Electronic Journal of
El Hombre y su Ambiente Department
ISSN: (Pending)

2012. Vol.1 (1): 21-31

Phenotypical and biochemical study of denitrification bacteria populations from Sontecomapan Lagoon, Veracruz, Mexico.

[*1]Ferrara-Guerrero MJ, [1]Angeles-Vázquez JR, [1]Morales-Nieto MG, [1]Figueroa-Torres MG, [2]Pagano M.

[1]Departamento El Hombre y su Ambiente. Universidad Autónoma Metropolitana-Xochimilco. Calzada del Hueso 1100, Col. Villa Quietud 04960, Distrito Federal.

[2]Centre d'Océanologie de Marseille, (LOPB), UMR 6535 - Centre d'Océanologie de Marseille Campus de Luminy Case 901. 13288 Marseille Cedex 09

*fgmd6735@correo.xoc.uam.mx .

ABSTRACT

The biogeochemical cycle of nitrogen consists of numerous nitrogen compounds transformations that are catalyzed by microorganisms. This cycle controls the biological productivity of the marine ecosystems and involves a series of oxide-reduction reactions, many of which are used in microorganism's metabolism. The microbial activity of nitrate reduction has been widely studied because it is the main mechanism of loss of the fertilizing role of nitrogen and because it is a process that contributes to the atmosphere N_2O enrichment. Owing to the fact that the denitrifying bacteria with microaerobic metabolism are a microbial group that has been little studied, this work contributes to the knowledge of their phenotypical and chemical diversity. Twenty-one bacterial isolates with denitrifying potential were obtained from bottom water and superficial sediments (0 to 5 mm depth) samples. All the physiological tests for the phenotypical characterization were performed at different pCO_2 (0, 2, 4 and 21%) in order to determine their oxygen preferences. Their cell membrane fatty acids methyl esters (FAME) were determined. The cluster analysis of the results grouped the 21 isolates in 4 groups or phenons by to their physiological diversity and difference in the chemical composition of their cell membranes. For its oxygen tolerance, they can be classified as bacteria with strict microaerobic metabolism and anaerobic airtolerant; 34 fatty acids in their cell membranes were identified, most of them were from the saturated ones group and only 12 of them from the unsaturated ones group.

Key words: Microaerobic bacteria, methyl esters, physiology, oxygen tolerance.

INTRODUCTION

In the marine ecosystems the nitrogen cycle controls the availability of nitrogen nutrients and the biological productivity along with the atmospheric fixation of the CO_2. In addition, in many ecosystems inorganic nitrogen is the most common limiting nutrient for the primary producers (Zehr and Ward 2002), due to the fact that microorganisms consume nitrogen in any one of its forms for its metabolic processes.

In general, either through contributions allochthonous or autochthonus, bacteria play a primary role in the nitrogen cycle, releasing inorganic nitrogen (NH_3^+) during the decomposition of organic matter. In this way, the bacteria can use Dissolved Inorganic Nitrogen (DIN) as efficiently as the organic matter and so they can even, compete with the phytoplankton by inorganic nitrogen (Kirchman and Wheeler 1998, Goldman and Dennette 2000, Zehr and Ward 2002). The bacteria also can occupy the DIN simultaneously while they release NH_4+ during organic matter decomposition. And they can be competing by NH_4^+, regenerating NH_4^+ or both, thanks to the different members of the microbial loop responsible for different processes (Kirchman 2000).

In the nitrogen cycle the bacteria and cyanobacteria catalyze the chemical conversions between different oxidation states of nitrogen, including nitrogen gas (N_2), ammonium (NH_4^+), nitrite (NO_2^-) and nitrate (NO_3^-); this process is known as nitrification and he involves bacteria of the genre *Nitrosomonas* that transform the NH_3^+ to

Received: June 1st 2012. Accepted: August 1st 2012. Published: December 1st 2012

Electronic Journal of
El Hombre y su Ambiente Department
ISSN: (Pending)

2012. Vol.1 (1): 21-31

NO_2^- using oxygen as oxidant, and *Nitrobacter* wich oxidizes the NO_2^- to NO_3^-. The nitrogen cycle is closed with the denitrifying bacteria (*Alcaligenes*, *Paracoccus*, *Pseudomonas*, *Moraxella*, *Spirillum*, T*hiobacillus* and *Bacillus*) which use the NO_3^- as an oxidizer for its metabolic processes instead of molecular oxygen, reducing nitrogen to its gaseous form (N_2). As much during the denitrification process as in the nitrification one takes place N_2O that is a greenhouse gas and a stratospheric NO source, relevant in the ozone layer destruction (Williams *et al*. 1978, Cervantes-Carrillo 2000, Spiro and Stigliani 2004, Mora-Revelo *et al*. 2007).

Finally, in the group of denitrifying bacteria also species exist that own the virtue of being microaerobic; i.e. that have an aerobic metabolism so far as airtolerant anaerobic one, and which use oxygen as hydrogen acceptor in the respiratory process of energy production but showing an optimal growth at low oxygen concentrations (Ferrara-Guerrero and Bianchi 1989). This microbial group has been little studied and in our country, so the present research aims provide a little knowledge of the diversity and physiology of microaerobic denitrifying bacterial population from the water column and the sediments of the Sontecomapan Lagoon, Veracruz (Mexico).

Sontecomapan Lagoon is located at the southeast of the State of Veracruz, at coordinates 18° 30 ' and 18° 34' N and the meridians 99° 00 ' and 99° 04 ' W, and it is divided in several zones: La Barra, from the beach to Roca Morro, the channel El Real, that when opening itself conforms the lagoon mainly, which is divided in three zones due to the deltas formed by the Coscoapan River (Fig. 1). The lagoon has an area of 12 km and a width of 1,5 km and average depth of 1,5 m, transparency of 60 cm, and is predominantly mesohalina (5 to 18 gL^{-1}) in the southern part, polihalina in the central region (25-30 gL^{-1}) and eurohalina (30-40 gL^{-1}) in the mouth of communication with the coast line. The sediments are mainly sandy-clays (Contreras 1985).

MATERIAL Y METODOS

The isolation and purification of the bacterial isolates were carried out from bottom water and superficial sediments samples (0 to 5 mm depth). The samples of bottom water were taken with a horizontal Van Dorn Bottle and conserved in

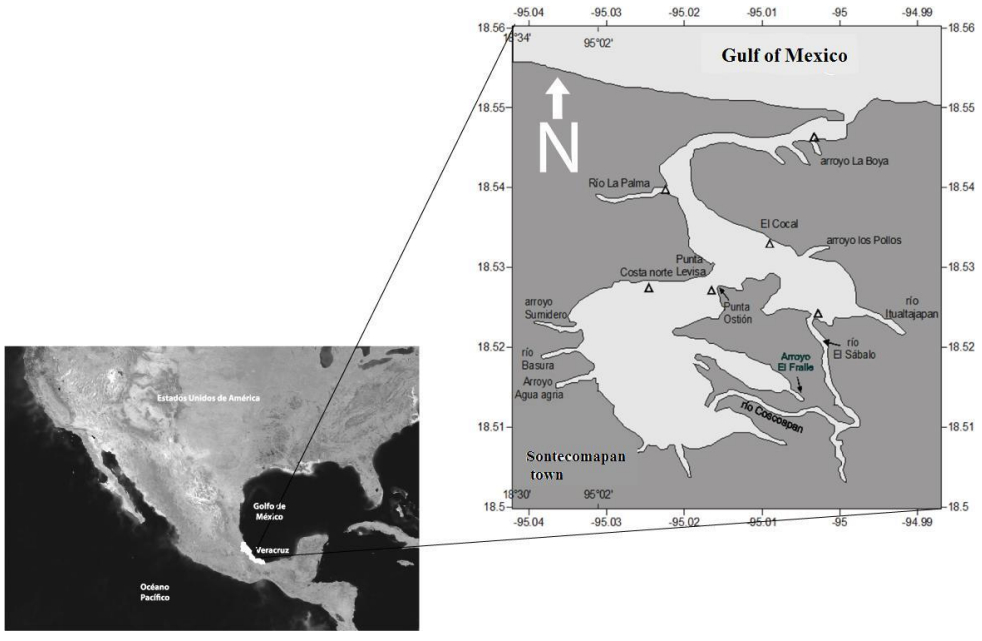

Fig. 1. Sampling areas location of the in the Sontecomapan Lagoon, Veracruz(Mexico).

Received: June 1st 2012. Accepted: August 1st 2012. Published: December 1st 2012

sterile glass vials containing glycerol at 20% at -18°C, until processed. The undisturbed sediment samples were taken by free diving with a 20 cm long and 5 cm diameter manual corer. First 5 mm of sediment were placed in a sterile vial containing 45 ml of glycerol at 20% sterile and stored at -18°C until processed.

Isolation of microaerobic denitrifying bacteria

Was carried out by the decimal dilutions series method (from 10^{-1} to 10^{-6}) using a saline solution (0,9% NaCl); an aliquot of a 1 ml of each decimal dilution was inoculated in 9 ml of nitrate marine broth (Bioxon) in triplicate and incubated 48h at room temperature. The presence of denitrifying bacteria was detected transferring 1 ml of the growth culture medium in a sterile test tube and adding a few drops of Griess A and B reagents (Ramirez-Gama 2003). In order to isolate the microaerobic denitrifying bacteria, from the tubes that presented growth of denitrifying bacteria, an aliquot of 1 ml was taken and inoculated in 9 ml of specific medium (g l^{-1}: 2 KNO_3, 0.5 $C_4H_4O_4Ca$, 10 NaCl; mg l^{-1}: 0.05 biotin, 0.5 thiamine, 0.1 $MgCl_2$, 0.05 $MnCl_2$, 5 ml sodium lactate, pH 7.3) contained in Hungate tubes (Bellco®) gasified with pO_2 of 4% balance N_2 (Ferrara-Guerrero et al. 1994), until obtaining pure isolates. The tubes were incubated at room temperature by 8 days. The purity of the isolates was verified by 100x phases contrast microscopy (Olympus Bimax60), and in Petri dishes with solid culture medium incubated under low pO_2 (4% O_2/96 N_2) atmosphere in anaerobiosis jars (BIOXON®).

Phenotypical study

All tests were realised in triplicate from 8 days of incubation in specific liquid and solid culture media incubated in low oxygenation atmosphere.

a) Morphological characteristics.

The colonial characteristics, the grouping and cellular morphology were observed, the response to the Gram dye the presence of spores and capsules, the motility in the 2, 3, 5 triphenyl-tetrasodium (TTC) medium at 0,2%, the capacity to accumulate poly-ß-hydroxybutirate (PHB) (Meynell and Meynell 1979).

b) Physiological characterization.

The tolerance to the oxygen presence was verified (pO_2 of 2,4 %, ± 0,3% rel., N_2 balance), aerobic conditions and oxygen absence (pCO_2 10% N_2 balance), salinity (0, 10, 20 and 60 ‰ of NaCl), and temperature (4, 25 and 40°C). The growth was measured by colorimetric method at a wavelength of 650 nm (Shimadzu spectrophotometer double ace of light).

c) Respiratory metabolism.

It was observed the presence of catalase (hydrogen peroxide at 30%), and cytochrome oxidase (Kovacs 1956) enzymes, the ability to oxidize and to ferment the glucose (Hugh and Leifson 1953) and to breathe nitrates and sulphates (Ramirez-Gama et al. 2003).

Analyse of fatty acids methyl esters (FAME).

The bacterial biomass for the fatty acid (FA) analysis was obtained on enriched solid specific growth medium and incubated at pO_2 of 4% for 8 days at room temperature. The cellular biomass was recovered by washing the Petri dishes with formaldehyde to 5%, the suspension was centrifuged to 700 g during 45 min at 4°C. The cellular button was re suspended twice in 10 ml of a physiological solution (0,85% NaCl) and then centrifuged again. The bacterial biomass obtained was stored at -70° C for 24 h (Ángeles-Vázquez 2007). The FA were identified by gas chromatography (Bading and Joung 1983), taking as reference the FAME (Fatty Acid Methyl Esters) standard of 37 components (Perez *et al.* 1997, Diaz-González *et al.* (2002). In order to realise the FA analysis, the cellular biomass was lyophilized for 3h at low vacuum (7 kPa) and a temperature of -50°C. Twenty milligrams of lyophilized product were methylated with 0,1 ml of sodium methoxide 0,5 N and 0,9 ml of nanograde petroleum ether. An aliquot of 1 µl was injected to the gas chromatograph (Perkin Elmer Auto system 9000) with a flame ionization detector and a capillary tube of 100 meters in long in silica fused with a high heat

Received: June 1st 2012. Accepted: August 1st 2012. Published: December 1st 2012

Electronic Journal of
El Hombre y su Ambiente Department
ISSN: (Pending)

2012. Vol.1 (1): 21-31

resistence HTS SEG (SUPELCO®) siloxane-carbonate base. Helium 4.6 was used as gas carrier.

Statistical analysis

With the results it was carried out a cluster analysis and the index of similarity and Euclidean distance of Ward, using STATISTIC® software program (1999).

RESULTS AND DISCUSSION

Twenty-one pure microaerobic bacterial isolates with denitrifying potential were obtained, which along with 2 microaerobic collection strains (*Azospirillum lipoferum* DSMZ 2291 and *Magnetospirillum magnetotactium* DSMZ 3856) and 3 anaerobic facultative ones (*Escherichia coli*, *Enterococcus* sp and *Bacillus subtilis* from the IMSS collection) (Table 1), 101 phenotypic tests and the FAME analyse of their cellular membranes were applied to them. A very high percentage of the isolates (96%) was able to grow both in aerobic as microaerobic conditions, but only 61,5 % of them grew under anaerobic conditions. The 92,3% of them former colonies of smooth appearance, 11,5% were incrusting ones, 57,7% with regular edges, 92%convex, 89% formed microcolonies and 81% had runny consistency, the predominant colour was beige (61,5%) and, in a smaller percentage (11,8%), white. Vibroides Gram negative forms predominated (65,8%) about coccobacilli (20,2%) and filamentous forms (13%), 54% were mobile, 11,5% sporulated ones and only 27% were able to accumulate the poly-ß-hydroxibutyrate (PHB) as reserve material.

All the bacterial isolates support tolerate from 0 to 60 ‰ and temperatures between 25 and 40°C, only 85% of them grew to 4°C; 96% of them were able to grow as much in conditions of oxygen saturation (pO_2 21%) as of microaerobiosis (pO_2 of 2 and 4%), and only 61,5% grew under anaerobic conditions (Fig.2). On the other hand, only 15,4% of the isolates showed a cytochrome oxidase and 54% catalase, everyone breathed nitrates and none sulphates, all oxidize the glucose and 92,3% ferment it.

In order to analyse the diversity of the denitrifying bacterial population present in the bottom water and the sediments from the lagoon of Sontecomapan, Veracruz, the method of numerical taxonomy was used. According to O'Brien and Colwell (1987),this method can simply be considered as "the grouping of organisms by statistical methods"; in which are used software's

Table 1. Origin of the denitrifying bacterial isolated.

Key	Zone	Level
Sf2	Costa Norte	Bottom water
Sf4	The Palma River	Bottom water
SD5	Costa Norte	Bottom water
SD7	Costa Norte	Bottom water
Sf8	Sábalo River	Bottom water
Sf10	Palma River	Bottom water
Sf11	Sábalo River	Bottom water
Ss13	Boya stream	Bottom water
Ss15	Palma River	Bottom water
Ssd16	Pollos stream	Sediment
Sf18	Basura River	Bottom water
Sf20	Costa Norte	Bottom water
Sf21	Line coast	Bottom water
Ss24	Palma River	Bottom water
Ssd26	Fraile estuary	Sediment
Ss27	La Barra	Bottom water
Ss28	Costa Norte	Bottom water
Sf30	Estero el Fraile	Bottom water
Ss31	The Cocal	Bottom water
Ss32	El Morro	Bottom water

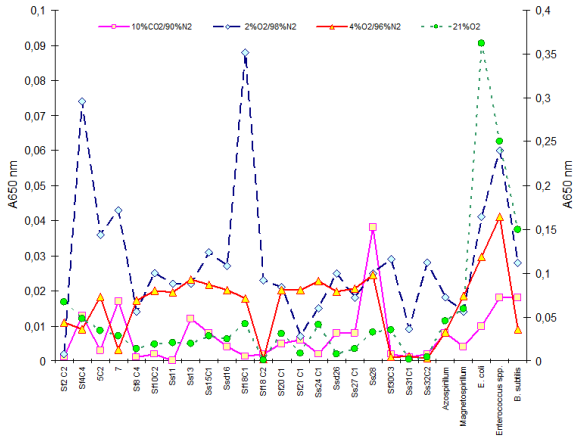

Fig. 2. Tolerance of the bacterial isolates obtained from water and sediment from Sontecomapan Lagoon, Veracruz, samples to different partial oxygen pressures (pO_2: 21, 2 and 4%) and anaerobic condition (pCO_2 10%).

Received: June 1st 2012. Accepted: August 1st 2012. Published: December 1st 2012

Electronic Journal of
El Hombre y su Ambiente Department
ISSN: (Pending)

2012. Vol.1 (1): 21-31

where all the tests realised for each organism are evaluated, based on the positive responses, giving as result the formation of groups or clusters based on the similarities existing between them.

The statistical cluster analysis developed from the results of all phenotypical and chemical tests (Pielou 1984), grouped the 21 isolates and 5 strains used as reference, in 5 phenons and 4 pairs (Fig.3).

a) The phenon 1 gathers 5 bacterial isolates from sediments as much from freshwater zones as from La Barra and the coastline (SD5C2, Ssd16, Ss24C1, Ss27 and Ss32C2, Costa Norte, los Pollos River, la Palma River, La Barra and the coastline, respectively). They form smooth, mucous and pigmented microcolonies, with regular and runny edges and pigmented. They are pleomorphic, Gram negative, are up to 5,5 µm in length, oxidize the glucose, they breathe nitrates, produce the enzyme catalase, and tolerate temperatures from 4 to 40°C and salinities from 0 to 60‰, and its best growth was obtained at low partial oxygen pressures (Fig 2). The FA that they have common is the types C8:0, C12: 0, C14: 0, C15: 0, C16: 0, C20: 0, C 20:3n3 and C24:1. This group has the minor FA diversity in its cell membranes.

b) The phenon 2 brings together the largest number of isolates, 4 of which were isolated from the bottom water of the Basura River (Sf18C1, Sf18C2), Costa Norte (Sf20C1), Fraile estuary (Sf30C3 y), and 2 from the superficial sediments from the Fraile estuary (Ssd26) and from the coastline (Ss32C2). The colonies that form are mucous, bright with regular edges and colour beige.

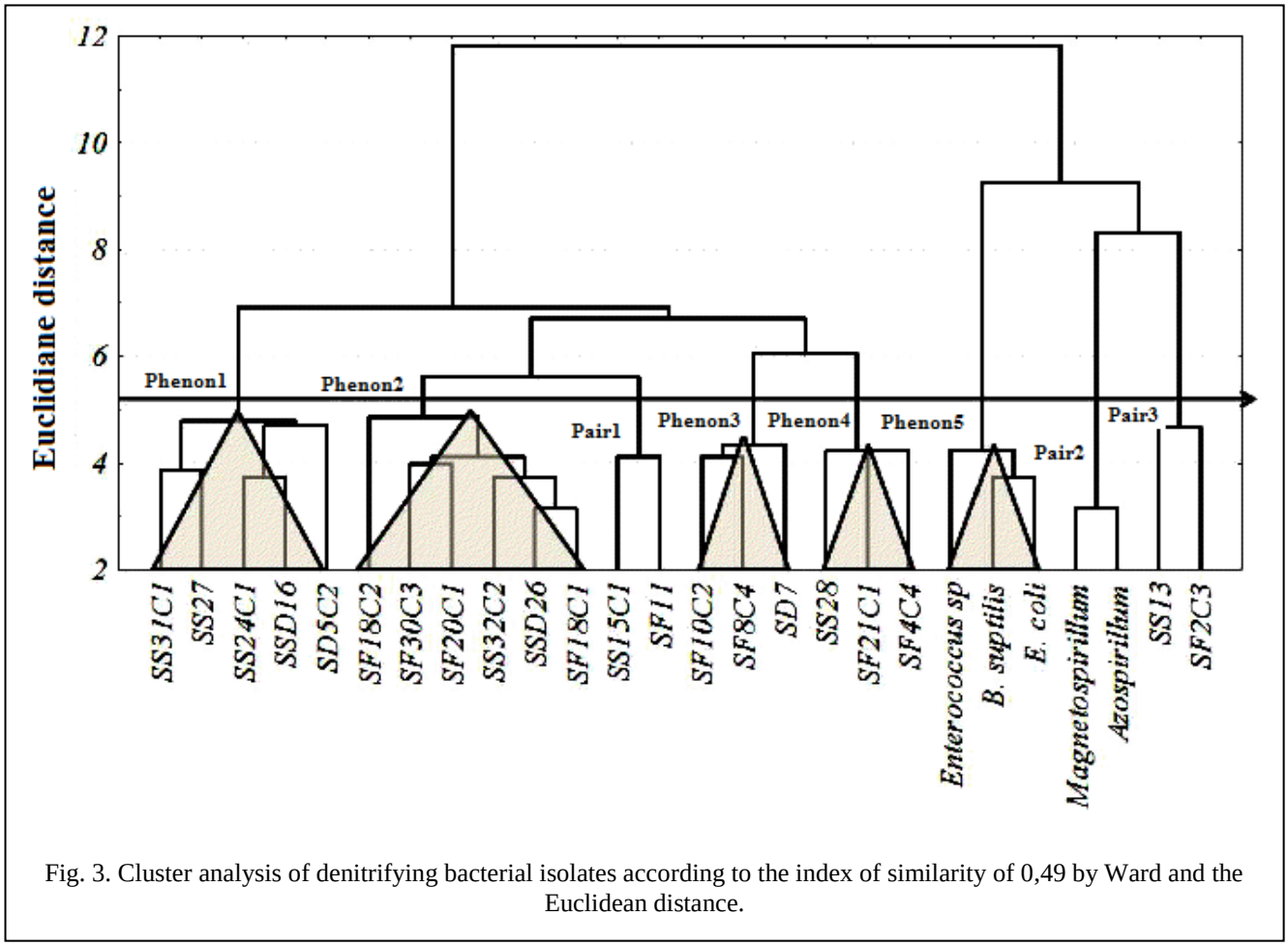

Fig. 3. Cluster analysis of denitrifying bacterial isolates according to the index of similarity of 0,49 by Ward and the Euclidean distance.

Received: June 1st 2012. Accepted: August 1st 2012. Published: December 1st 2012

They are pleomorphic rods, with cell dimensions that may reach up to 29 µm lenght, Gram negative; they present the enzyme catalase, oxidize the glucose and breathe nitrates. Best growth occurs in microaerobic conditions (Fig 2), and they tolerate salinities from 10 to 60‰ and temperatures from 25 to 40°C, and are motile. They share the FA C8: 0, C10: 0, C12: 0, C13: 0, C14: 0, C14: 1, C15: 0, C15: 1, C16: 0, C16: 1, C17: 0, C20: 0 and C24: 1; the fatty acids C18: 0, C18: 2n6c and C20: 3n6 are the most representative in this group.

c) The pair 1 brings together the isolates Sf11 and Ss15C1, isolated from bottom water and surface sediments from the mouths of El Sábalo and La Palma Rivers; they form mucous microcolonies, with smooth edges and beige pigment, and are coccobacilli with cellular dimensions from 0,9 to 2,9 µm long and from 0,5 to 1,5 µm of width. They are Gram negative, produce the enzyme catalase and breathe nitrates. They tolerate salinities from 0 to 60 ‰ and temperatures from 4 to 40°C, and their optimal growth is microaerophilic (pO_2 2 and 4%) (Fig 2). They present in its cell membranes FA C8: 0, C12: 0, C13: 0, C14: 0, C15: 0, C16: 0, C17: 0, C17: 1, C20: 0 and C24: 1. The FA separating this pair from the others are C18: 1n9t and C20: 2, which only found in the isolates that comprise this pair.

d) The phenon 3 bringd together the isolates SD7, Sf8C4 and Sf10C2 (sediments from Costa Norte and bottom water from the mouth of El Sábalo and La Palma Rivers, respectively), that form pigmented, smooth, shining and runny microcolonies. They are bacilli Gram negative, that present intracellular vacuoles and a length up to 5,5 µm long ; they ferment and oxidize the glucose, breathe nitrates, tolerate salinities between 10 and 60 ‰ and temperatures from 25 to 40°C; just as the pair 3 and phenon 4, its best growth is in low oxygenation conditions (Fig 2). The FA share are C8: 0, C12: 0, C14: 0, C14: 1, C15: 0, C15: 1, C16: 0, C17: 0, C20: 0, C20: 3n3, C20: 4n6, C22: 1n9 and C24: 1. This pair is the unique one that owns the fatty acids C20: 4n6 and C22: 1n9 in its cellular membranes.

e) The phenon 4 is formed by the isolates Sf4C4, from the bottom water from La Palma River mouth, Sf21C1 from the coastline bottom water, and Ss28 from the sediment from the Costa Norte site. They form pigmented mucous colonies of smooth consistency and regular edges. They are pleomorphic Gram negative cells with presence of vacuoles, and cellular dimensions from 29,2 to 0,9 µm long. They oxidize the glucose and breathe nitrates; they tolerate a wide range of salinities and temperatures from 25 to 40°C. Their optimal growths occur in anaerobic conditions and pO_2 of 2 and 4%, so this group is considered as airtolerant anaerobes (Fig 2). They share FA of the types C8: 0, C12: 0, C13: 0, C14: 0, C14: 1, C15: 0, C16: 0, C17: 0, C17: 1, C24: 1; and the fatty acids that differentiate them are the C10: 0, C11: 0 and C18: 3n6.

f) The phenon 5 gathers the reference strains of *Escherichia coli*, *Enterococcus* sp and *Bacillus subtilis* (Collection of the IMSS). They form smooth, runny, shiny and concave colonies, with regular edge, and water soluble beige pigment. They are sporulated bacilli and their cellular dimensions range from 1 to 5.5µm long (Holt *et al*. 1994). They oxidize the glucose, breathe nitrates, possess the enzyme catalase and only 66% of them present the enzyme cytochrome oxidase. They tolerate salinities from 0 to 60‰, temperatures from 4 to 40°C, and its optimal growth occurs at pO_2 of 21%, so are considered facultative aerobes (Fig 2). This phenon shares the greatest FA diversity C8: 0, C12: C13: 0, C14: 0, C14: 1, C15: 0, C15: 1, C16: 0, C16: 1, C17: 0, C17: 1, C18: 0, C18: 1n9c, C18: 1n9t, C18: 2n6c, C20: 0, C20: 2, C20: 3n3, C22: 0 and C24: 1. The FA that separate this group are C18: 2n6c and the C20: 2.

g) In pair 2 are grouped the two strains from collection *Azospirillum lipoferum* and *Magnetospirillum magnetotactium*, which are Gram negative spirilla that measure up to 29 µm, form smooth, opaque colonies, and produce water-soluble yellow pigment. They oxidize the glucose, breathe nitrates and possess an enzyme cytochrome oxidase. They tolerate salinities from 0 to 60gL^{-1} and temperatures from 4 to 40°C, and their optimal growth was achieved in microaerobic conditions. With regard to fatty acids profile, they share are: C14: 1, C15: 1, C16: 1 and C18: 3n3. With respect

Received: June 1st 2012. Accepted: August 1st 2012. Published: December 1st 2012

Electronic Journal of
El Hombre y su Ambiente Department
ISSN: (Pending)

2012. Vol.1 (1): 21-31

another groups, this one has only few fatty acids pattern and C18: 1n9c fatty acid was characteristic of this group, because it do not found it in another groups and phenons.

h) The pair 3 (Fig. 3) who consists from isolated bottom water (Sf2C3) and shallow sediment (SS13) localized between entrance of marine water and the lagoon (arroyo la Boya and north coast) (Fig.1), form fouling micro colonies, rough consistency, bacilli with vacuoles inside, negative Gram, who fermented and glucose oxidized form, breathe nitrates and present catalase enzyme. This group tolerate 0 to 60gL-1 salinity concentration and its optimum growth was found in microaerobic conditions (pO2 2 and 4%) (Fig. 2). The characteristic FA profile of their cell membranes of this isolated group were C12: 0, C14: 0, C14: 1, C17: 0, C24: 0 and C20: 5N3.

In the analysis of the methyl esters profile of the bacterial isolates cell membranes FA, 34 fatty acids were identified and the most representative ones were types C14: 1, C15: 1, C16: 0, C16: 1, C17: 1 and C18: 1n9c. In figure 4, it can be observed that the greatest percentage of fatty acids belongs to the saturated ones (C14: 1 Myristoleic, C15: 1 cis-10-Pentadecenoic, C16: 0 Palmitic, C16: 1 Palmitoleic, C17: 1 cis-10-Heptadecanoic), and only a small part of them belong to the group of the unsaturated ones (C18: 1n9c Oleic, C18: 2n6c Linoleic, C20: 3n3 cis-11,14, 17-Elcosatrienoic).

According to the results obtained in the phenotypical characterization of the pair 3 and the phenon 3, and according to the Bergey's Manual (Holt *et al*. 1994), the grouped isolates could belong to the genre *Pseudomonas*, since they are characterized for being straight and curved, Gram negative, chemio-organotrophic, non-fermenter bacilli that grow from 4 to 43°C, own catalase and normally oxidase enzymes, grow very well in media containing NO_3, and it has been reported that members of this genus inhabit in terrestrial and aquatic environments. Nevertheless, it should be noted that the genre *Pseudomonas* has been described as strict aerobe, and in the case of the bacterial isolates obtained during this investigation, the bacterial isolates of Pair 3 presented a respiratory metabolism slow strict microaerobic, and those gathered in phenon 3 an anaerobic air tolerant respiratory metabolism (Fig. 2). Due to the lack information aimed at studies on

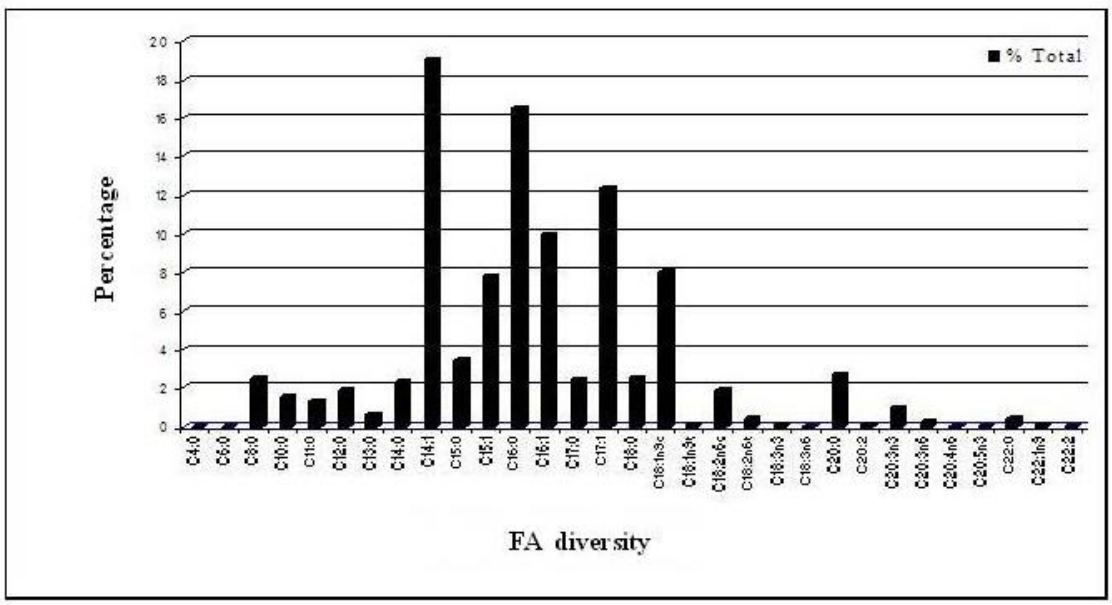

Fig. 4.Total percentage of fatty acids methylic esters of present in cell membranes of the 21 denitrifying bacterial isolates and the five reference stocks.

Received: June 1st 2012. Accepted: August 1st 2012. Published: December 1st 2012

Electronic Journal of
El Hombre y su Ambiente Department
ISSN: (Pending)

2012. Vol.1 (1): 21-31

microaerophilic bacteria, it is presumed that in this genre could find bacteria that possess the ability to grow better in microaerobic conditions.

With respect to bacteria grouped in the pair 1 and in phenons 1, 3 and 4, it was noted that their phenotypic characteristics are similar to the reported ones in the Bergey's Manual for the Spirilaceae family (Holt *et al.* 1994), since present cell shape spiral of 1.4 to 1.7 µm wide and of 14 to 60 µm long, without formation of coccoid bodies, Gram negative and motile; usually they are microaerobic, but can also grow aerobically in media containing certain growth supplements. For the Pair 1, both isolates were strict microaerobes. Phenon 4 was formed by bacterial isolates with anaerobic airtolerant respiratory metabolism, and phenons 1 and 2 by isolates with strict microaerobic metabolism. Also it is mentioned that some species pertaining to this family reduce the NO_3^- to NO_2^-; and the optimal growth temperature is 30°C, and they present positive oxidase and catalase negative. Nevertheless, it is necessary to highlight that none of the bacterial isolates grouped in these phenons has a cytochrome oxidase and catalase positive they are. It has been also reported, that the members of this family come from brackish zones, like the isolates conglomerated in the phenons 1, 2 and 4.

The analysis statistic of principal components performed only at the FA in the cell membranes of the 21 isolates and the 5 reference strains, threw the formation of 4 groups (Fig 5). The bacterial isolate SD5 was separated from group 1 because it presents less diversity of FA (4), of which two are also present in groups 1 and 2 (C8: 0 and C12: 0), and the two others (C11: 0 and C22: 1n9) are characteristic of this isolate (Table 2).

In group 1, isolates with greater diversity of saturated FA and two unsaturated ones (C20 meet (4n6 and C: 20:5n3) are grouped. The fatty acids C20: 4n6, C17: 1, C13: O and C20: 5n3 are characteristic of this group. In the group 2 were aggregated all bacterial isolates which presented the most frequent FA in their cell membranes.

Group 3 also presents a wide range of FA, but 3 of the 9 are characteristic of the group (C14: 1, C15: 1 and C16: 1). Three of the 6 FA that present the reference strains of group 4 are characteristically of them (C18: 3n3, C18: 1n9c and C18: 0).

Although in the cluster analysis the reference strains (*E. coli*, *Enterococcus* sp and *B. subtilis*) were grouped in phenon 5 because they presented different responses to the phenotypical tests applied, with regard to the rest of the isolates obtained in this research, the principal components analysis grouped them with the isolates of groups 2 and 3, because they present the same type of FA in their cellular membranes. Only the strains of *A. lipoferum* y *M. magnetotacticum* (both classified as strict microaerobic bacteria) were phenotypically and biochemically different from the rest of the isolates, due to the fact that the first is a diazotrophe

Table 2. Fatty acids with major % (weight/weight) in cell membranes of bacterial isolates conglomerated in the different groups formed in the main components statistical analysis.

Group	Conglomerates of bacterial isolates	AF with major % (weight/ weigh) in cell membranes
1	Ss11, Ss13	C12:0, C13:0, C14:0, C15:0, C17:0, C17:1, C20:0, C20:4n6, C20:5n3, C24:1
2	Sf2_C3, Sf4_C4, SD7, Sf8_C4, Ss15_C1, Ssd26, Ss24_C1, Ss27, Ss28, Ss31_C1, *Bacillus subtillis*, Ss332_C2	C8:0, C12:0, C14:0, C16:0, C20:0, C24:1
3	Sf10_C2, Sf18_C1, Sf18_C2, Sf20_C1, Ssd26, Sf30_C3, *Escherichia coli*, *Enterococcus* sp	C:120, C14:0, C14:1, C15:0, C15:1, C16:0, C16:1, C17:0, C24:1
4	*Azozpirilum lipoferum* DSMZ 2291 *Magnetospirillum magnetotacticum* DSMZ3856	C14:1, C15:1, C16:1, C18:0, C18:1n9c, C18:3n3
	SD5_C2	C8:0, C11:0, C12:0, C22:1n9

Received: June 1st 2012. Accepted: August 1st 2012. Published: December 1st 2012

Electronic Journal of
El Hombre y su Ambiente Department
ISSN: (Pending)

2012. Vol.1 (1): 21-31

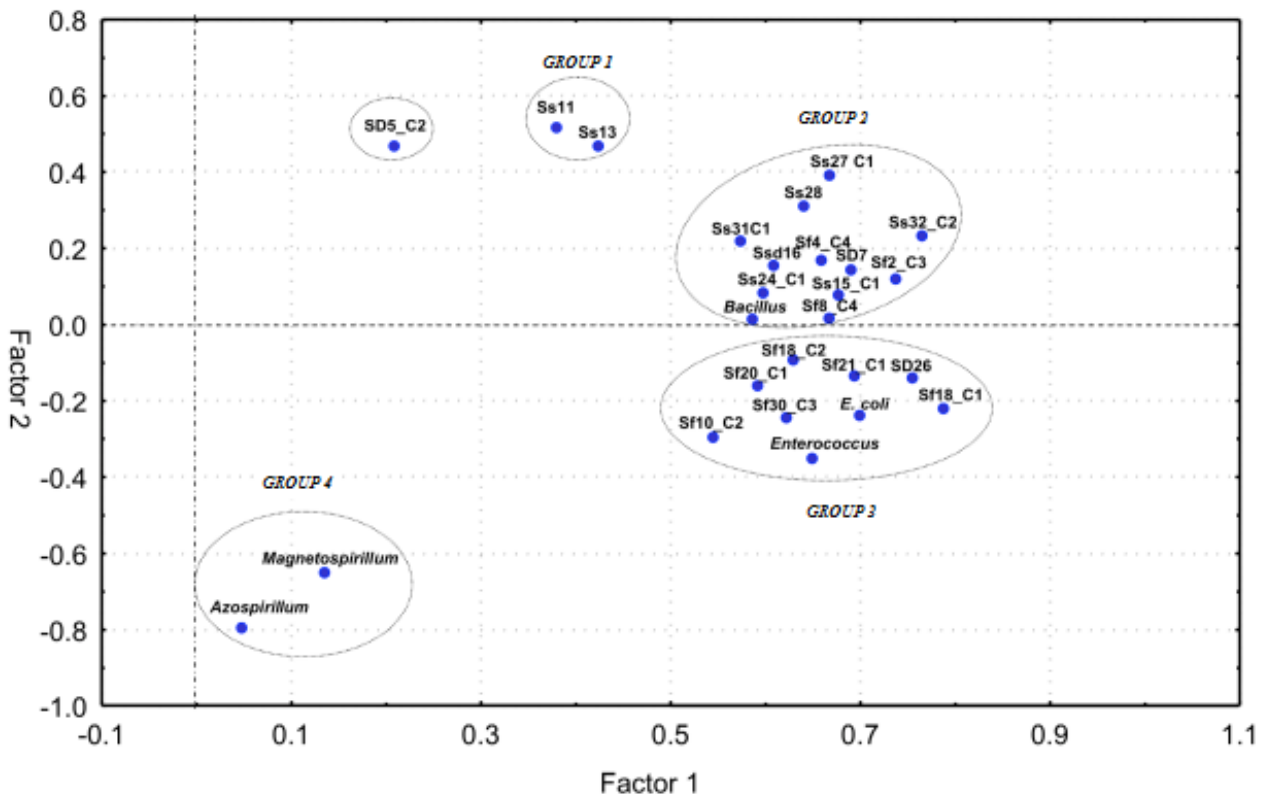

Fig. 5. Analysis of main components based on the diversity of fatty acids present in cell membranes of the 21 bacterial isolates and the 5 stocks of reference.

of terrestrial origin and the second is a spirillum inhabiting shallow fresh water bodies, with abundance of iron.

Generally, the FAME analysis of cell membranes showed that the largest percentage of fatty acids identified is saturated; this coincides with the reported by Song *et al.* (1999) who mentioned that, generally, the bacterial cell membranes are constituted, to a large extent, by saturated fatty acids. And O'Leary (1962) reported that the saturated fatty acids like the C16: 0, C16: 1, C18: 0, are encountered in the species *Pseudomonas aeuruginosa*, *Corynebacteruim diphtheriae* y *Lactobacillus acidophilus*. On the other hand, groups 1, 2, 4, 5 and SD5_C2 strain (Table 2), characterize themselves to present a hydrocarbonated chain constituted by carbons sp^3, which usually are solid and serve as support to the cell, and simultaneously are regulators of the osmoregulation processes (O'Leary 1962).

In summary, this type of chemical analysis, along with the molecular and biochemical analyses, is of great importance in the taxonomic classification of the different microbial groups. On the other hand, the analysis of the cell membranes FAME profiles is a useful tool that can be used as a biomarker of the presence of a bacterial group in natural environment, especially for those who by their physiological characteristics are hardly cultivable; it's as the case of the microaerobic bacteria.

CONCLUSSIONS

During this research it was confirmed that most of bacterial isolates from the bottom water and the superficial sediments from Sontecomapan

Received: June 1st 2012. Accepted: August 1st 2012. Published: December 1st 2012

Lagoon presented denitrifying capacity, had a strict microerobic respiratory metabolism or air tolerant anaerobic respiratory metabolism, since when they were in strict aerobic conditions its growth was very low.

The analyses of cell membranes FA methyl esters are an important tool for the taxonomic classification of this bacterial group; and it can be used in natural environments, as biomarkers of the presence of the different microaerobic denitrifying bacterial population.

BIBLIOGRAPHY

Angeles-Vazquez JR. 2007. Tasas de fijación de nitrógeno atmosférico y caracterización química y molecular de bacterias diazotrofas microaerofilicas procedentes de sedimentos. Tesis Maestria. Universidad Autónoma Metropolitana- Xochimilco. México D.F. 20-24 p.

Badings TF y C de Joung. 1983. Glass capillary gas chromatography of fatty acid methyl ester. A study of condition for the quantitative analysis of short a long-chain fatty acid in lipid. Journal of Chromatography 279:493-506.

Cervantes-Carrillo F, J Pérez-Trevilla, y J. Gómez. 2000. Avances sobre la eliminación biológica del nitrógeno de las aguas residuales. Revista Latinoamericana de Microbiología 42:73-82.

Contreras F. 1985**.** Lagunas costeras mexicanas. Centro de Ecodesarrollo. Secretaría de Pesca. D. F. 253 p.

Díaz-González G, R Gutiérrez, N Pérez, S Vega y León, M González, G Prado, G Urbán, A Ramírez y M Pinto. 2002. Detección de adulteraciones en la grasa de leche pasteurizada mexicana. Revista de Salud Animal 24: 54-59.

Ferrara-Guerrero MJ y A Bianchi. 1989. Comparison of culture methods for enumeration of microaerophilic bacteria in marine sediments. Reserch in Microbiology Institute Pasteur/Elsevier 140:255-261.

Ferrara-Guerrero MJ, DG. Marty y A Bianchi. 1993. Isolation and enumeration of anaerobic and microerophilic bacteria in aquatic habitat. p. 9-19. En Kemp PF, BF Sherr, EB Serr y J J Cole (eds). Handbook of methods in aquatic microbial ecology. CNRS Press. Boca Raton, Fla.

Goldman JC y MR Dennett. 2000. Growth of marine bacteria in batch and continuous culture under carbon and nitrogen limitation. Limnology and Oceanography 45: 789-800.

Holt JG, NR Krieg, HAP Sneath, JJ Staley y ST Williams. 1994. Bergey's manual of determinative bacteriology. Williams y Wilkins. U.S.A.

Hugh R y E Leifson. 1953. The taxonomic significance of fermentative versus oxidative metabolism of carbohydrates by various Gram negative bacteria. Journal of Bacterioogy. 66:24-26.

Kirchman DL y PA Wheeler. 1998. Uptake of ammonium and nitrate by heterotrophic bacteria and phytoplankton in the sub-Artic Pacific. Deep-Sea Research 45:347-365.

Kirchman DL. 2000. Uptake and regeneration of inorganic nutrients by marine heterotrophic bacteria. p. 261-288. En Kirchman D L (ed.). Microbial ecology of the oceans. Wiley-Liss. New York.

Kovacs N. 1956. Identification of *Pseudomonas pyoeyana* by oxidasa reaction. Nature. 178:703.

Meynell GG y E Meynell. 1979. Theory and practice in experimental bacteriology. 2°. Ed. Cambridge University Press. G.B. 347p.

Mora-Revelo SG, F Gavi-Reyes, JJ Peña-Cabriales, J Pérez-Moreno, L Tijerina-Chávez y H Vaquera-Huerta. 2007. Desnitrificación de un fertilizante de lenta liberación y urea + fosfato monoamónico aplicados a trigo irrigado con agua residual o pozo. Revista Internacional de Contaminación Ambiental. http://scielo.org.mx/scielo.php? visitado 9/11/2012

O'Brien M y R Colwell. 1987. Characterization test for numerical taxonomy studies. In Methods in microbiology. vol 19. Academic Press. London.

O'Leary WM. 1962. Fatty acids of bacterial. Journal of Bacteriology 26:421-447.

Pérez F, G Díaz, G Prado, T Gutiérrez, L González y R Acacia. 1997. Manual de técnicas de análisis por cromatografía de gas–líquido para la determinación de ácidos grasos, triglicéridos y esteroles. Universidad Autónoma Metropolitana-Xochimilco. México DF.

Pilou EC. 1984. The interpretation of ecological data. Wiley-Interscience. NY. 254 p.

Ramírez-Gama MB, CC Mejia, MO Velázquez, RGTsuzuki, GLVierna, y I Müggenburg. 2003. Manual de prácticas de microbiología general. Facultad de Química, UNAM. México. 290 p.

Song B, MM Häggblom, J Zhou, JM Tiedje y NJ Palleroni.1999. Taxonomic characterization of denitrifying bacteria that degrade aromaticcompounds and description of *Azoarcus*

Received: June 1st 2012. Accepted: August 1st 2012. Published: December 1st 2012

toluvorans sp. Nov. and *Azoarcus toluclasticos* sp. nov. International Journal of Systematic Bacteriology 49:1129-1140.

Spiro T.G. y WM Stigliani. 2004. Química medioambiental. 2a. Ed. Pearson Prentice Hall. Madrid. 504 p.

Williams DR, JJ Rowe, P Romero y RG Eagon. 1978. Denitrifying *Pseudomonas aeruginosa*; some parameters of growth and active transport. Applied and Environmental Microbiology 36:257-263.

Zehr JP y BB Ward. 2002. Nitrogen cycling in the ocean: new perspectives on processes and paradigms. Applied and Environmental Microbiology 68: 1015-1024.

Received: June 1st 2012. Accepted: August 1st 2012. Published: December 1st 2012