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TAXONOMY AND DISTRIBUTION OF HYDROCARBONONCLASTIC BACTERIA FORM THE IXTOC-I AREA.

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RÉSUMÉ - L'impact des activités pétrolières a été mesuré grâce à une étude systématique de la structure des populations hétérotrophes dégradant le pétrole. L'indice bactéries dégradant le pétrole - bactéries hétérotrophes (saprophytes) a été employé pour suivre quantitativement l'évolution dans le temps de ces populations. Des méthodes de taxonomie numérique ont été appliquées afin de constituer des écotypes bactériens des souches capables de dégrader le pétrole en mer.

Mots clés : bactéries dégradant le pétrole, contamination par le pétrole, golfe du Mexique, bactéries marines.

ABSTRACT - A bacteriological monitoring program has been established in order to evaluate the impact of oil activities. The ratio of heterotrophic-hydrocarbonoclastic bacteria was used to follow the evolution in time of both populations. Numerical taxonomic methods were employed in order to define ecotypes of marine oil degraders.

Key words : oil pollution, oil degrader, hydrocarbonoclastic bacteria, Gulf of Mexico, marine bacteria.

INTRODUCTION

The ixtoc oil field is located in the southern Gulf of Mexico, off-shore of "Laguna de Términos" and the Grijalva-Usumacinta river system. Thus, the area is influenced by river input as well as by ocean waters coming from the Caribbean and the Loop currents. These influences are reflected in the physical, chemical, geological and biological characteristics of the area.

The seasonal influence of ocean and river waters, make this area a dynamic boundary with different geological and biological factors such as the deltaic and carbonated sedimentological provinces (Lynch, 1954), and the benthic fauna (Soto, 1979, Yanez-Arancibia and Sànchez-Gil, 1983).

The Campeche bank in the southern Gulf of Mexico, is responsible for more than half of Mexico's oil production and a third of the shrimp catches in Mexican waters. Therefore since the onset of this decade (Botello and Castro, 1980) a multidisciplinary monitoring program has been established on the continental shelf of the Campeche Bank.

This work is a study of the annual and seasonal changes in the bacteria community. It was carried out using the concentrations of heterotrophic and oil degrading bacteria in the water and in the sediment habitats. In additions, oil degrading bacteria, which were isolated in March 1980, are characterized using numerical taxonomic methods.

MATERIALS AND METHODS

Water from a depth of 1 m and sediment samples were collected with special 2 L : sterile bottles and Smith-McIntyre grab respectively. All the samples were processed immediately, on board the B/O "Justo Sierra". A series of ten fold dilutions were made with artificial sea water (Lymann and Fleming, 1940), according to the concentration expected. These dilutions were seeded onto Zobell 2216-E medium (Oppenheimer and Zobell, 1952) using the spread plate method, for the enumeration of heterotrophic bacteria. The most probable number technique, described by Mills *et al.*, (1978), in 5-tube series, was used for the quantitative determination of hydrocarbonoclastic bacteria, using 20 ml screw cap bottles containing 0.1 % Ixtoc-I crude oil and artificial sea water, enriched with phosphate and nitrate. Enumerations were performed after incubations lasting 48 h for the heterotrophic group, and 2 months for the oil degraders. The incubation temperature was 28± 2°C for both methods.

From one of the cruises (March, 1980), we isolated and purified 204 strains of oil degraders from water and sediment samples (Fig. 1) from stations 6 (27 Strains), 10 (35 strains), 11 (30 strains), 12 (35 strains), 13 (41 strains) and 32 (36 strains). A morpho-physiological study was carried out with the pure cultures, giving a total of 101 tests on each of the 204 strains. The methods used are based on the work of Stanier *et al.* (1966), and on those reported by Bensoussan and Bianchi (1983).

A basic medium composed of 15 g of agar, 1000 ml of artificial sea water (op. ci.) and 2 g of a selected organic source containing carbon as energy was used to test the metabolic activity of the strains. 4 sugars, 5 fatty acids, 4 hydroxyacids, 4 organic acids, 10 alcohols, 15 aminoacids, 6 amines and phenol were tested as organic substrates.

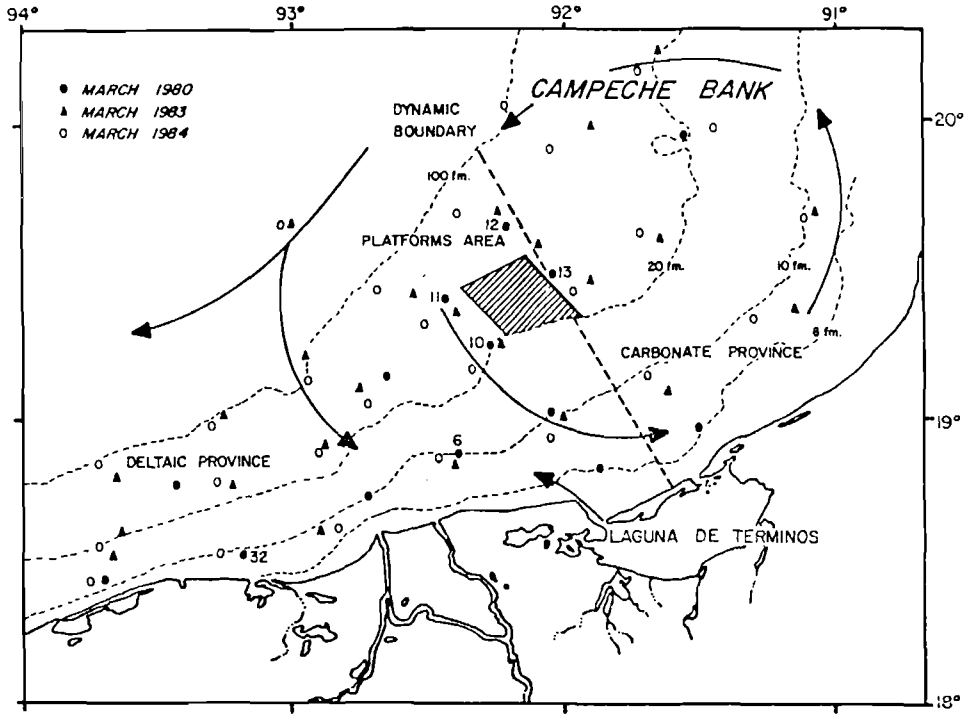

Figure 1 : Localization of the sampling stations for the different cruises.

Exoenzymes were produced and fermentations were carried out according to the methods reported by Colwell and Williams (1970).

Grams stains, morphological observations and maintenance of cultures were made using cultures growing on Zobell medium.

Numerical methods were applied in the analysis of the data, employing the Jaccard S_{sj} similarity coefficient, and the unweighting average linkage as a cluster method.

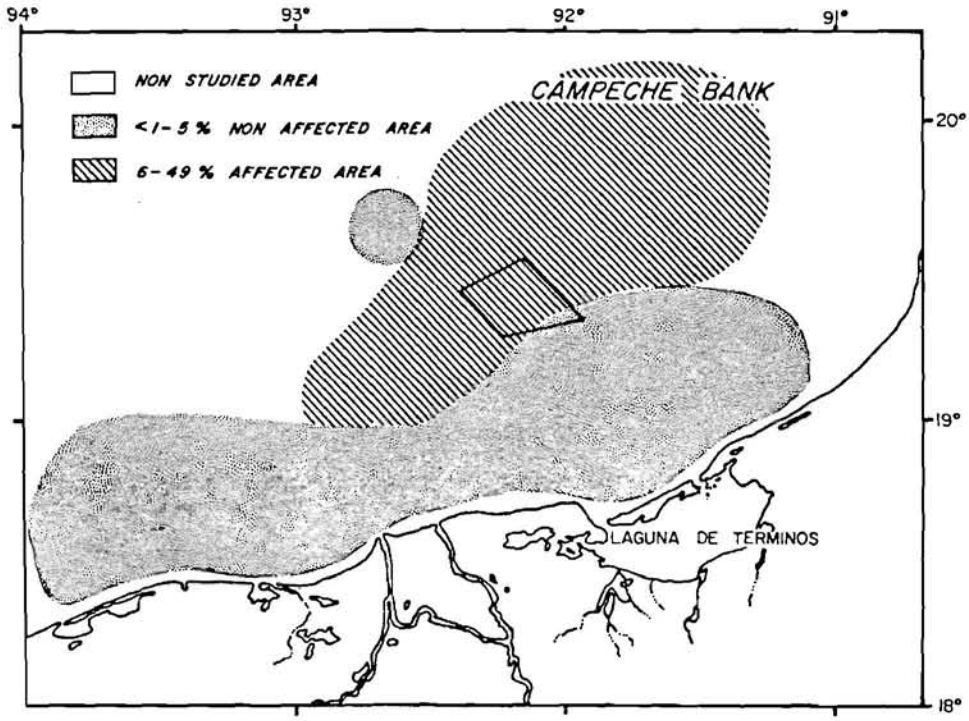

Figure 2 : Hydrocarbonoclastic/heterotrophic bacterial ratio in surface water for March 1980.

RESULTS AND DISCUSSION

Figures 2, 3 and 4 represent the quantitative evolution of the ratio oil degrading/heterotrophic bacteria in the surface water, during March 1980, March 1983 and March 1984, respectively. In these figures, we have delimited areas according to the percentages of hydrocarbonoclastic bacteria into the heterotrophic population.

Most of the zone studied was considered to be not affected by oil spills because high hydrocarbonoclastic bacteria concentrations were restricted to limited areas. Such a condition is probably due to weathering of the crude oil inputs by the synergism of biotic and abiotic factors in the area. The affected area was always found around the oil platforms and its extension seems to be conditioned by the coastal circulation. The platform area appears to be the principal source of antrophogenic hydrocarbons but its impact in the area is relatively limited.

The sediment habitat generally gives very small ratios. We found that the percentages of the oil degraders were 25 % or more (Lizarraga-Partida *et al,* 1983), only in the oil field zone.

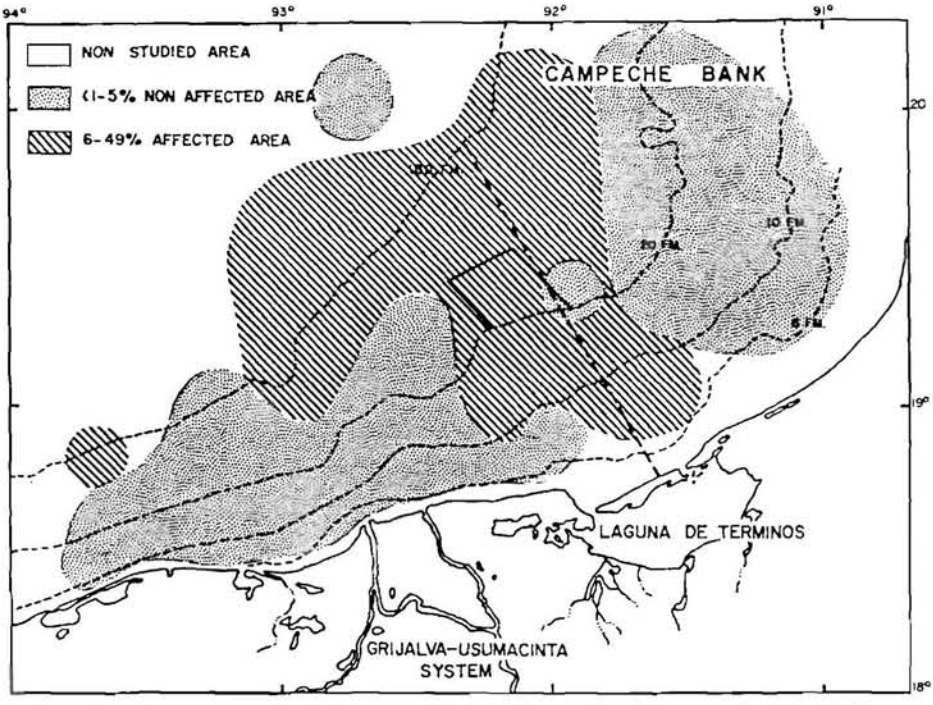

Figure 3 : Hydrocarbonoclastic/heterotrophic bacterial ratio in surface water for March 1983.

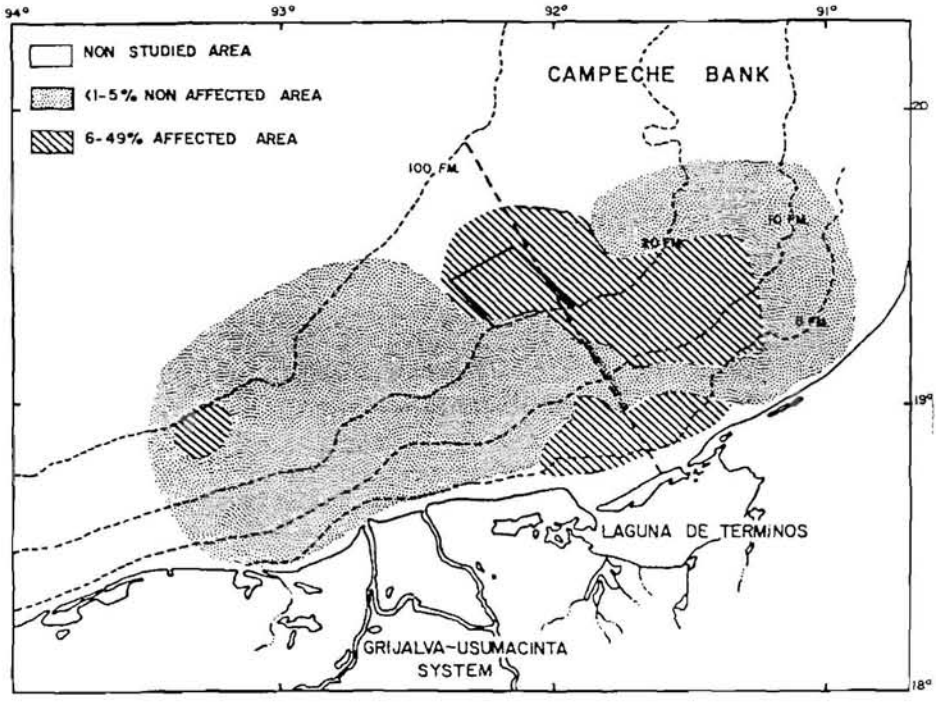

Figure 4 : Hydrocarbonoclastic/heterotrophic bacterial ratio in surface water for March 1984.

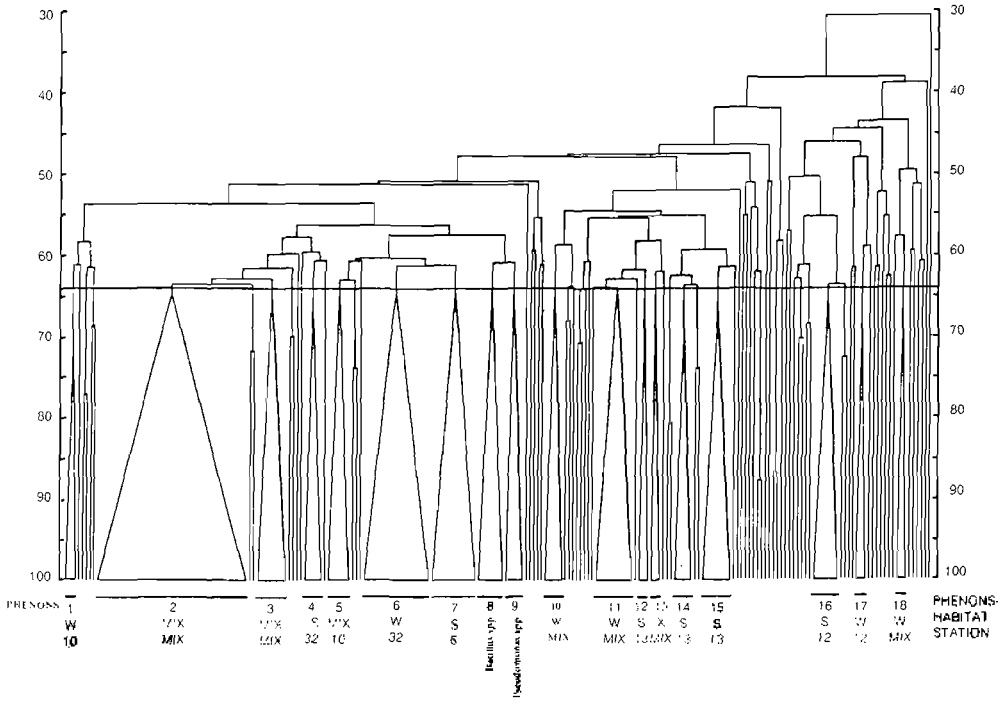

Figure 5 : Dendrogram of sediment and water hydrocarbonoclastic bacterial isolates. (W) water (S) sediment (mix) mixed habitats.

In Figure 5 we present the dendrogram of the morphological, physiological and nutritional data of the water and sediment oil degraders, which was obtained by numerical methods.

The cutting line was defined, with the "nomen species" criterium (Bianchi, 1971). Similarity was 64 % (S_{sj}), 18 phenons were formed, with at least 3 strains per phenon. Two of these phenons (8 and 9) correspond to reference strains of *Bacillus spp* and *Pseudomonas spp.*

Eight phenons showed a predominance of strains isolated from surface water (1, 5, 6, 10, 11, 13, 17 and 18) ; in five phenons we found a predominance of isolates from sediment (4, 7, 14, 15 and 17). The phenons 2 and 3 have a mixed population of these habitats.

Geographically, the majority of the phenons show a dominance of strains isolated from one sampling point, especially at the sediment but we also have certain phenons with strains from several stations, or from both habitats.

The phenon number two is particulary interesting, because of its mixed population from different habitats and sampling stations. The oil degrading bacteria belonging to this phenon showed utilization of a wide range of a carbon sources and an extensive enzyme activity. Its wide distribution in the area suggests that this group is representative of the coastal bacteria. Thus, it may be considered as an inoculum in the platform area.

Because some of the phenons were dominated by strains isolated from a specific sampling

station, it is suggested that even with the mixing of river and ocean waters, and the suspension of sediments in the water column by heavy winds, some groups of bacteria are able to remain of a location without significative perturbations. Also, it is suggested that an adaptation of the bacteria exists from coastal to ocean environment, where there is an important chronic oil pollution. Gram negative, fermentative, strains tend to be more conspicuous in areas with chronic oil inputs and according to other morphological and physiological characteristics they belong to the genus *Vibrio spp.* nevertheless, some GC % or hybridation analysis have been performed on those *Vibrio* like organisms.

Grimes *et al,* (1984) report that *Vibrio* species are the dominant bacteria in the Puerto Rico dumping site. Thus, it appears that this genus may play an important role in the degradation of pollutants, even more important that reported for *Pseudomonas.*

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