Chemotactic Response of Zoospores of Five Species of Phytophthora

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ABSTRACT

Chemotaxis of zoospores of five species of *Phytophthora (P. cactorum, P. capsici, P. cinnamomi, P. citrophthora, P. palmivora)* was studied. Zoospores of the five species responded positively to a wide range of chemicals including vitamins, phenolic compounds, nitrogenous bases of nucleic acid, nucleotides, growth regulators, sugars, organic acids, and amino acids. A distinct, directionally oriented attraction to amino acids was observed. Several chemicals (e.g., vitamins, nucleotides, organic acids, and growth regulators) caused accumulation of zoospores by trapping and immobilization without evoking a directional movement of zoospores.

A quantitative assay of chemotaxis was made with four amino acids (arginine, aspartic acid, glutamic acid, methionine). Both chemotactic index and threshold molarity for chemotaxis indicated that zoospores of *P. cactorum, P. capsici*, and *P. palmivora* were considerably more chemosensitive to the four amino acids than zoospores of *P. cinnamomi* and *P. citrophthora*. Aspartic and glutamic acids were better chemotactic agents than arginine and methionine. The chemosensitivity of both arg- and met- auxotrophs of *P. capsici* to arginine and methionine was less than that of the wild type.

Since positively charged molecules were more attractive to zoospores than negatively charged molecules, ionic structure of the amino acid molecule is important in determining its chemotactic activity. Zoospores suspended in aspartic or glutamic acid solution responded positively to either amino acid, indicating that the chemoreceptors in zoospores are likely to be nonspecific. Various metabolic inhibitors and surface-active agents failed to prevent chemotaxis at a concn that did not affect motility of zoospores.

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Additional key word: chemoreception.

Zoospores play an important role in the life cycle of *Phytophthora*. They may serve to initiate a new generation or function as an effective inoculum in spreading the pathogen (13). The tactic responses of zoospores toward plant roots have, in many cases, been attributed to chemotaxis induced by the stimulative chemicals in the root exudates (5, 10, 11, 14, 18, 20, 29). The study of Troutman and Wills (26) also implicated electrotaxis as a cause for zoospore accumulation on plant roots.

Zentmyer (27) observed a pronounced attraction of zoospores of *Phytophthora cinnamomi* to the region of elongation of avocado roots, an area of active root exudation. He also provided evidence of host specificity with regard to zoospore-plant interaction. Various investigators have demonstrated chemotaxis of zoospores to compounds in the root exudate, particularly amino acids, sugars, and organic acids (3, 5, 11, 18, 21, 28). Chang-Ho and Hickman (5) reported that the cationic fraction (amino acids) of the root exudate exhibited all the characteristics shown by the root exudate in causing accumulation of zoospores of *Pythium aphanidermatum*. Spencer and Cooper (24) noted strong attraction of zoospores of two *Pythium* species to 0.5 and 0.75% glutamic acid solution, but slight attraction to fructose and glucose. Zentmyer (28) observed attraction of zoospores of three species of *Phytophthora* to a number of amino acids particularly aspartic and glutamic acids. Recently, Bimpong and Clerk (2) reported attraction of zoospores of *Phytophthora palmivora* to several amino acids and sugars.

Although chemotaxis of zoospores is a widespread and significant phenomenon, the quantitative aspects have received relatively little attention with two possible exceptions (5, 18). No quantitative study of chemotaxis of zoospores of *Phytophthora* has yet been published. The objectives of the present study were to quantify chemotactic response of zoospores of five species of *Phytophthora* and to investigate the possible nature of chemoreception of *Phytophthora* zoospores. A brief report of this work has been published (16).

MATERIALS AND METHODS.—*Isolates.*—Five species of *Phytophthora* from the culture collection of the Department of Plant Pathology, Univ. Calif., Riverside, were included in the study. They were: *P. cactorum* (Leb. & Cohn) Schroet. (P-472) isolated from pear; *P. capsici* Leonian (P-504) from pepper; *P. cinnamomi* Rands (SB-216-1) from avocado; *P. citrophthora* (R. E. Sm. & E. H. Sm.) Leonian (P-316) from lemon; and *P. palmivora* (Butl.) Butl. (P-255) from cacao. Two auxotrophic mutants of *P. capsici*, deficient for arginine and methionine (arg-, L-10; and met-, P-505-6) previously isolated by Timmer (25) and Castro (4), were also included.

Media.—Clear V-8 juice broth (CV-8) and clear V-8 juice agar (CV-8A) were used for growing all isolates of *Phytophthora*. The liquid medium (CV-8) was prepared by adding 5 g of $CaCO_3$ to 354 ml of Campbell's V-8 juice (Campbell Soup Co., Camden, N.J.) and centrifuging the mixture at 3,000 *g* for 15 min. The supernatant was diluted (1:9, v/v) with deionized water and was used for sporangium

production. The solid medium (CV-8A) was made by diluting the supernatant (1:4, v/v) with deionized water and adding 15 g/liter of Difco agar.

Zoospore production.—All isolates were maintained on CV-8A in 60 × 15-mm petri dishes. For sporangium production, inoculum disks were cut from the growing margin of a 7-day-old culture and placed in a petri dish containing 20 ml of CV-8 liquid medium. Mycelial mats, developed after 5 days of growth at room temperature (24 ± 2 C), were washed three times with sterile deionized water to remove nutrients. The dish, containing deionized water, was then placed under a 40-W fluorescent cold daylight lamp for 24-48 hr to induce sporangium formation. Abundant sporangia were formed by all species except *P. cinnamomi* after this period of illumination. To achieve synchronized release of zoospores, mycelial mats bearing sporangia were chilled at 15 C for 15-20 min. They were then returned to room temperature and zoospores were released within 20-30 min.

To obtain sporangia from *P. cinnamomi*, the fungus was grown in liquid CV-8 medium for 24-36 hr, after which the mycelial mat was washed with distilled water and incubated in nonsterile soil extract, or in a sterile mineral solution (6).

Chemotaxis test.—A capillary root model technique similar to that of Royle and Hickman (20) was used in chemotaxis experiments under standardized conditions. Chemical solutions for tests were mixed in equal proportions with 1% purified Difco agar at 40-50 C. Solutions of aspartic and glutamic acids at 10^{-1} M concn were gradually heated to dissolve the chemicals completely. Capillary tubes were filled by capillarity. Each of the capillary tubes (prepared from Drummond's standardized 2-lambda micropipettes) was cut in half after being filled with chemical, and stored on clean glass slides in a moist chamber until ready for use. Unless otherwise stated, all amino acid solutions were adjusted to pH 3 with 1.0 N HCl or KOH prior to being mixed with agar. There was no appreciable change in the pH after agar was incorporated. Deionized water agar acidified to pH 3 was used as a control.

Although workers in the past often placed capillary tubes in a petri dish containing a zoospore suspension, this method was not satisfactory because the chemical diffusion gradient and the pattern of zoospore accumulation were too easily disturbed by manipulation for microscopic observation. An observation cell (Fig. 1) was made from a modified glass slide to facilitate the chemotaxis study. Two glass spacers (1-mm thick) were glued on the two sides of a glass slide and a cover glass was placed on top to make a chamber of approximately 12 × 22 × 1 mm. By virtue of capillary action and surface tension, a relatively constant volume of zoospore suspension was held inside the chamber. A capillary tube was then introduced from each side of the chamber. This cell could be moved conveniently and placed on a microscope stage for observation without disturbing the capillary tubes. To prevent desiccation of the suspension, the cells were placed in a moist chamber between observations.

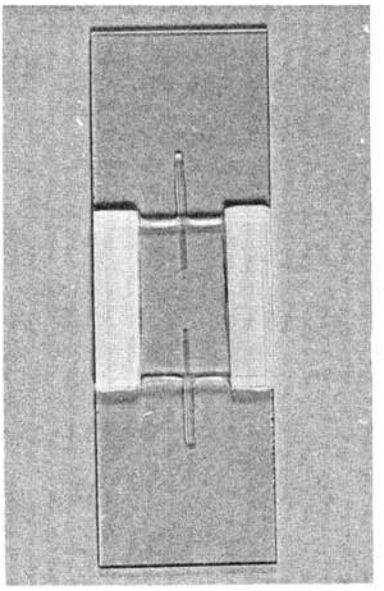

Fig. 1. Cell device used for chemotaxis study showing arrangement of the two capillary tubes in the chamber on glass slide (see text for description).

Zoospore behavior near the mouth of the capillary tubes was observed for the initial 10 min, 30 and 60 min,and at 1-hr intervals thereafter. Most chemotactic responses were completed in the first 3-5 hr when quantitative readings were recorded. Quantitative estimation of chemotaxis was similar to that of Rai and Strobel (18). The number of zoospores that encysted at the end of the capillary tube as a result of chemotactic response in a ×100 field (about 2.6 mm^2 area) was counted. Readings of the randomly encysted zoospores were also taken from regions (same area of ×100 field) 6-8 mm from the end of the capillary tube, where there was no apparent influence by the chemical in the capillary tube. The chemotactic index was calculated by dividing the number of zoospores encysted in the field near the end of the capillary tube by the number of zoospores randomly encysted in a similar field beyond the influence of the solution in the capillary. A chemotaxis index of 1.0 indicates no chemotactic response, and values >1.0 indicate some degree of chemotactic response.

The amino acids arginine, aspartic acid, glutamic acid, and methionine were assayed at 10^{-5}, 10^{-4}, 10^{-3}, 10^{-2}, and 10^{-1} M. The concn of zoospores was maintained at 4×10^4/ml. Unless otherwise stated, all chemotaxis experiments were carried out in sterile deionized water.

RESULTS.—*Chemotaxis of zoospores for various chemicals.*—In a preliminary study, chemotaxis of zoospores of *Phytophthora cactorum, P. capsici, P. cinnamomi, P. citrophthora*, and *P. palmivora* was tested against vitamins, phenolic compounds, nitrogenous bases of nucleic acids, nucleotides,

TABLE 1. Chemotaxis of zoospores of five species of *Phytophthora* to various amino acids at pH 3

Amino acid (0.05M)	Chemotactic response[a]				
	P. cactorum	*P. capsici*	*P. cinnamomi*	*P. citrophthora*	*P. palmivora*
Alanine	+	+[b]	+	+/–	+[b]
Arginine	++[b]	++[b]	++[b]	++[b]	++[b]
Aspartic acid	+++[b]	+++[b]	+++[b]	+++[b]	+++[b]
Asparagine	+[b]	+[b]	+[b]	+[b]	+[b]
Glutamic acid	+++[b]	+++[b]	+++[b]	+++[b]	+++[b]
Glutamine	+[b]	+[b]	+[b]	+[b]	+[b]
Histidine	+[b]	+[b]	+[b]	+[b]	+[b]
Leucine	++[b]	++[b]	+[b]	+[b]	++[b]
Methionine	++[b]	++[b]	++[b]	++[b]	++[b]
Phenylalanine	+/–	+/–	+[b]	+[b]	+/–
Proline	+/–	+[b]	+[b]	+	+[b]
Serine	+[b]	++[b]	+[b]	+[b]	++[b]

[a] Final zoospore response showing various degrees of zoospore accumulation based on the following arbitrary grading system: – indifference (same as water agar control), +/– very slight accumulation, + slight accumulation, ++ moderate accumulation, +++ strong accumulation.

[b] A directionally oriented movement of zoospores due to attraction of chemical was observed.

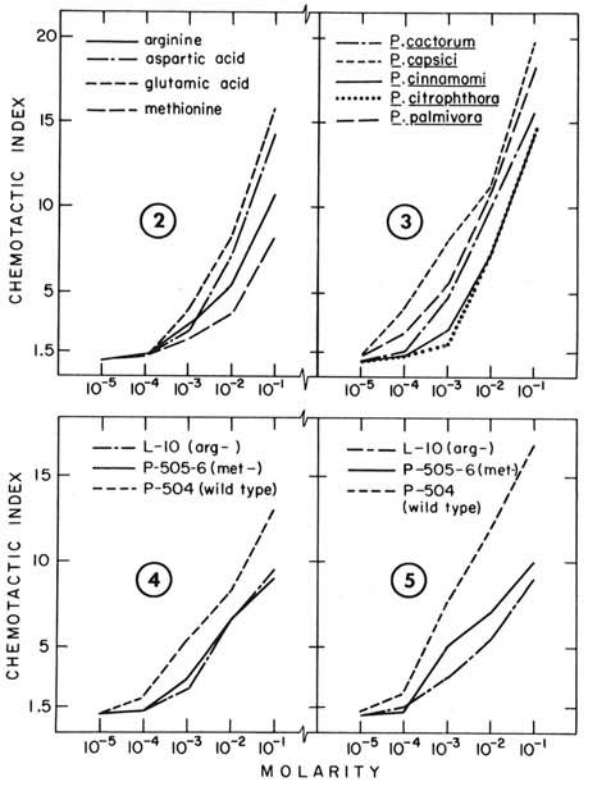

FIG. 2-5. 2) Chemotactic response of *Phytophthora cinnamomi* (SB-216-1) zoospores to various concns of four amino acids adjusted to pH3 in deionized water. **3)** Chemotactic response of zoospores of five *Phytophthora* spp. to various concns of aspartic acid adjusted to pH3 in deionized water. **4)** Chemotactic responses of zoospores of arginine-deficient mutant (L-10), methionine-deficient mutant (P-505-6), and wild type (P-504) of *Phytophthora capsici* to various concns of arginine adjusted to pH3 in deionized water. **5)** Chemotactic responses of zoospores of arginine-deficient mutant (L-10), methionine-deficient mutant (P-505-6), and wild type (P-504) of *Phytophthora capsici* to various concns of methionine adjusted to pH3, in deionized water.

growth regulators, sugars, organic acids, and amino acids.

Many of chemicals tested induced accumulation of zoospores, but the amino acids were the most effective (Table 1). Both a directionally oriented movement of zoospores toward the capillary mouth, and subsequent encystment and germination of zoospores occurred. Positive orientation of the germ tubes towards the capillary mouth was observed in most cases. In contrast to amino acids, some chemicals such as vitamins, nucleotides, organic acids, and growth regulators caused an accumulation of zoospores by trapping and immobilization of zoospores near the capillary mouth, but not a directionally oriented movement.

The amino acids arginine, aspartic acid, glutamic acid, and methionine which induced the most consistent and prominent attraction for zoospores of all five species of *Phytophthora*, were selected for further quantitative study.

Quantitative assay of chemotaxis.—For zoospores of all five species of *Phytophthora* (Fig. 2) the

TABLE 2. The threshold molarities of four amino acids for chemotaxis of zoospores of five species of *Phytophthora*

Species & isolates	Threshold molarity[a] (X 10^{-5})			
	Arginine	Aspartic acid	Glutamic acid	Methionine
P. cactorum (P-472)	1.9	3.2	3.1	1.6
P. capsici (P-504)	2.2	1.2	1.4	1.6
P. cinnamomi (SB-216-1)	13.0	17.0	12.0	20.0
P. citrophthora (P-316)	32.0	21.0	23.0	20.0
P. palmivora (P-255)	4.2	1.8	2.8	2.4

[a] The molarity giving a chemotactic index of 1.5.

chemotactic index increased with increasing concn of arginine, aspartic acid, glutamic acid, and methionine, indicating a quantitative relationship between chemotactic response and concn. In general, aspartic and glutamic acids were more effective chemotactic agents than arginine and methionine for all species of *Phytophthora* as indicated by a higher chemotactic index (Fig. 2). In water agar controls the chemotactic index was 1.0 ± 0.2.

At a concn of up to 10^{-2}M, all of the amino acids induced a distinct directionally oriented movement of zoospores toward the source of chemicals, indicating a true chemotactic attraction. The attracted zoospores eventually encysted and germinated. However, at 10^{-1}M concn, zoospores moved sluggishly and encysted rapidly as they approached the capillary mouth.

A difference in the degree of sensitivity was observed in the chemotactic index among zoospores of different species of *Phytophthora* toward a given amino acid. This difference in chemo-sensitivity was more prominent at low concn than at high concn, as indicated by the response to aspartic acid (Fig. 3). The difference in chemo-sensitivity among species held true for arginine, glutamic acid, and methionine as well.

To more precisely compare the chemo-sensitivity of the different species, a threshold molarity for chemotaxis was determined for each chemical. This threshold molarity was designated as the molar concn of chemical which induced a chemotactic index of 1.5. The threshold molarity was determined by extrapolation on a semi-logarithmic scale. A considerably higher threshold molarity of any of the four amino acids (Table 2) was required to attract zoospores of *P. cinnamomi* and *P. citrophthora* than to attract zoospores of *P. cactorum*, *P. capsici*, and *P. palmivora*, indicating that zoospores of the former two species were less chemo-sensitive than zoospores of the latter three species.

Chemotaxis of two auxotrophic mutants of *P. capsici* was also tested (Figs. 4 and 5). Both L-10 (arg-) and P-505-6 (met-) auxotrophs were less chemo-sensitive to either arginine (Fig. 4) or methionine (Fig. 5) than the wild type (P-504). This decrease in chemo-sensitivity appeared to be nonspecific since L-10 responded at a lower chemotactic index not only to arginine but also to methionine, and P-505-6 responded similarly. The threshold molarity of arginine and methionine for chemotaxis (Table 3) was about 8 times higher for the arginine auxotroph and about 7 times higher for the methionine auxotroph, than for the wild type.

Chemotactic acitivity of amino acid solutions at different pH values.—To determine whether different ionic forms of an amino acid affect its chemotactic activity for zoospores, solutions of arginine, aspartic acid, glutamic acid, and methionine (10^{-2}M) were adjusted to the pH at the various pK values for each. Acidified or alkaline agar controls were included for comparison. Chemotactic responses of zoospores of *P. capsici* and *P. palmivora* are presented in Table 4.

At both extreme pH values (pH <2.3 or >9.5) of the water agar, motility of zoospores was decreased as they approached the periphery of the capillary mouth, which might increase the number of randomly encysted zoospores in that area. This was taken into consideration when the chemotactic index was computed. Hence, if the chemotactic index of the water agar control at certain pH was significantly raised above 1.2, the chemotactic index for the appropriate chemical at the corresponding pH was corrected by dividing it by that of the control. There was a considerable difference in the chemotactic index between pK_1 and pK_2 of the four amino acids (Table 4). In general, the four amino acids were more attractive to zoospores at acidic pH than at alkaline pH. At pK_1 all amino acids were positively charged and induced the highest chemotactic index for zoospores.

Possible nature of chemoreceptors.—It is generally assumed that zoospores detect a chemical by means of the chemoreceptors on their membrane (3). To

TABLE 3. The threshold molarities of two amino acids for chemotaxis of wild type and deficient mutants of *Phytophthora capsici*

Isolate	Threshold molarity[a] (× 10^{-5})	
	Arginine	Methionine
L-10 (arginine–)[b]	18.0	13.0
P-505-6 (methionine–)	15.0	12.0
P-504 (wild type)	2.2	1.6

[a] The molarity giving a chemotactic index of 1.5.
[b] Minus sign following "arginine" and "methionine" indicates that the isolate is deficient in that amino acid.

TABLE 4. Chemotaxis of zoospores of *Phytophthora capsici* and *P. palmivora* to four amino acids at various pH values

pH	Chemotactic index[a]	
	P. capsici	*P. palmivora*
Arginine		
2.0 (pK_1)	8.6	8.2
9.0 (pK_2)	1.5	1.4
12.5 (pK_3)	1.4	1.2
Aspartic acid		
2.1 (pK_1)	11.5	11.3
3.9 (pK_2)	11.2	10.8
9.8 (pK_3)	1.2	1.3
Glutamic acid		
2.1 (pK_1)	12.5	11.0
4.1 (pK_2)	6.0	6.4
9.5 (pK_3)	1.2	1.2
Methionine		
2.3 (pK_1)	12.2	8.4
9.2 (pK_2)	1.4	1.6

[a] Corrected by appropriate water-agar controls, see text for details.

TABLE 5. The threshold molarities of aspartic and glutamic acids for chemotaxis of zoospores of two species of *Phytophthora* suspended in 10^{-3}M of either aspartic or glutamic acid[a]

Zoospores suspended in	Threshold molarity[b] (× 10^{-5})			
	Aspartic acid		Glutamic acid	
	P. capsici	*P. palmivora*	*P. capsici*	*P. palmivora*
10^{-3}M aspartic acid	17.0	17.0	19.0	16.0
10^{-3}M glutamic acid	13.0	14.0	15.0	15.0
Deionized water	1.2	1.8	1.4	2.8

[a] Zoospores were suspended in the amino acid solution for 10 min before the chemotaxis experiment was conducted.
[b] The molarity giving a chemotactic index of 1.5.

determine whether these chemoreceptors are specific in nature, the following experiments were conducted. Zoospores were suspended in 10^{-3}M of either aspartic or glutamic acid solution for 10 min in an attempt to desensitize the chemoreceptors that might be responsible for the detection of either of these amino acids. Capillary tubes containing either aspartic or glutamic acid at various concns (10^{-5} to 10^{-1}M, pH 3) were then placed in the zoospore suspension. Subsequent observation of chemotaxis revealed that under such conditions, zoospores still responded positively toward capillary tubes containing either amino acid. However, a generally lower chemotactic index was observed throughout the same concn range of chemicals as compared with the experiments in which zoospores were suspended in deionized water. A considerable difference in the threshold molarity for chemotaxis (Table 5) was also noticed. Zoospores suspended in either aspartic or glutamic acid were about 5-14 times less chemo-sensitive to these two amino acids than those suspended in deionized water.

Chemotaxis in the presence of various metabolic inhibitors and surface-active agents.—The following compounds, which included various known metabolic inhibitors, antibiotics, and surface-active agents, were incorporated into the zoospore suspension to determine whether they affected chemotaxis of zoospores: bacitracin, chloramphenicol, *p*-chloromercuribenzoate, cycloheximide, 2,4-dinitrophenol, EDTA·Na_2, filipin, iodoacetic acid, N-methylmaleimide, neomycin SO_4, nystatin, penicillin G, pimaricin, polymycin B SO_4, sodium azide, sodium barbital, sodium dedocyl SO_4, streptomycin SO_4, tetracycline HCl, Tween 80, urea, and vancomycin HCl. At a concn which showed no adverse effect on motility of zoospores (the highest from a series of 10-fold increments), none of the chemicals prevented chemotaxis of zoospores toward a known chemotactic agent (e.g., aspartic or glutamic acid). However, subsequent germination of zoospores was somewhat affected by most compounds, especially cycloheximide, N-methylmaleimide, and pimaricin.

DISCUSSION.—This study demonstrated the ability of zoospores of *Phytophthora* to respond positively to a wide range of chemicals, such as vitamins, phenolic acids, nitrogenous bases of nucleic acid, nucleotides, growth regulators, sugars, organic acids, and amino acids. Since most of these compounds are common components of the root exudate of many plants (19, 22), this nonselective response is of significance in that it would enhance the chances of zoospores to secure a suitable host root.

In studying chemotaxis, it is important to differentiate the cause of accumulation of the motile organisms. As has been pointed out by Clayton (7), proper interpretation of the patterns of accumulation of motile organisms requires a microscopic study of individual organisms in which the effect of the reagent (chemical) upon motility is noted. The present study of chemotaxis indicated that accumulation of zoospores around the capillary mouth could be due to a directionally oriented attraction as exemplified by most amino acids, or trapping and gradual immobilization of zoospores as indicated by the response to various vitamins, nucleotides, organic acids, and growth regulators. Harris (12) maintained that only the former (directionally oriented movement) reflects true chemotactic response. The attraction of zoospores of *Phytophthora* toward amino acids is consistent with results obtained by several other workers on zoospores of various Phycomycetes (2, 5, 17, 21, 24).

In the attempt to quantify chemotaxis of *Phytophthora* zoospores, the present study has revealed some interesting aspects of zoospore response. By use of the chemotactic index as a basis for comparison, there were quantitative differences in the chemotactic response among different species of *Phytophthora* to the amino acids, arginine, aspartic acid, glutamic acid, and methionine.

The chemotactic responses of two auxotrophic mutants (arg- and met-) of *P. capsici* as compared with the wild type were quantitative rather than qualitative. The decrease in the chemo-sensitivity of the deficient auxotrophs was nonspecific. Unfortunately, a good chemotactic behavioral mutant of *Phytophthora* is not presently available for a study of the mechanism of chemoreception in this fungal system. Such mutants, however, are available for study of chemotaxis in *Escherichia coli* (1).

Although a low concn (10^{-2}M or lower) of amino acids distinctly attracted zoospores, a high concn (e.g., 10^{-1}M) appeared to immobilize the zoospores somewhat as they approached the capillary mouth. This phenomenon is not unique to zoospores of *Phytophthora*; a similar observation was also reported by Cooper and Fuller (9) who found that zoospores of *Allomyces* were immobilized in 2×10^{-1}M mixture of L-leucine and L-lysine but were attracted to them at a lower concn.

Ionic structure of the amino acid molecule is important in determining its chemotactic activity. Positively charged molecules were more attractive to zoospores than negatively charged molecules. This

was true for the four amino acids tested. It seems also that an appropriate balance between certain ionic forms of carboxyl (COOH) and amino (NH_3^+) groups in amino acids is important for the chemotactic activity.

Chemoreceptors of zoospores of *Phytophthora* are likely to be nonspecific since zoospores suspended in either aspartic or glutamic acid still responded positively to capillary tubes containing either amino acid, although under such conditions, a decrease in chemo-sensitivity was noted compared with tests in deionized water.

Failure of various metabolic inhibitors and surface-active agents to prevent chemotaxis of zoospores might suggest that chemotaxis is not linked directly to active metabolism of the spores. However, technical difficulty exists in establishing conclusive proof of the chemotactic effectiveness of a particular agent, in that motility must not be affected when chemotaxis is suppressed. Failure to observe inhibitory effects of compounds on chemotaxis could be either because motility and chemotaxis were simultaneously affected above a certain concn, or the action of the agent was not expressed under the experimental conditions. Interestingly in our observation, some agents (e.g., cycloheximide, N-methylmaleimide, and pimaricin) at certain concns did not affect motility or chemotaxis of zoospores, but did suppress germination of zoospores.

Although several hypotheses have been advanced to explain chemotaxis in various organisms (8, 15, 23), the mechanism of chemotaxis of zoospores is still by and large unknown. Carlile (3) postulated that in fungal zoospores, two or more spatially separated chemoreceptors provide information on the direction of chemical gradient by recording different intensity of stimulation. In the course of chemotaxis, the zoospore turns in the direction of those receptors experiencing the greater stimulation until the chemoreceptors are stimulated symmetrically with respect to the long axis of the zoospore; it then proceeds directly up the chemical gradient. Obviously, there is still much to be learned about the cause of chemoreception in zoospores.

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