacteriology 160:480-482.

Schropp SJ & JR Schwarz. 1982. Nitrous oxide production by denitrifying microorganisms from the eastern tropical north Pacific and the Caribbean Sea. Geomicrobiology Journal 3: 17-31.

Shapiro A, D Dilello, M Loudis, D Keller & S Hutner. 1977. Minimal requirements in defined media for improved growth of some radioresistant pink tetra-cocci. Applied Environmental Microbiology 33: 1129-1133.

Sieburth J McN. 1968. Observations on planktonic bacteria in Narragansett Bay, Rhode Island; a résumé. Bulletin of Misaki Marine Biological Institute, Kyoto University 12: 49-64.

Sieburth J McN, JP Willis, KM Johnson, CM Burney, D Lavoie, KR Hinga, DA Caron, FW French, WP Johnson & PG Davis. 1976. Dissolved organic matter and heterotrophic microneuston in the surface microlayer of the North Atlantic. Science 194: 1415-1418.

Sieburth J McN. 1971. Distribution and activity of oceanic bacteria. Deep Sea Research 18:1111-1112.

Sieburth J McN. 1979. Sea Microbes. New York. Oxford University Press, 491 p.

Soto Y, J Robeson & P García-Tello. 1984. Incidencia y características metabólicas de bacterias de superficie hidrofóbica presentes en el neuston de pozas litorales. Revista de Biología Marina, Valparaíso 20: 127-137.

Williams PM, AF Carlucci, SM Henrichs, ES Vleet, SG Horrigan, FMH Reid & KJ Robertson. 1986. Chemical and microbiological studies of sea surface films in the southern Gulf of California and of the west coast of Baja California. Marine Chemistry 19: 17-98.

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