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NUMERICAL TAXONOMY OF VIBRIONACEAE ISOLATED FROM BIVALVES AND FISHES IN VALPARAISO COASTS, CHILE

(Taxonomía numérica de Vibrionaceae aisladas desde bivalvos y peces en zonas costeras de Valparaiso, Chile)

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Key words: Numerical taxonomy, Vibrionaceae, bivalves, fishes.
Palabras clave: Taxonomía numérica, Vibronaceae,bivalvos, peces.

SUMMARY

*A taxonomic study has been carried out on 123 strains of the Vibrionaceae family isolated from bivalves an fishes in Valparaiso coats. All strains were characterized with 86 phenotypic tests and clustering using S_{SM} coefficient and the UPGMA linking analysis. The strains were clustered into three phena at a 80% similarity level. These strains were identified as **Aeromonas hydrophila, Vibrio** sp. and **Plesiomonas shigelloides**.*

RESUMEN

*Se realizó un estudio taxonómico de 123 cepas de la familia Vibronaceae aisladas desde bivalvos y peces en las costas de Valparaiso. Todas las cepas fueron caracterizadas con 86 test fenotípicos que se agruparon usando el coeficiente S_{SM} y el análisis de union de UPGMA. Las especies fueron agrupadas en tres fenones con un nivel de similitud del 80%. Estas especies fueron identificadas como **Aeromonas hydrophila, Vibrio** sp. y **Plesiomonas shigelloides**.*

INTRODUCTION

Members of the Vibrionaceae family comprise one of the predominant bacterial group in marine environment and constitute a considerable part of marine heterotrophic bacterial population (Kaneko & Colwell 1973). Microorganisms which belong to Vibrionaceae family are ubiquitous in estuarine and marine waters and sediments Kaper et al. 1973). They are associated with nitrogen fixation (West et al. 1985), degradation of specific organic pollutants (West and Colwell 1984) and colonization of surfaces and internal organs of invertebrate and vertebrate marine animals (Huq et al. 1983; Colwell & Grimes 1984; Prieur et al. 1990). Under stress conditions, some of the species that are commensal to marine animal can behave as pathogens, causing severe economic loss for to the marine culture trade (Sindermann 1970).

In this paper we describe the phenotypic characteristics of 123 strains of the Vibrionaceae family that were isolated from sea food in Valparaíso coasts, Chile, which are of great interest for the shellfish and fish commerce.

MATERIAL AND METHODS

Samples.

Withing June and November 1994, 10 samples of bivelves and fish were collected in the chilean coastal area

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comprising Caleta Membrillo (Valparaiso) and Con Con, V Región.

Bacterial strains.

The 123 strains were isolated from shellfish (***Mesodesma donacium, Pyura chilensis*** and ***Ameghinomya anticua***) and fish (***Merluccius gayi).*** The isolates were obtained from enrichments in alkaline peptone water (pH 8.6), followed by inoculation in agar TCBS (Difco) (APHA 1992). The following reference strains were included: ***Vibrio parahaemolyticus*** ATCC 17802, ***Vibrio fluvialis*** ATCC 33809, ***Vibrio cósticola*** NCMB 701, ***Aeromonas hidrophyla*** ATCC 7966, ***Aeromonas caviae*** ATCC 15468 and ***Aeromonas sobria*** CIP 7433.

The cultures were maintained on Tryptic Soy Agar (TSA) with 3% NaCl at 30°C.

Characterization of the isolations.

86 phenotypic characters including morphological, physiological, biochemical, nutritional tests and sensibility to the 0/129 compound at a concentration of 10 or 15 (g. were determined for each strain on media containing 1% NaCl, and at an incubation temperature of 30°C. Details of the procedures have been previously described (Veron 1975; Lee et al. 1979; Ventosa et al. 1982; Quesada et al. 1984; Wets & Colweell 1984)

Numerical analysis.

A total of 48 characters were selected for numerical analysis. Positive and negative results were coded as 1 and 0 respectively; no comparable or missing data were coded as 9. Strain similarities were estimated with Simple Matching (S_{SM}), (Sokal and Michener 1958) coefficient and clustering was achieved by the unweighted pair-group method of association (UPGMA) (Sneath & Sokal 1973). The test error was estimated by examining 12 strains in duplicate (Sneath & Johnson 1972). The computation was performed by the MINT program (Rohlf 1985).

RESULTS AND DISCUSSION

Each strain was examined for 86 characters. All strains were Gram-negative, motile, oxidase, catalase and nitrate reduction positive; and able to ferment glucose. They produced acid from ribose, glucose, trehalose, fructose, galactose, maltose and glicerol, utilized glucose as the sole carbon source and grew at pH 10 and 37°C

Negative characteristics for all strains were: H2S production, urease, phenylalanine deamination; acid from inulin, sorbose, xilose, adonitol and dulcitol; utilization of: malonate, L-lysine, L-methionine, L-valine, L-triptophan, L-phenylalanine, L-cysteine and adenine as the sole carbon source. Production of difusible pigment, swarming, luminiscence, growth in 10% NaCl were not observed.

These characteristics were excluded from the final data matrix, since they were not differentiating value. Taxonomic resemblance among the 123 strains examined was accordingly based on 48 characters. The probability for test error was estimated to be 3,0 % by the method of Sneath and Johnson (1972).

The results of numerical taxonomic analysis of all 123 strains, grouped by S_{SM} coefficient and UPGMA clustering yielded the dendrogram shown in Fig. 1. The majority of the strains could be grouped into three phena at a 70% similarity level. Table 1 lists the main features of the tree phena. The Jaccard coefficient (S_J) was also used. It gave a similar clustering pattern, but the clusters formed around a 55% similarity level.

Cophenetic correlation coefficient was 0.859 for S_{SM}/UPGMA phenogram. Phena were identified by comparing properties of the strains with published diagnostic keys and tables.

Phenon A: the 32 strains of this phenon clustered at a 85% similarity level. They are isolated from fish and molluscs. This phenon has been identified as ***Aeromonas hydrophila*** since includes the type strain ***A. hydrophila*** ATCC 7966 and the phenotipic characteristics of the microorganisms belonging to this group agrees with the description of this strain carried out by Popoff (1984). However, there are some differences: esculine hydrolysis, acid production from salicine and Voges-Proskauer test, which are considered positive by Popoff (1984). Our results also agree with the description of ***A. hydrophila*** reported by Austin et al. (1989).

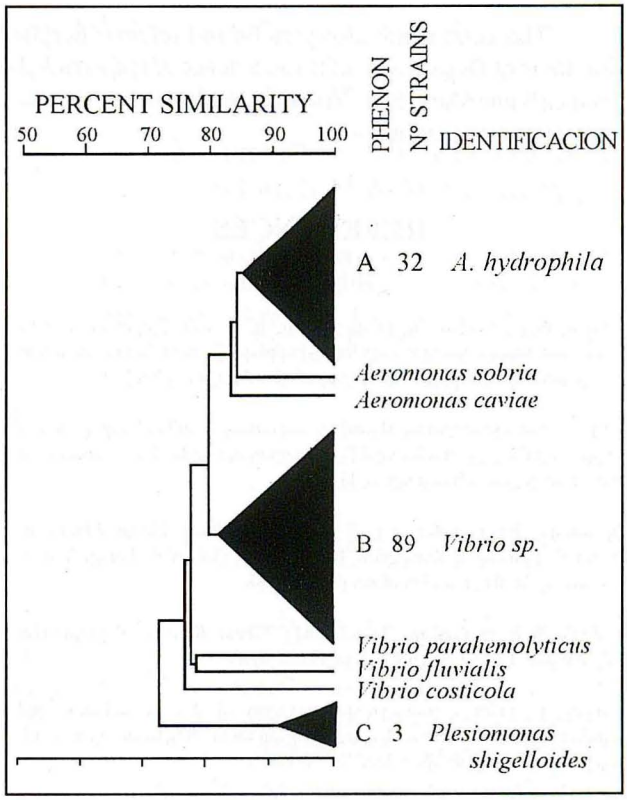
Phenon B: At a similarity level of 82% most of strains (89) grouped in phenon B. Distinctive characters of the strains of marine ***Vibrio*** included in this group are: motile, oxidase-positive, a fermentative type of metabolism, sensibility to 0/129, NaCl requirements for growth, arginine dehidrolase negative and ornitine and lisine descarboxi-lase positive. These phenotipic characteristics agree with the description of genus ***Vibrio*** carried out by Bauman et al. (1984). All the strains included in this phenon were isolated from molluscs and seem to be very related with the marine environment and their fauna.

Phenon C: The three strains of phenon C grouped at a 93% similarity level. The microorganisms included in this phenon are arginine, ornitine and lisine decarboxilase positive, they ferment the inositol and the lactose, they don't produce amilase, lipase, gelatinase, desoxorribo-

Table 1. Phenotypic characteristic of Vibrionaceae strains.

Phenon	A	B	C
N° of strains	**32**	**89**	**3**
Arginine decarboxilase	100	0	100
Lysine decarboxilase	32	100	100
Ornithine decarboxilase	0	100	100
Arginine dehydrolase	100	0	100
Degradation of : Casein	90	100	0
Tween 80	100	100	0
Aesculin	4	0	0
Production of : Amylase	100	100	0
Gelatinase	100	100	0
Deoxyribonuclease	100	100	0
Lecithinase	100	100	0
Acid from: L- arabinose	95	100	0
L- rhamnose	4	100	0
D- cellobiose	72	0	0
D- lactose	73	0	100
Sucrose	100	100	0
Inositol	0	0	100
Manitol	100	100	0
Sorbitol	85	0	0
Salicine	32	85	0
D- manose	100	100	0
Haemolysis	96	100	0
Indole	93	95	100
Voges-Proskahuer	32	0	0
Methyl red	41	0	0
Gas glucose	96	0	0
Utilization as sole carbon source: L- arabinose	95	100	0
D- cellobiose	72	0	0
Galactose	91	100	100
Succinate	63	100	0
Gluconate	94	100	0
Glysine	16	100	0
Glutamate	94	100	0
L- arginine	97	100	0
L- histidine	97	100	100
L- proline	72	100	0
L- asparagine	100	100	0
Aspartate	50	100	0
L-serine	100	100	0
L- threonine	38	100	0
Ethanol	0	88	0
Growth at: 4°C	97	0	0
45°C	25	0	100
Growth in % NaCl: 0	100	0	100
3	25	100	100
6	0	100	0
8	0	100	0
Susceptibility to 0/129 150 ug	0	100	100

Figure 1. Simplified dendrogram showing clustering of 123 strains of *Vibrionaceae*, based on the Simple Matching coefficient and unweighted pair group method with averages (UPGMA) clustering.



nuclease neither caseinase, they also utilize very few compounds like only source of carbon, nitrogen and energy. These characteristics are identical with the description of the genus ***Plesiomonas*** (Schubert, 1984).

The present taxonomic study of isolates from marine foods was focused to microorganisms of the Vibrionaceae family. The results on strains identification agrees with these carried out by other authors (Esteve,1995; Kaznowki et al. 1989; Austin et al. 1989; Ortigosa et al. 1989). Although our strains differ in some phenotipic characteristics from the reported by these authors. They are included within the genera ***Vibrio, Aeromonas*** and ***Plesiomonas***.

Our study demonstrates that the genus ***Vibrio*** is the most abundant (89 strains) of the total of isolates studied (123). They constitute a very homogeneous group in their phenotipic characteristics and all of then have a specific requirement of NaCl for optimal growth.

The Vibrionaceae family is an habitual component in the microbiote of bivalve molluscs and fish, and this is an important ecological niche for this family such as

reported by other authors (Pricur et al. 1990; Ortigosa et al. 1989; West et al. 1986; Wong et al. 1992) .

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