

*Nota Científica***Atypical bacteria accompanying the scallop *Argopecten purpuratus***

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ABSTRACT. A simple bacteriological analysis for accompanying culturable microflora from different regions of the body of *Argopecten purpuratus* showed a rather atypical bacteria considering that farming is done in the sea-water column. High numbers of *Vibrio* strains, Gram-positive cocci, and sporeformers were present. Also members of the Enterobacteriaceae family were isolated.

Key words: scallops, bacteria, Gram-positive, sporeformers, *Vibrio*, Enterobacteriaceae.

Bacterias atípicas en el ostión *Argopecten purpuratus*

RESUMEN. Se realizó un análisis sencillo de la bacterioflora cultivable acompañante de diferentes regiones del cuerpo del ostión *Argopecten purpuratus* la cual se mostró compuesta de bacterias atípicas para la columna de agua. Se presentó un número alto de cepas de *Vibrio*, coccus Gram-positivos y formadoras de esporas. También fueron aisladas cepas de la familia Enterobacteriaceae.

Palabras claves: ostión, bacteria, Gram-positiva, esporulados, *Vibrio*, Enterobacteriaceae.

The knowledge of the most frequently accompanying groups of bacteria and their numbers in aquaculture farming invertebrates can help the understanding of better conditions to be held in modern artificial cultures (Llanos *et al.*, 1993). Also the importance of natural flora was shown by Nogami and Maeda (1992) to repress the development of *Vibrio* spp. and pigmented bacteria in cultures of the larvae of *Portunus trituberculatus*. The same authors said that microorganisms at the interphase of the organisms and environment are important in equilibrium and health. By comparison with an established pattern it could be possible to be aware of the quality of water in which the molluscs are living and nourishing (Prieur, 1984). Also the presence of contaminating allochthonous bacteria can make an advertisement of culture conditions (Plusquellec *et al.*, 1994). It can also help

before it can spread all through the culture. With the same idea Schwegman (1991) established the normal accompanying flora for a marine vertebrate, a dolphin.

As a result of these kind of scientific analysis it can be saved the investment and the hard work of many people. What is most important is that in this way we can avoid to commit again the same error in future cultures. Nevertheless studies of the normal flora of marine cultures is not frequently financed by investors. Here we present the results of a case of aquaculture from a Chilean breeding site in which we suspect through the knowledge of accompanying bacteria an improper culture place.

Because we know the importance of scallops in Chilean aquaculture we decided to investigate the accompanying bacterial flora on these bivalve molluscs from a particular marine aquaculture,

finding what we call an atypical bacterial flora.

Isolation, tentative identification, and counts of viable bacteria was done from the following regions of the scallop: inter-valvar liquid (Ilq), gills (Gl), gonads (Gd), digestive gland (Dg), intestine (Int), and the mantle (Mtl). Different organs of 30 specimens of the bivalves were separated with a scalpel. Each group of organs was weighed before putting them in Marine Broth (yeast extract 0.5 g; peptone 15 g; sodium chloride 25 g; sea water 0.75 L and distilled water 0.25 l) in relation of 1/10. Intervalvar liquid was measured with a pipet and diluted in the same way. Each organ was grinded in a sterile warring blender of which 0.1 mL were plated in Marine Agar 2216 (Difco). Incubation was done at 20°C in a low temperature incubator for 14 days. Colony forming units were count in plates with 30 to 300 colonies. From the same plates, fifty randomly selected colonies proceeding from each organ or region of the scallops were isolated. Enterobacteria strains were grown in MacConkey agar (Difco) at 37°C and applied oxidasa test according to Kovacs (1956). A total of 200 strains were randomly selected for identification. Not less than 30 colonies of each

organ or part were submitted to identification according to identificative scheme of Oliver (1982).

Counts gave high numbers of viable bacteria in the intestinal contents of this mollusc, reaching $5 \cdot 10^6$ CFU ml⁻¹, lesser quantities were found in the rest of the organs. According to Prieur (1984) and Prieur *et al.* (1990) the intestine of molluscs is a reservoir and concentration place for bacteria. Tentative identification of bacterial groups showed the presence of *Vibrio* strains as the most important in all the organs and in the inter-valvar liquid. This genus present values above 34% (Table 1). *Vibrio* is a normal accompanying bacteria for molluscs, nevertheless in unapropriate culture conditions it is known this strain can develope a pathogenic bacteriosis. For the *Pseudomonas* group the highest value detected was 23% in the intestines. Remarkable is the presence of Enterobacteriaceae in all parts studied, specially in the inter-valvar liquid and digestive gland with a 22% and 17% respectively. This is an important aspect. Plusquellec *et al.* (1994) found *Salmonella* in bivalve shellfish. A high number of Gram-positive bacteria non spore forming is present, mainly coccoids cells, free or as

Table 1. Percentage of *Pseudomonas*, *Vibrio*, Enterobacteriaceae and miscellaneous groups of bacteria in different organs of *Argopecten purpuratus*. Ilq: Intervalvar liquid, Gl: Gills, Gd: Gonads, Dg: Digestive gland, Int: Intestine, Mtl: Mantle.

Tabla 1. Porcentaje de *Pseudomonas*, *Vibrio*, Enterobacteriaceae y bacterias misceláneas en diferentes órganos de *Argopecten purpuratus*. Ilq: Líquido intervalvar, Gl: Branquias, Gd: Gónadas, Dg: Glándula digestiva, Int: Intestino, Mtl: Manto.

	Ilq	Gl	Gd	Dg	Int	Mtl
<i>Pseudomonas</i>	14	5	13	14	22	8
<i>Vibrio</i>	55	50	43	58	42	34
Enterobacteriaceae	22	15	11	17	12	12
Miscellaneous	9	30	33	11	24	46
Total	100	100	100	100	100	100

Table 2. Percentage of Gram stain groups and sporeformers in diferent organs of *A. purpuratus*. Ilq: Intervalvar liquid, Gl: Gills, Gd: Gonads, Dg: Digestive gland, Int: Intestine, Mtl: Mantle.

Tabla 2. Porcentaje de los grupos de tinción Gram y formadores de esporas en diferentes órganos de *A. purpuratus*. Ilq: Líquido intervalvar, Gl: Branquias, Gd: Gónadas, Dg: Glándula digestiva, Int: Intestino, Mtl: Manto.

	Ilq	Gl	Gd	Dg	Int	Mtl
Gram-negatives	78	20	80	100	95	75
Gram-positives	11	60	10	0	5	18
Sporeformers	11	20	10	0	0	7
Total	100	100	100	100	100	100

staphylococcal arrangement (Table 2). Important is the presence of this group in gills (60%). Also the spore forming bacilli are important in gills (20%) and in the inter-valvar liquid (11%). Notably Gram-positive strains are more frequently present in sediments and molluscs adapted to a benthic way of life as with *Mesodesma donacium* (Muhlhauser and García-Tello, 1971; Zazopulos *et al.*, 1991).

Our results suggest that these groups of scallops submitted to this simple bacteriological analysis reveal that they are in contact with waters possessing an important allochthonous microflora. We find a high percentage of Gram-positive spore forming bacilli and non-spore forming cocci, uncommon in marine sea-water column. Taking into account Enterobacteriaceae isolated we can say that this is rather a sediment and sewage microflora. We have not done water oxygen analysis, but we was informed by the breeding center that was a very low value. Most probably producing a stressed condition to the culture.

Finally we have to conclude that our results showed the importance of selecting the appropriate place to establish an aquaculture and how simple bacteriological tests can help in this kind of decision (Orozco-Borbon *et al.*, 1994).

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