RESEARCH PAPER PRESENTATIONS
VE. MA—9

COMPARISON OF METHODS FOR ENUMERATION OF VIBRIO PARAHAELOMYLOCUS FROM SEAFOODS

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ABSTRACT:

A comparative study of the recovery of V. parahaemolyticus inoculated into fish homogenates using glucose salt teepol broth (GSTB) salt polymyxin broth (SPB) containing different concentrations of polymyxin, salt water yeast extract broth (SWYE) and nutrient broth containing 0.5% cosin yellow (NBYE) was carried out. Enumeration of V. parahaemolyticus from naturally contaminated fish and prawns used these enrichment broths were also carried out and the results analysed statistically. The results indicate that direct plating on TCBS is superior to MPN technique when V. parahaemolyticus counts in the sample are high. Among the enrichment broths used, SWYE and SPB with 0.25 ug polymyxin yielded slightly better recoveries than GSTB and NBYE.

VE. MA—10

STUDIES ON HALOPHILIC BACTERIA ISOLATED FROM SALTED FISH

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Spoilage and discolouration of salted fish by halophilic bacteria is a major problem leading to a heavy economic loss to the fishing industry. We have attempted to isolate halophilic bacteria from salted fish and study their morphological, cultural, biochemical characters and their antibiotic sensitivity, with a view to devising a method for controlling salted fish spoilage.

From the six species of salted fish, twenty isolates of halophilic bacteria including two extreme halophiles of Genus Halobacterium, ten moderate halophiles and eight slight halophiles were obtained. Ten of the isolates were selected for further characterization. Most of the isolates were resistant to penicillin-G, while all were sensitive to streptomycin-sulfate (40Mgm/ml) and tetracycline (50Mgm/ml). The bacteriological analysis of brine, salt and the selection and use of effective antibiotics for preservation can help in controlling salted fish spoilage. This report should be useful in providing the methodology for similar type of studies as there is no readily available reference of such work done in India.
14th INTERNATIONAL CONGRESS OF MICROBIOLOGY, 7-13 SEPTEMBER, 1986, MANCHESTER, ENGLAND.

PG2-21 Sanbar Lake : Chemical Composition of the Brines and Studies on Archaeobacterial Haloalkaliphiles.
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The red, viscous, alkaline brines from the solar evaporation pans of the Sanbar Lake, India were studied with respect to their chemical composition and presence of extremely haloalkaliphilic bacteria. The brines showed pH values of 9.5 ± 0.2 and a total salt content more than 30%. Six strains of red, extremely haloalkaliphilic bacteria were isolated. All the isolates showed obligate requirement for sodium as the major cation. Magnesium ions were required in traces for maintaining morphological structure and pigmentation. Biochemical characterization was carried out. Archaeobacterial lipids and bacteriochlorophylls are present in all the isolates.

PG2-22 Growth of callus cultures and plants inoculated with nitrogen-fixing cyanobacterium Anaabaena variabilis.
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Preferences of mutual growth for calli and plants in artificial associations with cyanobacteria were determined. Experiments were performed during cultivation on the nitrogen-deficient or nitrogen-free media BS. The gain of associated tobacco calli exceeded by 25-30% that of the callus mono-culture. The 3-fold increase in biomass growth of lucerne callus in co-culture with cyanobacteria compared with that in monoculture was shown. Cyanobacteria in tobacco shoots regenerated from mixed culture prevented the cell degradation which was characteristic of tissues of nonassociated shoots. Lucerne plants with cyanobacteria inoculated into rhizosphere demonstrated normal growth, whereas uninoculated plants degraded on the medium without combined nitrogen. Inoculated lucerne plants have grown in sterile soil for 5 months, cyanobacteria being multiplied on the roots and in the soil. Cyanobacteria in associations with plant tissues formed heterocysts and exhibited acetylene-reducing activity. The results obtained have shown the possibility to produce effective artificial nitrogen-fixing systems.


The production of antibiotic substances by marine bacteria has been suggested as a relevant factor in the bactericaidal properties of seawater. In this work, we have studied the activity of some antibiotic-producing strains against different bacterial pathogens which cause important diseases in marine fish species.

A total of 20 producer strains were screened by the double-layer method, finding that all were active against different strains of Vibrio anguillarum, V. luimpini, Arsenia hydrophila, A. salmonicida, A. sobria, Pasteurella piscicida and Edwardsiella tarda. Half of the strains demonstrated activity also against V. vulnificus and V. carlsonii. When a producer strain and a bacterial fish pathogen were cultured together, with natural seawater as culture medium, a rapid inhibition of the fish pathogen was observed.

These results suggest that antagonism can also take place in natural ecosystems. Hence, a possible use of these strains as a biological control of bacterial diseases in reared marine fish species need to be investigated.


Worm grown in pure culture, the rumen fungi Neocallimastix frontalis and Prononas communis digested chopped barley straw to a greater extent than Rhizomucor albus, one of the predominant cellulolytic bacteria of the rumen. Co-culture of the fungi or B. albus with methanogenic bacteria enhanced the digestion of straw straw; in general, associations with Methanobrevibacter smithii were more stable and active than those with Methanobacterium barkeri or Methanobrevibacter rumenii. Co-cultures of N. frontalis with B. albus or with R. plateiformis were markedly less active in digesting straw than the fungus in pure culture; in contrast, co-cultures of N. frontalis and R. mucilagines were more active than either organism in pure culture. The cellulolytic activity of N. frontalis was enhanced by co-culture with the lactate utilizing bacterium Veillonella alcalescens, and with some strains of Anaerobacter ciceri.
MM-6

ISOLATION OF ARCHAEBACTERIAL HALOPHILES FROM SOLAR EVAPORATION ENVIRONMENT

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Six strains of archaebacterial halophiles were isolated from the brine samples collected from solar evaporation pans at Tata Chemicals Limited, Mithapur, Gujarat State. Strains MSW 1, MSW 2, MSW 3, were disc-shaped and grew optimally at 15% (w/v) NaCl concentration. Strains MSW 4, MSW 5, MSW 6 were rod-shaped, with an optimum salt requirement of 25% (w/v). All strains possessed bacterioruberins and archaebacterial lipids. All strains were assigned to the genus Halobacterium.
mapped by conjugation to an arc of the *Streptomyces venezuelae* chromosome lying between hisA6 and strA6 and opposite to adeA10. Phage SV1-mediated transduction indicated that all of the *cm1* mutations were located in a single gene cluster between the cotransducible auxotrophic markers, *cys-28* and *pdxB*. The apparent relative order of mutation sites showed little correlation with the expected sequence of their associated lesions in the biosynthetic pathway. However, potential regulatory mutations *cml-6*, *cml-9*, and *cml-11* were all located to one side of *cml-12*. Another phenotypically similar mutation, *cml-7* was mapped on the opposite side of *cml-12*. Also *cml-5* and *cml-8* (affecting hydroxylation of p-aminophenylalanine) could be adjacent, although *cml-4*, which apparently affected the same reaction, was separated from these loci by at least the same site of *cml-12*.

Five new mutants blocked in chloramphenicol biosynthesis were isolated by localized mutagenesis of DNA in the region near *cys-28* and *pdxB* loci. Contransduction mapping of two of these mutations subsequently confirmed their location near *cys-28* and *pdxB*.

**Abstracts of Posters**

**Environmental Microbiology**

(ENV)

**ENV-1**

**ISOLATION OF AN EXTREMELY HALOALKALIPHILIC COCCOID BACTERIUM FROM SAMBHAR SALT LAKE, RAJASTHAN**

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A strain of novel coccoid haloalkaliphile was isolated from brine sample of Sambhar Salt Lake, Rajasthan. The isolate designated as strain 4F was an extremely haloalkaliphilic coccus growing optimally at ~25% NaCl and high pH (8.5-11.0). The isolate was identified as a strain belonging to the new genus *Natronococcus* and differs from *Natronococcus occultus* NCMB 2192 in its ability to hydrolyse starch and utilize several carbon and nitrogen sources. This strain possessed archaeabacterial diether core lipids. The antibiotic sensitivity of this strain was also carried out.
ANTAGONISTIC INTERACTION WITHIN THE ARCHAEBACTERIAL HALOALKALIPHILES

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Antagonism among archaebacterial halophiles due to the production of halocin H. mediterranei ATCC 33500 has been reported. We report the antagonistic interactions among archaebacterial haloalkaliphiles due to the production of a bacteriocin like substance. The antagonism among archaebacterial haloalkaliphiles was detected during the isolation of these organisms from the brine samples of Sambhar Salt Lake, Rajasthan. Two strains which inhibited the growth of closely related strains in mixed population were isolated and were identified as strains belonging to the new genus Natronobacterium. The strains were designated as 1S and 3S respectively.

The inhibitory effect of extracellular water-soluble agent produced by these organisms was tested on several strains.

Experiments carried out indicate that such interaction is not due to phages but due to the effect of a bacteriocin like substance, which is designated as natronocin.

THE LIPIDS OF EXTREME HALOPHILES AND HALOALKALIPHILES FROM INDIAN HYPERSALINE ENVIRONMENTS

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The lipids of extremely halophilic bacterial strains from Tata Chemicals Limited, Gujarat and of extremely haloalkaliphilic bacterial strains from Sambhar Salt Lake, Rajasthan were characterized by thin layer chromatography. The major polar lipids in most of the strains were phosphatidylglycerol (PG), phosphatidylglycerophosphate (PGP) and phosphatidylglycerosulphate (PGS). The polar lipid patterns were qualitatively similar to those reported by several groups. The non-polar lipids consists mostly of squalenes, vitamin-MK8, and bacterioruberin with traces of β-carotene, lycopene and retinal in some of the strains. The absorption spectra of the pigment extracts showed maxima at 370, 388, 494 and 527 nm similar to that of bacterioruberina. The diether core lipids characteristic of archaebacterial halophiles and haloalkaliphiles were also detected.

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ENV-4
ANTIBIOTIC SENSITIVITY OF ARCHAEBACTERIAL HALOPHILES AND HALOALKALIPHILES

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Several strains of archaebacterial halophiles and haloalkaliphiles isolated from Indian hypersaline environments alongwith reference cultures were tested for sensitivity to sixteen antibiotics by the disc method. Most of the strains were sensitive to bacitracin, novobiocin and erythromycin. Some of these strains were sensitive to chloramphenicol, streptomycin and tetracycline. Studies on antibiotic sensitivity patterns within the two groups were carried out to evaluate its use for chemotaxonomic analysis.

ENV-5
MICROBIAL DEGRADATION OF DYESTUFF WASTE

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Effluents arising from dyes and dyestuff industries contain a number of chemicals used as raw materials and traces of the dyes manufactured which find their way to reach the environment. Since many of these chemicals are known to be toxic to living systems, it is an urge to treat the wastes. In the absence of suitable physical or chemical methods of treatment, biological method becomes a novel approach.

Laboratory studies were carried out to degrade the dyestuff waste containing methyl violet, rhodamine B, nigrosine, chrysoidine, phenol and aniline as the major pollutants exerting organic load of 2000 to 8000 mg/L COD (Chemical Oxygen Demand), 425 to 1200 mg/L TOC (Total Organic Carbon), 18 to 830 mg/L phenol and 126 to 325 mg/L ammoniacal nitrogen. Biomass of the microorganisms growing exclusively on the waste was developed from cattle dung and used as an inoculum to degrade 2.5 L waste in 5L glass bottles at 4 days batch detention and under aeration at ambient temperature. The data collected during a period of 112 days of experiment showed 75% degradation in COD, 89% in TOC, 98% in phenol and 54% in ammoniacal nitrogen. The predominant microbial species involved in the degradation were Pseudomonas alcaligenes and P. mendocina.