STUDIES ON THE BACTERIAL FLORA ASSOCIATED WITH SOME MARINE GREEN ALGAE

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SUMMARY

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The thallus surface of the marine green algae *Ulva reticulata, Chaetomorpha antennina* and *Enteromorpha clathrata* were screened for the occurrence of nitrogen fixing heterotrophic bacteria. The viable counts obtained with Brown's N-free medium was more than that obtained with other isolation media indicating the occurrence of a huge population of N-fixing heterotrophic bacteria on the surface of the thalli of these algae.

Ten isolates were selected for further studies based on the relative ability of the isolates to grow under N-free conditions. These isolates were characterized and idenfified. They belonged to seven genera namely, *Cytophaga, Pseudomonas, Bacillus, Flexibacter, Vibrio, Alcaligenes* and *Acinetobacter*. These isolates belonged to four different groups, namely Group IV, V, XV and XVIII according to the classification outlined in Bergey's Manual of Determinative Bacteriology (1994). Though, the occurrence of these genera in marine environment is known, their presence as associative symbionts of the marine chlorophycean macro algae is recorded for the first time.

Most of the isolates were aerobic, gram negative and were either coccoid or bacilli in appearance. A few were pigmented. Only two isolates (Bacillus) were gram positive aerobic sporulating rods.

The experimental bacterial strains showed variations in their tolerance to sodium chloride and heavy metals in the medium. *Cytophaga* sp. *Bacillus* (UL-24), *Vibrio* (CH-22), *Alcaligenes* (EM-11) and *Acinetobacter* (EM-33) were

extremely salt tolerant while the other genera showed slow growth. *Bacillus* strains (UL-13 & UL-24) showed resistance to Zinc. Old cultures showed slow recovery in the presence of heavy metals. Most of the heavy metals inhibited the growth of the isolates.

Extracts of the isolates *Bacillus* (UL-24), *Pseudomonas* (UL-12), *Vibrio* (EM-24), *Alcaligenes* (EM-11) and *Flexibacter* (CH-11) exhibited antagonism against *E.Coli*, *Staphylococcus aureus*, *Klebsiella pneumoniae* and *Salmonella typhi*. Alcoholic extracts were more effective than acetone and buffer extracts.

The experimental isolates responded variously in their sensitivity to the antibiotics Penicillin, Kanamycin, Ampicillin, Streptomycin, Amikacin, Tetracycline, Chloramphenicol and Gentamycin.

Galactose supported growth of the isolates under nitrogen free conditions. Under these conditions *Bacillus* sp. (UL-13) utilized all the different sugars tried in the present study.

Nitrogen fixation in terms of total nitrogen content increased with age in all the isolates and cessation of nitrogen fixation was not observed for any of the isolates upto a period of 96 hrs. *Bacillus* sp. (UL-24), *Acinetobacter* sp (EM-33), *Flexibacter* sp. (CH-11) and *Bacillus* sp. (UL-13) fixed more nitrogen/ml culture than other isolates. Nitrogen fixation in relation to carbohydrate utilization also showed similar results for different isolates.

Nitrogenase activity (Acetylene reduction) could not be detected in *Cytophaga* sp. (UL-21), *Bacillus* sp. (UL-24), *Vibrio* sp. (CH-22) and *Vibrio* sp. (EM-24). The rest of the isolates except *Pseudomonas* sp (EM-12) had high levels of the enzyme.

The available data in the present investigation indicated the occurrence of an associative symbiosis between marine macro algae and nitrogen fixing heterotrophic bacteria. The microsymbiont probably provided the nitrogen for the algae which grow in the nitrogen limiting marine environment.