1. INTRODUCTION

Since the time of its introduction by Waksman, the term 'antibiotic' has been a common household word. Antibiotics are among the most widely prescribed drugs not only in human medicine but also in veterinary field. Furthermore, they are often used to protect plants from infection, to decontaminate the shells of eggs, and to improve weight gain and feed conversion in a variety of animals. Many antibiotics are essentially used in the elucidation of specific cellular functions and in genetic engineering in selecting easily determined genetic markers.

Antibiotics are low molecular weight microbial metabolites that at low concentrations inhibit the growth of other microorganisms. The molecular weight of these substnces is at most a few thousand daltons. By 'low concentrations', generally we mean values of <1 mg/ml (Lancini and Parenti 1982).

To date, more than 8000 antibiotics have been isolated and described. Determination of the chemical structures of many of them indicates that the antibiotics form a very heterogenous group chemically. This large variety of molecules is produced by an array of widely diverse microorganisms. The taxonomic distribution of the antibiotic producing strains is neither uniform nor random. About two-thirds of the antibiotics described are in fact produced by strains that are members of only one bacterial group, the actinomycetes, and especially by one genus of this group, the *Streptomyces*. Other important species are: the species of the genera *Bacillus* and *Pseudomonas* among the bacteria, further some groups of fungi, mainly from the neighbourhood of the genus *Penicillium* (Höfle and Reichenbach 1990).

The production of antibiotics is not species specific. The same antibiotic can be produced by strains belonging to different taxonomic groups. For example, cycloserine has been isolated from both a *Streptomyces* and a *Pseudomonas* strain. On the other hand, strains classified taxonomically as members of the same species can produce different antibiotics. A classic example is *Streptomyces griseus*. Streptomycin (an aminoglycoside), novobiocin (a glycoside with a complex aromatic moiety), cycloheximide (aromatic structure derived from acetate), viridogrisein (a depsipeptide), griseoviridin (a lactone), candicidin (a polyene), and grisein (a sideromycin) are produced by different strains of this species. However, as a general rule, greater the taxonomic difference between two microorganisms, the lower is the probability that they will produce the same antibiotic molecule (Lancini and Parenti 1982).

Actinomycetes have been and still are being investigated intensively. They still yield new compounds, but, of course, the amount of work required as well as the costs to find new substances are rising continuously, because more and more often known compounds are rediscovered. It would thus be of considerable interest if new groups of producers could be identified. To date, hundreds of thousands of microbial strains have been screened for antibiotics. However, not all the types have been discovered, and that the chance of finding new chemical structures would be much greater if we would examine unusual, rare microorganisms. The rationale behind this opinion is that the structures of microbial metabolites are expressions of the genetic characteristics of the producing species (Lancini and Parenti 1982).

Myxobacters are among those rare groups which produce copious amounts of antibiotics, but have not been adequately screened for their antimicrobial activities. Myxobacters are a rich source of not only antibiotics but also other potentially useful secondary metabolites (Reichenbach *et al.* 1984). It were the myxobacters that we chose to investigate.

Myxobacters are the fruiting gliding bacteria (McCurdy 1989). They are very remarkable microorganisms with the most sophisticated life cycles known in bacteria. One of the peculiarities is that the vegetative rods move by gliding along the surface of substrate. Hence, myxobacter colonies (swarms) look considerably different from those of ordinary bacteria; they spread over the surface of the substrate relatively rapidly, and therefore remain thin, film-like, sometimes hardly visible. The most spectacular aspect in the life of myxobacters is their striking social behaviour. This behaviour reaches a dramatic climax in the formation of fruiting bodies. As soon as the depletion of nutrient starts, the vegetative rods start to assemble at certain points within the swarm. Knob-like structures containing 10^5-10^6 cells arise, and from these masses fruiting bodies develop. In the most complex forms, the cell mass excretes a slime stalk which shifts the knob upwards. Then the knob begins to decay into individual portions, and each portion differentiates into a sporangiole. In the simplest forms, the fruiting body is merely a naked drop of slime, not much beyond the mass of aggregated rod-cells. Myxobacter fruiting bodies measure between 0.1-0.5 mm, and are thus well recognizable with the naked eye, particularly as they are almost always brightly coloured and

appear in large numbers. Within the maturing fruiting bodies, a second morphogenetic process takes place. The vegetative cells shorten, fatten and become dessication-resistant resting cells, called either myxospores or microcysts; they enable the organisms to overcome unfavourable environment (Höfle and Reichenbach 1990).

Thirty-two species of myxobacters are known. They are distinguished mainly by morphological characteristics, but some useful chemosystematic markers are also known (McCurdy 1989).

Regardless of their exotic life cycle, the myxobacters are neither rare in nature nor difficult to find. They live mainly in soil, but also inhabit rotting plant material, the bark of trees, and the dung of herbivores. They are found in all climatic zones, from the tropics to the antarctic, at all altitudes, in fresh water and swamps as well as in steppes and deserts (Höfle and Reichenbach 1990).

Although very common in soil, myxobacters can not be isolated by the usual dilution and plating techniques. Due to the slime matrix of the swarm, the myxobacter cells do not easily disperse when the soil sample is shaken in a common diluent, and therefore the organisms are highly underrepresented in number when the suspensions are streaked out. In addition there are no selective isolation media, and strain purification may become very tedious. Another problem is an almost total lack of knowledge of the physiology of these organisms. It is therefore difficult to find out a satisfactory enrichment medium for myxobacters. Thus they are often overgrown by other soil organisms because they grow relatively slowly, and are easily

overlooked because their colonies are delicate, spreading swarms (Reichenbach and Dworkin 1981).

Only a few years ago, it was turned out that myxobacters are a treasury of secondary metabolites (Reichenbach and Höfle 1989). But, this group has not thoroughly been screened. The main reasons for this omission may have been that pure cultures are not generally available, and that only a few laboratories are routinely working with these organisms which consequently often are regarded as exotic and difficult to handle (Reichenbach *et al.* 1984). The results of screening programme with myxobacters for antibiotics are encouraging as Reichenbach and his colleagues found 30 different basic structures with roughly 120 structural variants. A substantial set of data on secondary metabolites from myxobacters has already been accumulated, and some useful generalizations can be made.

Survey of myxobacters in Indian soils is quite inadequate. In India, survey works on myxobacters have been reported during 1947 to 1971 (Singh 1947; Agnihothrudu *et al.* 1959; Singh and Singh 1971). The main objectives of the present investigation had been the following:

1. Enrichment and isolation of myxobacters from soils.
2. Characterization of the isolates with a view to identify their taxonomic status.
3. Studies on the occurrence and distribution of myxobacters in Indian soils.
4. Screening the myxobacterial isolates for antimicrobial activities.

5. Selection of a potent antibiotic producing strain for isolation and characterization of the antibiotic produced by it.

6. Determination of optimal conditions for production of the antibiotic.

7. Isolation and purification of the active principle from culture filtrate.

8. Determination of the physicochemical and biological properties of the antibiotic.