MOTILITY AND CHEMOTAXIS IN *ACTINOPLANES**

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ABSTRACT

The present review deals with motility and chemotactic response of *Actinoplanes* zoospores to various substrates *in vitro* and in natural habitats. Response of zoospores to chemical stimuli by moving towards some organic materials and different types of fungal propagules has been shown. Role of motility and chemotaxis in soil microbial ecological processes has been suggested.

INTRODUCTION

THE members of *Actinoplanes* were discovered by Couch[1,2] and grouped in the family Actinoplanaceae. *Actinoplanes* are hyphae-forming, gram-positive and non-acid fast zoosporic actinomycetes. A majority of these organisms exhibit an orange or yellow colour when grown on a variety of media. Aerial mycelium is not present in most of *Actinoplanes* isolates. Because of the un-predictable nature of these organisms, only a few detailed studies have been made on their life cycles. The details of life cycle of a typical *Actinoplanes* have been described by Couch[1,3] and Bland and Couch[4].

Actinoplanes are universal in occurrence and widely isolated from Phillipines, US, Italy, India, Australia and Japan[3,5–8]. Though the distribution of these microorganisms is reported under different habitats such as forest litter[9], beach sand[10], aquatic environment[11] and various soils[12,13], nothing is known about the role of these microbes in decomposing the organic materials in soil. Much more work is needed before the actual role of these microorganisms in soil ecology can be ascertained.

The chemistry, biological characteristics and mechanism of action of antibiotics produced by some species of *Actinoplanes* have been reviewed recently[13–15]. However, the phenomenon of motility and chemotaxis in these organisms is not fully investigated. Therefore, in this review, some properties of motility and chemotaxis of *Actinoplanes* to various substrates are discussed without reference to other members of the family Actinoplanaceae possessing motile spores.

ZOOSPORES

Zoosporic actinomycetes are found in the holocarpic genus *Dermatophilus* and in the sporangia-forming members of *Actinoplanes*, *Ampullariella* and *Spirilospora*[14,16]. Sporangia of *Actinoplanes* are formed above the surface of pallisade hyphae. They are usually globose to langeniform and seldom cylindrical[17–20] (table,1 figures 1 and 2). Sporangia vary in size, shape and number of zoospores contained in them. No flagellate zoospores were seen in the sporangia. The details of developmental processes in the formation of sporangia are given

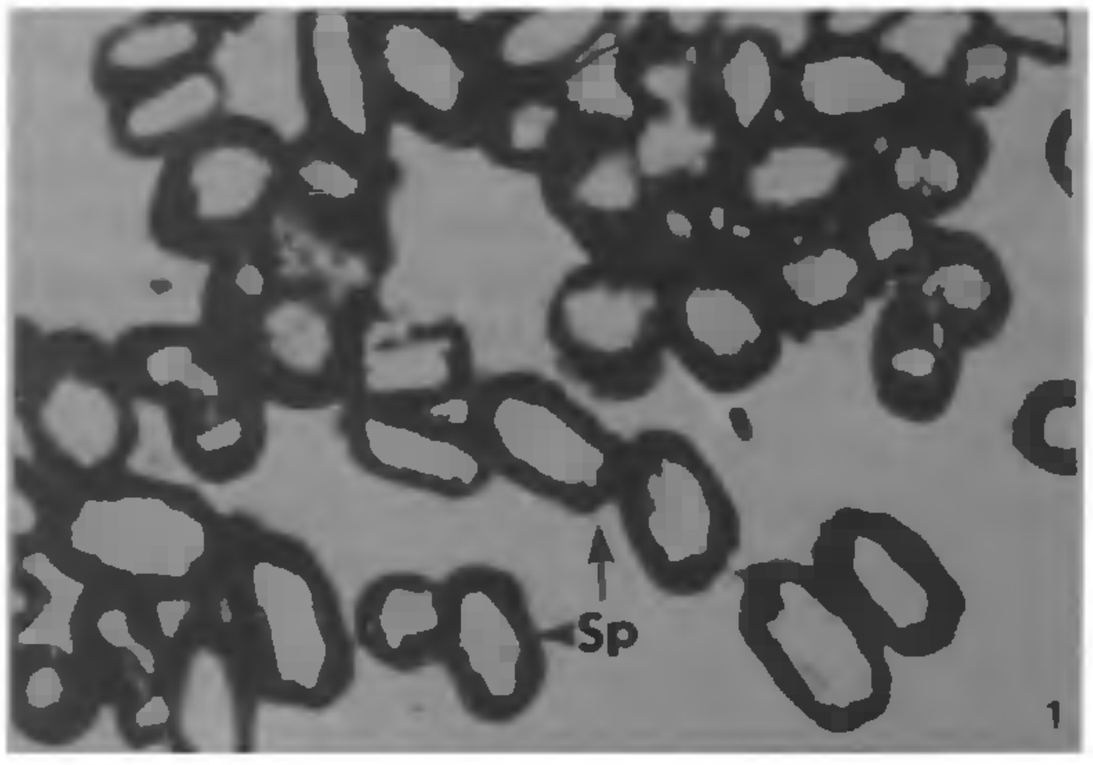

Figure 1. Bright field view of the surface of the colony showing sporangia (Sp) (×509).

*Dedicated to Professor J. L. Lockwood on his 62nd birthday.

Table 1 *Characteristics of some Actinoplanes zoospores**

Species	Shape	Size (μm)	Motility		Arrangements in sporangia
			A	B	
A. philippinesis	Spherical	1.0 – 1.2	+	NI	Coils
A. missouriensis	Sub-spherical	1.0 – 1.2	+	+	Coils
A. brasiliensis	Rod	1.2 – 2.5	+	NI	Coils
A. rectilineatus	Globose	1.5 – 2.0	+	NI	Rows
A. italicus	Oval	1.2 – 2.0	+	NI	Coils
A. utahensis	Sub-spherical	1.0 – 1.2	+	+	Coils
A. armeniacus	Rows	1.0 – 1.5	+	NI	Coils
A. jantinogenes	Spherical	1.4 – 1.8	+	NI	Coils
A. deccanensis	Spherical	1.0 – 1.5	+	NI	Coils
A. liguriae	Spherical	1.5 – 2.0	+	NI	Coils
A. ferrugineus	Globose	0.9 – 1.0	+	NI	Coils

* Modified from Parenti and Caronell (13), with additional data from other studies (7, 12, 24). A. *in vitro*; B. in soil; NI. not investigated; +. present.

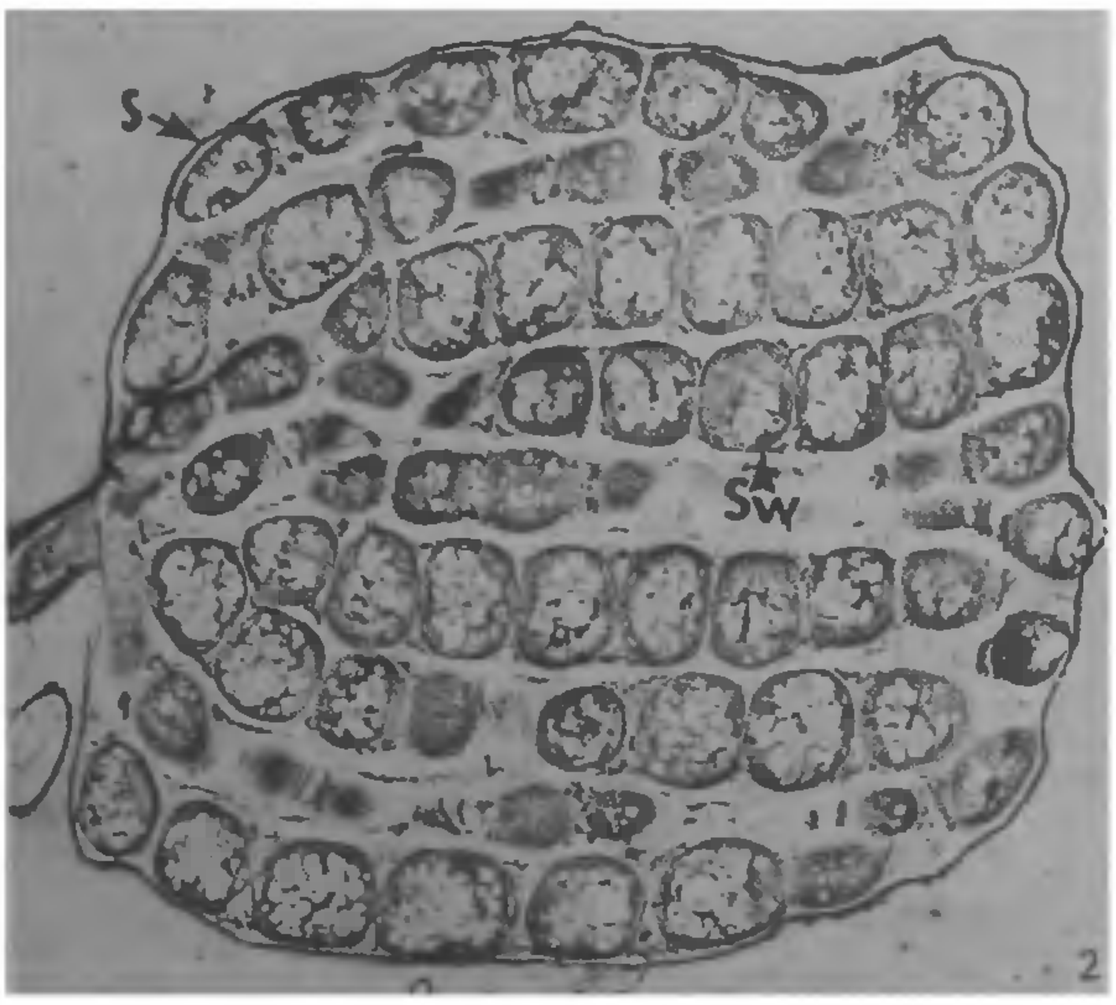

Figure 2. Section through a sporangium. Transmission electron microphotograph. S, sheath (×9164).

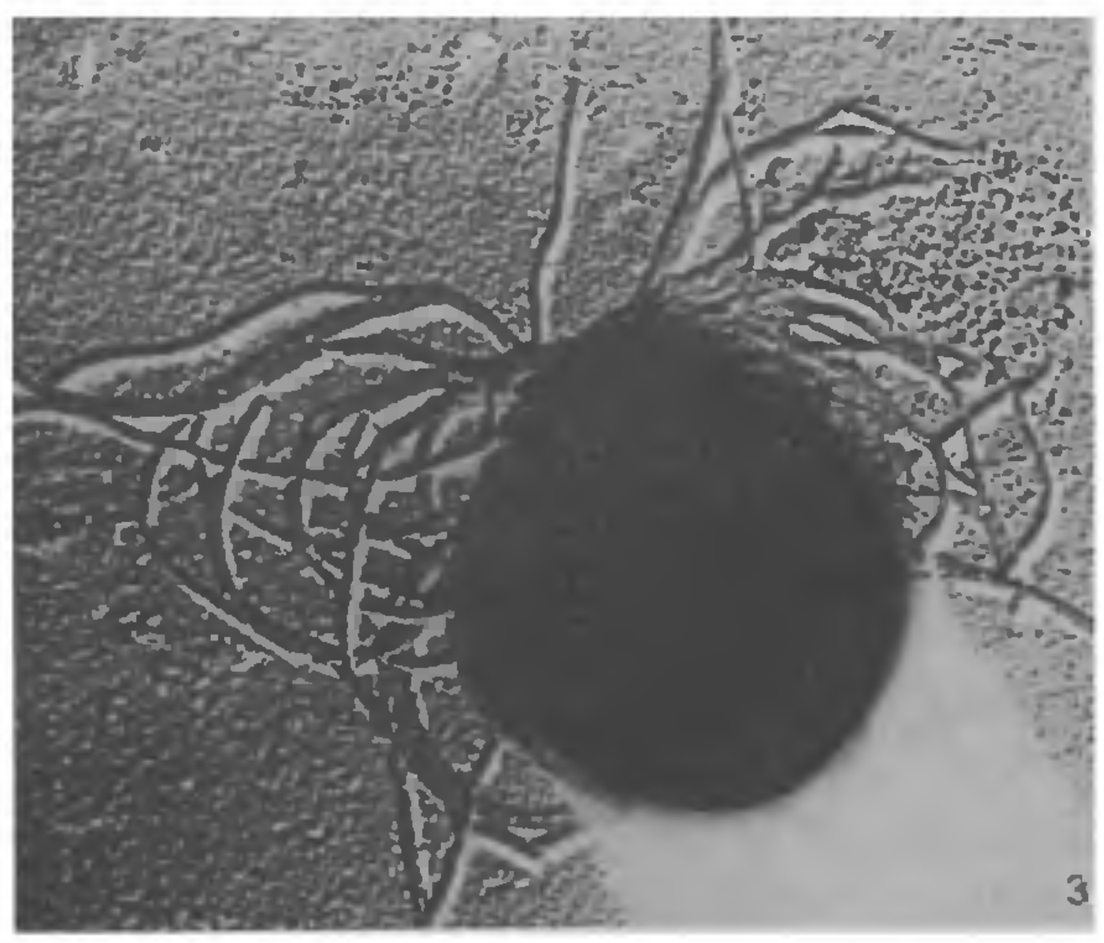

Figure 3. A zoospore (shadowed preparations). Transmission electron microphotograph (×27364).

in recent reviews[13,14,21]. Electron microscopic observations of some *Actinoplanes* strains have revealed the morphological and histological details of sporangia and zoospores and clarified their functional significance (figures 1, 2 and 3). The sporangia contain round to ovoid or irregular to globose (1 to 2 μm diam) zoospores (table 1). The number of flagella per zoospores varies from 1–40 which often form polar tufts[17,19,21,22]. Generally, the surface of flagella is not smooth but helically striated indicating its mode of formation by the coiling of subfibrils[17]. The flagella are made up of 5–6 subfibrils arising from a band-like disk situated at the inner membrane surface[23]. Unlike the proteins present in the motile bacterial cells, those of *Actinoplanes* zoospores may belong to different types since the flagella remain active for shorter duration. Zoospores consist of granular cytoplasm with fibrillar nuclear substances and some electron-light areas probably representing storage materials.

Details of zoospore releasing mechanism from mature sporangia were investigated by Higgins[18]. Flagellation and zoospore release are often triggered soon after (10–60 min) flooding the mature sporangia with water or buffer (pH 7.0) or 0.85% NaCl solutions. The addition of wetting agents such as tween-80 (1 μg/l) enhanced zoospore release probably by altering the properties of hydrophobic layers of the sporangial sheath. However, Palleroni[12] reported that wetting agents are not necessary for the acceleration of release of zoospores in *A. brasiliensis*. Mechanical agitation of the sporangia can cause release of unflagellated zoospores which become motile after 30 min of resting period. Generally, the zoospores swim actively when released. Motility can also be seen within the sporangium. Perhaps, 2–3 fold swelling of zoospores inside sporangium plays a major role in the process of dehiscence. Higgins[18,19] emphasized that swelling, and not motility, is responsible for the release since unflagellated non-motile zoospores can be released from the sporangium. There is no conclusive evidence of swelling of intersporal substances during zoospore release. After dehiscence, zoospores germinate within 4 hr of incubation at 37°C. Germination requires organic nutrients such as glucose and casein amino acids. Zoospore germination is stimulated by 6–azauracil[19]. Practically, nothing is known about the regulation of sporulation or germination.

MOTILITY

Unlike the motile bacteria, but similar to certain zoosporic lower fungi such as *Phytophthora* and *Pythium*, motility in *Actinoplanes* is a transient phase in the life cycle. The presence of flagellated motile stage in the life cycle of these microorganisms suggests an aquatic habitat for them. This need not be true. Careful studies have revealed that the mechanism of zoospore motility is similar to bacteria. The active chemotaxis recently demonstrated for *Actinoplanes* suggests an important role for motility with their natural competitors[12,24].

To what extent the external nutrients influence motility is not clear. In most species, zoospores can remain motile for 3–4 hr under ordinary conditions in the absence of any stimulators of energy generation. This can, however, be stimulated by the amino acids and other compounds such as casaminoacid (1%), L-arginine hydrochloride (6 mM) and urea (0.01 M)[25]. The number of flagella does not play a significant role in motility because a single well emerged flagellum may be sufficient for active movement. In mechanically deflagellated zoospores, flagellation can be induced in the presence of actinomycin D, chloramphenicol or streptomycin[18]. But whether or not motility can be restored in such reflagellated zoospores is not yet clear. Progressive increase in the motility was associated with decrease in osmatic pressure[18]. Motility can be inhibited by metabolic poisons such as *p*-chloromercuribenzoate, sodium iodoacetate, 2-iodoacetate, sodium azide and 2,4-dinitrophenol. The essential requirements for maintaining normal motility are: (a) pH value ranging between 7.0–7.8, (b) energy source for stimulation, (c) oxygen and (d) temperature between 28–35°C.

Virtually nothing is known about the motility of zoospores in soil or in other natural habitats. Like the motility of bacteria, motility of zoospores would be expected to depend upon soil colloids, soil moisture and diameter and continuity of soil pores. A coarse-textured soil held at 0 and −10 mb matric potential was found most suitable for active movement of zoospores[24]. No studies have been made on the movement of zoospores in different soils of physico-chemical characteristics. Temperature ranging from 25–38°C and pH 6.5–8.0 were found suitable for the movement of zoospores in a coarse-textured soil at 0 mb matric potential (D.K. Arora, unpublished results). Like the motility of zoospores of *Phytophthora* spp[26,27], zoospores of *Actinoplanes* apparently require high matric potential value for the movement in soil than the nematodes[28] and bacteria[29,30].

CHEMOTAXIS

Chemotaxis is the movement of an organism towards chemical attractants and away from chemical repellents. In the recent past, the genetics and biochemistry of chemotaxis and motility of bacteria have been extensively investigated[31,32]. Chemotaxis of other microorganisms such as slime molds and zoospores of algae and fungi to different substances was comprehensively studied by several workers[26,33]. Recently, chemotaxis of some motile bacteria towards fungal resting structures have also been shown[30,34]. Apparently, very little information is available concerning the attraction of zoospores of *Actinoplanes* to various organic or inorganic compounds or other potential substrates though the motile phase of these organisms gives the opportunity for migration to appropriate ecological locations and thus play a significant role in the search for a suitable host. Investigations by Palleroni[12] and Arora[24] have shed some light on the chemotactic response of motile zoospores to various substances.

Motile zoospores of *Actinoplanes* are capable of responding to chemical stimuli by moving towards certain chemicals. Palleroni[12] studied the chemotactic response of zoospores of *A. brasiliensis* to bromide and chloride salt solutions. Compounds like succinate, galactose and glutamate were found mild repellent but KCl, KBr and seawater showed significant positive chemotaxis. Other strains of *Actinoplanes* tested showed no positive chemotaxis to halides or organic molecules. No single attractant or repellent was noticed to be active for all species. In dense suspension, zoospores of *A. brasiliensis* showed an apparent microaerophilic tactic response. The author suggests that attraction to the halides is fortuitous and only reflects the broad specificity of a receptor whose primary stimulants have yet to be discovered. Based on a combination of the aerotactic behaviour of the zoospores and attraction of chloride ions, Pelleroni[35] invented a technique to isolate *Actinoplanes* from soil. This simple method was found less time-consuming than the conventional baiting technique.

Living fungal propagules can also attract motile zoospores of *Actinoplanes*[24]. For example, exudates from different types of fungal propagules (conidia of *Curvularia lunata*, sclerotia of *Macrophomina phaseolina*, chlamydospores of *Fusarium solani* and oospores of *Pythium ultimum*) can attract zoospores of *A. missouriensis* and *A. utahensis in vitro* and in coarse-textured soil (table 2, figure 4). Varying extents of zoospore movement in soil, mixed with fungal resting structures, were observed at 0, −10, −50 and −100 mb matric potentials. Soil at 0 mb matric potential invariably attracted greater number of zoospores than soil held at lower matric potentials. Movement of zoospores in soil was significantly less at distances greater than 8–10 mm from the

Table 2 *Chemotaxis of Actinoplanes zoospores to various attractants*

Species	Attractants	Reference
A. brasiliensis	KCl; NaCl; $MgCl_2$; $CaCl_2$; $CsCl_2$; NH_4Cl; KBr; KI; K_2SO_4; KNO_3; glucose; mannitol; yeast extract; pollen grains; sea water	Palleroni[12]
A. missouriensis	Glucose; mannitol; maltose; β-D-fructose; trehalose; exudates or propagules of *Cl*, *Mp*, *Pu*, *Fs*, *Ps*.	Arora[24]
A. utahensis	Oospores of *Pu* and *Ps*	Arora; Fig. 4.

Cl, *C. lunata*; *Mp*, *M. phaseolina*; *Fs*, *F. solani*; *Pu*, *P. ultimum*; *Ps*, *Phytophthora* sp.

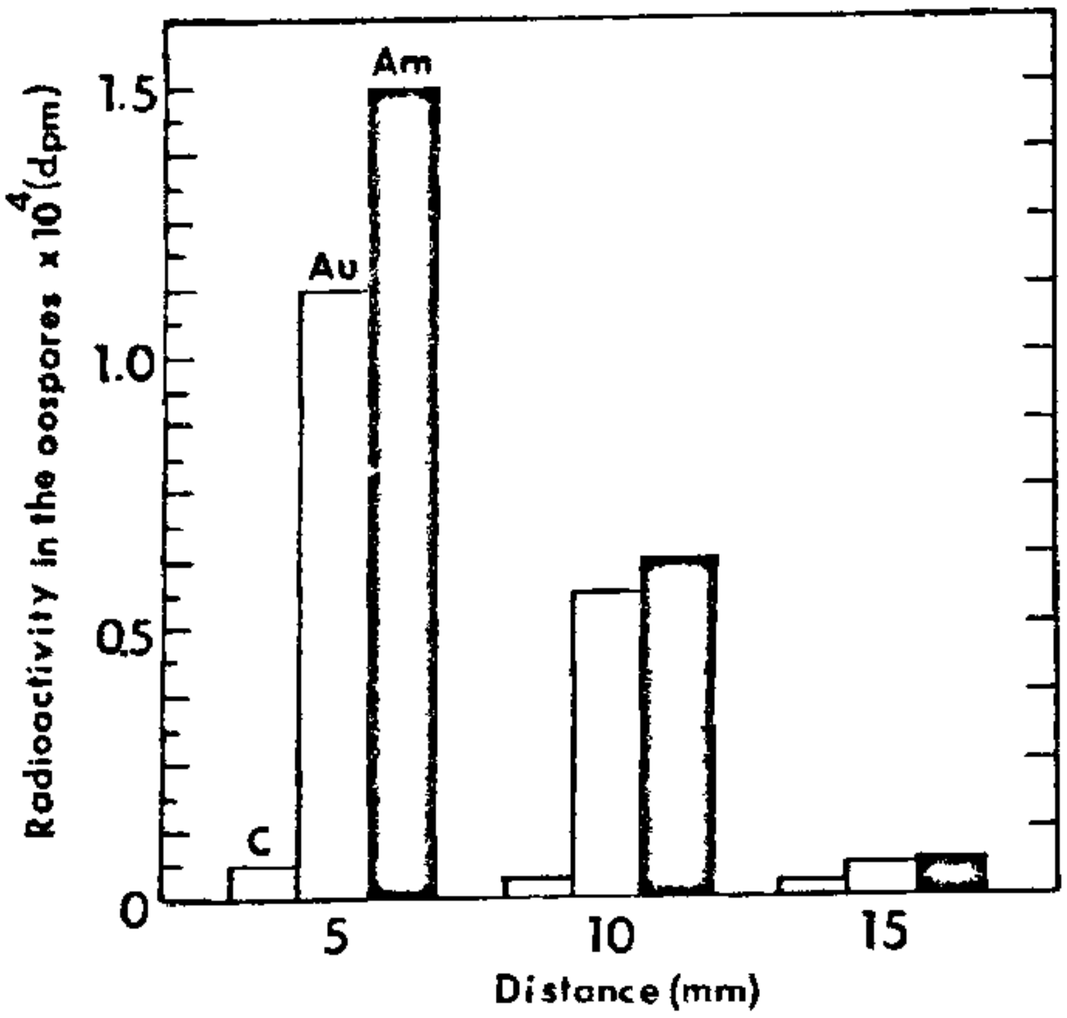

Figure 4. Chemotactic response of *A. missoriensis (Am)* and *A. utahensis* (*Au*) zoospores toward oospores of *P. ultimum* in soil at 0 mb matric potential. Oospores were placed at 5, 10 and 15 mm from a source of ^{14}C-labelled motile zoospores. C, control (soil without oospores).

source of motile zoospores. Accumulation of zoospores in response to different types of fungal propagules and exudates *in vitro* and in soil was generally in the order of conidia > sclerotia > chlamydospores > oospores Chemotaxis of zoospores of *A. missouriensis* and *A. utahensis* to some caron nutrients was also examined by the capillary-tube assay (table 2). The relative response of zoospores to glucose, mannitol and maltose was 10–30 fold greater than that of capillaries filled with sodium-phosphate buffer solution (pH 7.0)[24]. Chemotaxis is dependent on temperature. A 7–10 fold increase in the number of zoospores accumulation in the capillary was obtained when the temperature was raised from 25 to 33°C.

The tactic responses of zoospores toward fungal propagules may be mainly attributed to chemotaxis induced by stimulative chemicals in the exudate of fungal propagules. In general, response of zoospores to thin-walled conidia was greater than the thick-walled chlamydospores, sclerotia and oospores. One reason for this may be that thin-walled conidia exude more organic nutrients than thick-walled and relatively metabolically inactive chlamydospores, sclerotia or oospores. The relationship between chemotaxis and relative amount of nutrients exuded by fungal propagules is not investigated; though they seem to be mutually interdependent. It seems obvious that compounds exuded could have been derived, in part at least, from the energy pool of fungal propagules and thus may consist of many monosaccharides, sugar alcohols, amino acids and amino sugars[36].

In contrast to the behaviour of *Escherichia coli*[37] or *Spirillum volutans*[38], no peculiar migratory bands were observed in the capillaries filled with fungal exudate or oxidizable carbon source[24]. Chemotaxis of motile microorganisms to energy source may be independent of the capacity of such compounds to serve as energy source[39]. For example, very mild or no attraction of *A. brasiliensis* zoospores by a variety of growth stimulants such as cyclic-AMP, L-proline, glycerol and yeast extract was observed[12], though the zoospores were able to metabolize some of these compounds. In contrast to Palleroni[12], utilization and chemotactic response of some *Actinoplanes* species to ^{14}C-labelled fungal exudates and organic compounds was observed (D.K. Arora, unpublished results).

The presence of motility and chemotactic behaviour of zoospores to various substrates in soil suggest some role of these organism in microbial ecological processes such as food location, predator-prey interaction between organisms, symbiosis, aggregation and several other functions in community ecology. This phenomenon has not yet been studied by microbial ecologists. Recently, species of *Actinoplanes* were reported to be hyperparasites on some pathogenic soilborne fungal propagules and considered as potential biocontrol agents[40,41]. For example, Sneh *et al*[40] and others[41] reported parasitism of oospores of

Phytophthora megasperma var *sojae*, *Pythium cactorum* and *Aphanomyces euteiches* by the species of *Actinoplanes* in water-saturated soil. Parasitism of *P. megasperma* var *sojae* oospores in soil by *A. missouriensis* was facilitated by 0 mb matric potential, soil temperature 15–30°C and soil pH varying from 6.6–8.0[41]. In the above investigations soil moisture was sufficiently high to allow the movement of zoospores in water-filled pores and to detect their host via chemotactic response. Therefore, niche provided by fungal propagules may be significant for such zoospores in view of paucity of substrate available in soil. Thus, motile zoospores can manage, by means of their chemotactic response mechanisms, to reach and accumulate in the areas of favourable metabolic conditions such as sporosphere of fungal propagules. After accumulation on sporosphere, zoospores could have a role in the establishment of mycostasis further leading to complete colonization and lysis.

The role of *Actinoplanes* in antibiosis in soil or in other natural habitats is not known though most of these organisms are antibiotic producers[14]. The increase in population of *Actinoplanes* in chitin or fungal cell wall amended soil remains uninvestigated, yet chemotaxis seems to play an important role in such soils for colonization of pathogenic fungal propagules.

GENERAL REMARKS

Despite the significance of actinomycetes in soil ecology, little is known about the chemotactic response of *Actinoplanes* to various attractants or living microbial propagules. The physiological, biochemical and ecological basis of sporulation, motility and chemotaxis requires intensive investigations before the role of these microorganisms in microbial community ecology can be ascertained. Thus, it is difficult to draw a general conclusion about this little understood subject. The reasons for the over-sight of microbiologists about this subject are indeed surprising.

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