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71

HEAVY METALS EFFECT ON BACTERIAL COMMUNITIES FROM COASTAL SEDIMENTS.

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ABSTRACT - We have studied the effects of heavy metals on bacterial communities of sea sediments. Samples were taken from a very polluted zone near Barcelona.
Bacterial populations were estimated by the plate count method, using culture medium amended with heavy metals (Hg, Cd, Pb, Cr and Cu).
We have also made a numerical taxonomy study with a hundred resistant strains, using 129 unit characters.

Key words : heavy metals, bacteria, numerical taxonomy.

RÉSUMÉ - Nous avons étudié les effets des métaux lourds sur les communautés bactériennes des sédiments marins. Les échantillons ont été pris sur une zone très polluée par des effluents domestiques et industriels à côté de Barcelone.
Les peuplements bactériens ont été évalués par la méthode du comptage sur plaques utilisant un milieu nutritif et ajoutant quelques proportions des métaux lourds.
Nous avons aussi étudié la taxonomie numérique sur une centaine de souches résistantes utilisant 129 caractères unitaires.

Mots clés : métaux lourds, bactérie, taxonomie numérique.

INTRODUCTION

In previous studies carried out in our Institute, high levels of pollutants were found in the estuaries of Bésos and Llobregat rivers. The major pollutant content corresponds to organic matter, heavy metals and hydrocarbons. As a result of high levels of organic matter present; large populations of microorganisms exist in the sediments.The fate of heavy metals acts as a selection agent on those communities, supporting the growth of heavy metal-resistant strains.
This work deals with the evaluation of those bacterial communities and the determination of their taxonomic status.

MATERIALS AND METHODS

Samples were taken in the "METALS I" cruise, during April 1984 on board of the research ship "Garcia del Cid". Samples sites are described in Figure 1. Sediments were collected with a Van Veen dredge and stored in sterilized glass flasks at 4°C.

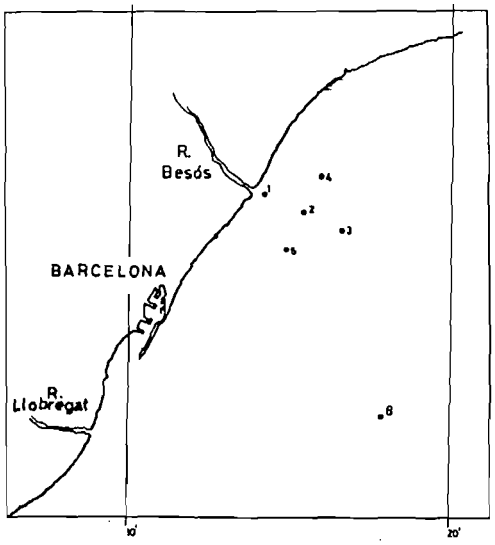

Figure 1 : Situation of sample sites near Barcelona.

Counting and isolation of resistant strains

Serial dilutions of sediments with 10^{-6} as the dilution factor maximum, using sterile sea water from the sampling zone. 0,1 ml of each dilution was spread on MH culture medium (Vallespinos & Tejero, 1977), having the following content : Yeast extract, 1 g/l ; Peptone, 5 g/l ; $FePO_4$, 0,01 g/l ; KNO_3, 1 g/l ; Filtered sea water, 600 ml ; Deonized water, 400 ml ; Agar, 15 g/l ; amended with heavy metals. Control plates without metal were also inoculated. Concentrations of heavy metals used in this study were : Hg (10 mg Hg/l, $HgCl_2$), Pb (400 mg Pb/l, Pb $(NO_3)_2$), Cd (5 mg Cd/l, Cd $(NO_3)_2$), Cu (300 mg Cu/l, $CuSO_4.5H_2O$), Cr (300 mg Cr/l, $K_2Cr_2O_7$). Three replicates of each diluted solutions were made and incubated for 7 days at 20 °C.
483 resistant strains were isolated and maintained in stock culture on MH plates.

Characterization of resistant strains

One hundred strains were randomly selected for numerical taxonomy studies. Each strain was tested for a total of 129 unit characters :
- Cellular morphology : lenght, width, shape of cell, spore formation, Gram strain, motility and cell arrangement.
- Colonial characteristics : size, shape, density, pigments.
- Physiology : growth at different temperatures, pH and salinities
- Biochemistry : O/F test, gas production from carbohydrates, Kliger test, oxidase, catalase, degradation of urea, starch, DNA and Tween 80, deamination of phenylalanine, indol test, Voges-Proskauer test, Simmons citrate, Mc Conkey agar, methyl red test and nitrate reduction.
- Resistance to the metals (Hg, Pb, Cr, Cu, Cd) and antibiotics (penicillin, carbenicillin, tetracylin, streptomycin, rifampicin, ampicillin, chloramphenicol and trimethoprime-sulfamethoxazole).
- Compounds used as the sole source of carbon : mannitol, methanol, ethanol, isopranol, buthanol, glucose, galactose, sucrose, starch, ribose, glycogen, lactose, maltose, ascorbic acid, formic acid, acetic acid, lactic acid, pyruvic acid, tartaric acid, cystine, glycine, arginine, ornithine, asparagine, lysine, tryptophan and tyrosine.

Computer analysis

Date were analyzed using the MINT program for numerical taxonomy (involving the Jaccard's similarity coefficient and the U.P.G.M.A. method of clustering).

Heavy metal analysis

The heavy metal content of sediments was analyzed as described by Modamio (1983).

RESULTS

Heavy metal levels in sediments are presented in Table 1. It seems there are no significant differences between the percentages of metal resistant in sediments, and these percentages are similar to control site ones (Table 2). Cadmium resistant bacteria became the most important fraction, followed by mercury resistant ones. Lead resistant strains were isolated only in the sediment 1, although their quantities were very small. We found no strains resistant to chromium and copper, because of the high levels of metals used.

The dendrogram obtained from numerical taxonomy analysis shows four large clusters as follows:
Cluster 1: 53 strains with a cophenetic value of 0.515. Most of them are Gram negative, oxidase positive rods. They have not been able to use the compounds tested as sole source of carbon. These strains are resistant to the five metals assessed and are sensitive to the antibiotics.
Cluster 2: Five strains with a cophenetic value of 0.486. They are not sporulated Gram positive rods. In the O/F test, the medium is made alkaline in aerobic conditions. The strains are capable of using almost all the compounds tested as a sole source of carbon (CSSC). They can reduce nitrate and are sensitive to the five metals and antibiotics.
Cluster 3: Three strains with a cophenetic value of 0.513. They are not sporulated Gram positive rods. They can growth with almost all CSSC. Oxydase, urease and starch hydrolisis tests are positive. They can reduce nitrate and are resistant to metals and sensitive to antibiotics tested.
Cluster 4: Three strains with a cophenetic value of 0.474. Gram positive rods. In the O/F test the medium is acidified in aerobic and anaerobic conditions. They can grow with almost all CSSC. They are oxidase positive, can use citrate, reduce nitrate and produce fluorescent pigments. They are resistant to penicillin, carbenicillin and ampicillin.

Sediment	Hg μg/g	Cd μg/g	Pb μg/g	Cr μg/g	Cu μg/g
1	5.70	1.02	306.1	149.6	123.0
2	2.89	0.65	167.0	127.7	88.3
3	0.90	0.27	89.9	53.5	45.3
4	1.92	0.30	144.3	84.6	69.4
5	3.00	0.74	208.9	183.0	124.8
6	0.16	0.06	32.0	12.0	12.9

Table 1 : Heavy metals levels in sediments from Besos delta.

Sample	Metal	Percentages
1	-	100
	Hg	5.8
	Cd	12.9
	Pb	0.16
2	-	100
	Hg	15.44
	Cd	20.7
3	-	100
	Hg	7.86
	Cd	21.77
4	-	100
	Hg	9.1
5	-	100
	Hg	6.7
	Cd	30.6
6	-	100
	Hg	5.62
	Cd	19.03

Table 2 : Percentage of resistant strains

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