Negative Chemotaxis of Gametes and Zoospores of *Allomyces*

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Gametes and zoospores of *Allomyces macrogynus* and *Allomyces arbuscula* were repelled by H⁺, K⁺, NH₄⁺ or Na⁺ as well as by Ca²⁺, Mg²⁺ and La³⁺. This negative chemotaxis, which was monitored with a chemotaxis bioassay, occurred no matter what anionic counter-ion was used. The use of a swim-out assay showed that repulsion was similar for all cations and resulted in a band of zoospores that migrated farther into the tube with time. When zoospores allowed to adapt to 10-fold dilutions of 10 mM-KCl were challenged with 10 mM-KCl and their motile behaviour monitored in swim-out assays, it was found that the higher the initial concentration of KCl incubated with the cells, the less repulsion occurred when these cells were challenged with 10 mM-KCl. In addition when zoospores were incubated in a 10 mM solution of one repellent (i.e. KCl, NH₄Cl or CaCl₂) and then challenged in swim-out assays with a 10 mM solution of another repellent (i.e. KCl or HCl) to determine if the cationic sites of interaction were common or different, the results indicated that the sites were the same since challenging with another cation did not cause zoospore repulsion. The repulsion mechanism was studied further by mixing 10 mM-KCl and the male pheromone attractant sirenin. These experiments, using the chemotaxis bioassay, showed that in a mixed population of male gametes and zoospores, the male cells were attracted towards the source of the sirenin while all the zoospores were repulsed by KCl. Thus it appears that the mechanism for attraction is different from repulsion or that the attraction mechanism can override the repulsion behaviour.

INTRODUCTION

A phenomenon noted in many prokaryotic and eukaryotic organisms is negative chemotaxis, where cells are repulsed by or ‘avoid’ certain harmful or potentially dangerous environments. Bacteria, including *Escherichia coli* and *Salmonella typhimurium*, are able to sense a variety of dangerous compounds such as acetate and indole and can bias their motility pattern so as to swim away from such harmful agents (Tsang *et al.*, 1973). Eukaryotic flagellated and ciliated cells also demonstrate a similar behaviour. The avoidance reaction in *Paramecium* (Kung & Saimi, 1982) and the photophobic response in *Chlamydomonas* (Schmidt & Eckert, 1976) are but two examples where low molecular mass cations can bring about the repulsion of cells. In the fungi, although repulsion of zoospores has been demonstrated in *Phytophthora* (Allen & Harvey, 1974; Cameron & Carlile, 1980), such behaviour has not been noted in the posteriorly uniflagellate groups (Lange & Olson, 1979).

The gametes and zoospores of the aquatic water mould *Allomyces* are posteriorly uniflagellate cells and all appear fairly similar ultrastructurally although the male gametes are only about half the size (5 μm) of the female cells or zoospores. The motile cells differ physiologically in that the female gametes produce a pheromone called sirenen that attracts the male gametes (Machlis, 1973; Pommerville, 1977, 1981) and the male gametes produce a pheromone that attracts the
female cells (Pommerville, 1977; Pommerville & Olson, 1987). The zoospores, which are not attracted to either pheromone, are attracted to a few amino acids whereas the gametes do not show this behaviour (Machlis, 1969).

In Phytophthora low molecular mass cations will stimulate negative chemotaxis (Allen & Harvey, 1974; Cameron & Carlile, 1980). We have, in this study, examined the effects of similar cations on motility of gametes and zoospores of Allomyces macrogynus and Allomyces arbuscula.

METHODS

Preparation of gametes and zoospores. Gametes were derived from the pure male (mal-1-4) and female (f-1 lys-1) strains of A. macrogynus Burma 3-35 (35°C) (Olson, 1984) while zoospores were prepared from A. macrogynus Burma 3-35 (23°C) or A. arbuscula CR21. Gametangia were scraped from agar cultures, rinsed with distilled water onto a 10 μm Nitex screen, and resuspended in distilled water. Following release of gametes, the cell number was adjusted to 100 cells μl−1 with distilled water. Zoospores were prepared in a similar manner to that described for the gametes except that zoosporangia were scraped from agar cultures and, after rinsing in a dilute salts (DS) solution (Machlis, 1973), were resuspended in DS. Following release of zoospores, the cells were gently pelleted (1000 g, 5 min) and resuspended in distilled water.

Solutions. Working solutions of known molarity were prepared from 20 mM stock solutions using analytical grade HCl, KCl, NH₄Cl or NaCl in distilled water and the pH of the solutions measured. In addition, other solutions were prepared from KNO₃, K₂SO₄, K₂CO₃, CaCl₂, MgCl₂ and LaCl₃ and the pH determined. The pH of all solutions was between 5.5 and 5.8, except for HCl (pH 2.3) and K₂CO₃ (pH 11.1).

Chemotaxis assay. A bioassay to examine chemotaxis has been described by Pommerville (1977, 1978) and is used here with minor modifications (Pommerville, 1987). Briefly, 1% (w/v) Noble agar was prepared in distilled water, heated, and 2.8 ml added to each 60 x 15 mm Petri dish. After the agar had solidified, a 1.5-mm well punch (American Scientific Products) was used to make two wells 1 mm apart. Cells (gametes or zoospores) within a micropipette were placed into one well of the bioassay and a solution containing the cation to be tested was added to the adjacent well. Coverslips were added to prevent evaporation and bioassays were observed with a dissection microscope. Cell motility was observed for 20 min in this assay.

Swim-out test. Glass microslides (rectangular capillary tubes 0.2 mm x 2 mm x 50 mm long; Vitro Dynamics) were marked at 1 mm intervals and filled with gametes or zoospores. One end of the microslide was sealed with clay and each microslide placed in the bottom of a 100 x 15 mm Petri dish. A solution containing the cation to be tested was added to the Petri dish to just cover the microslide. Cell behaviour was observed with a dissection microscope and the movement of cells (i.e. repulsion or swimming-out) was observed at 5 min intervals over 20 min. As a control, microslides containing motile cells were placed in Petri dishes and flooded with distilled water. Values reported were identical for three separate experiments of each cation tried.

Indicator test. The diffusion of H⁺ through a microslide was estimated by filling a microslide with a 0.01% solution of bromocresol green following the method of Allen & Harvey (1974). The microslide was placed in a Petri dish containing 10 mM-HCl. As the HCl entered the microslide the change in colour of the indicator solution (blue to green) and the position of this colour boundary were recorded for comparison with the sites where the cells congregated (cell band positions) during the swim-out tests.

RESULTS

Repulsion of cells by cations

The ability of male and female gametes or zoospores to swim away from several cations was examined qualitatively using the chemotaxis bioassay (Pommerville, 1977, 1987). Cells in distilled water were placed in one well and 10 mM solutions of HCl, KCl, NH₄Cl, or NaCl in the other. Within 10 min, the entire population of cells had been repulsed to that side of the well farthest from the diffusing cation. Controls using distilled water showed random motility. Thus the repulsion illustrated a similar type of cell movement as described for male gamete chemotaxis (see Fig. 6 in Pommerville, 1977) except all cells were repulsed by cations. Na⁺ was slightly less effective in this repulsion. During the period of observation, the cells remained active and motile in all assays except those involving 10 mM-HCl (see below).

Since these experiments did not rule out the possibility that Cl⁻ was triggering repulsion, additional bioassays were run using 10 mM solutions of different potassium salts (i.e. KNO₃, K₂SO₄, K₂CO₃). In each case repulsion was as dramatic as that with KCl.
Negative chemotaxis of Allomyces

Fig. 1. Recession of the colour boundary with time (□) and the repulsion of A. arbuscula zoospores (○) from the mouth of a microslide. The colour boundary was a change in colour (blue to green) of a 0.01% bromocresol green dye contained within a microslide and placed in a Petri dish containing 10 mM-HCl. Zoospores in distilled water were contained within another microslide and repulsed with 10 mM-HCl.

Fig. 2. Repulsion of zoospores (in distilled water) in microslides flooded with solutions of 10 mM-HCl (○), 10 mM monovalent cations (K⁺, NH₄⁺ or Na⁺; results identical) (●), 10 mM divalent and trivalent cations (Ca²⁺, Mg²⁺ or La³⁺; results identical) (□), or distilled water (△).

Zoospore behaviour in swim-out assays

Indicator tests on HCl diffusion using bromocresol green gave nearly identical results to that reported by Allen & Harvey (1974). The position of the colour change with time and the repulsion by 10 mM-HCl of A. arbuscula zoospores in distilled water are indicated in Fig. 1. When microslides containing zoospores in distilled water were flooded with a solution of 10 mM-HCl, -KCl, -NH₄Cl, or -NaCl, the zoospores did not swim out of the tube but rather retreated farther into the tube as a band of cells (Fig. 2). Although the band of zoospores repelled by HCl moved up the tube at a rate similar to that for the movement of the indicator dye colour change (Figs 1 and 2), the repulsion of zoospores by the other monovalent cations was at a slower rate than that by HCl (Fig. 2). In control tests, microslides containing zoospores in distilled water were flooded with distilled water. The zoospores in these microslides swam out of the tubes and remained motile (Fig. 2).

Some di- and trivalent cations (Ca²⁺, Mg²⁺ and La³⁺) were also tested. When microslides with zoospores in distilled water were flooded with a 10 mM solution representing one of the cations, repulsion of zoospores occurred as seen by their movement farther into the tube (Fig. 2). This repulsion was identical to that for the monovalent cations. Similarly, in the chemotaxis bioassay (with male or female gametes, or zoospores), 10 mM solutions of these cations gave results identical to those with 10 mM-KCl (see above).

Further experiments to consider the sensitivity of zoospores to ions were carried out using the chemotaxis bioassay. Different concentrations of KCl (0.01–10 mM) were placed in one well and gametes or zoospores in the other. Only 10 mM-KCl was effective in causing repulsion. With lower concentrations the gametes or zoospores continued to swim about normally for the 30 min duration of the experiment. Identical results were observed for NH₄⁺, Na⁺, K⁺, Ca²⁺, Mg²⁺ and La³⁺.

Effects of cations on motility

In the chemotaxis bioassay, although the zoospores were repelled by 10 mM-HCl, most of the cells eventually lost motility. This effect was studied further by mixing equal volumes of a solution containing the cation to be tested and a zoospore suspension from A. macrogyrus.
Table 1. Effect of cations on A. macrogynus zoospore motility

The effect on motility of other cations was tested using solutions of KCl, NH₄Cl, NaCl, CaCl₂, MgCl₂ and LaCl₃ in the concentration range 0.01–10.00 mM. None of these affected motility, irrespective of whether the zoospores were preincubated in distilled water or DS.

<table>
<thead>
<tr>
<th>Cation</th>
<th>Final concn (mM)</th>
<th>Distilled water</th>
<th>DS</th>
</tr>
</thead>
<tbody>
<tr>
<td>H⁺ (as HCl)</td>
<td>0.01</td>
<td>Motile</td>
<td>Motile</td>
</tr>
<tr>
<td></td>
<td>0.1</td>
<td>Motile</td>
<td>Motile</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>Non-motile (60–120 s)</td>
<td>Motile</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>Non-motile (30–60 s)</td>
<td>Motile</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>Non-motile (30–60 s)</td>
<td>Non-motile (30–60 s)</td>
</tr>
</tbody>
</table>

Fig. 3. Saturation of cationic sites on zoospores of A. macrogynus. Zoospores were incubated in 10 mM-KCl or dilutions of 10 mM-KCl and then challenged in swim-out assays with 10 mM-KCl. KCl concentrations: 5–10 mM (○), 0.1–1 mM (□), 0.01 mM (▲). △, Zoospores incubated in distilled water.

Within 30–120 s after addition of cells, all motility was lost when using final HCl concentrations of 10, 5 or 1 mM (Table 1). Final concentrations of 0.1–0.01 mM were without effect on motility during the 20 min examination. Observations of the non-motile cells by phase contrast microscopy showed that they had not encysted but rather had lysed in the presence of high concentrations of HCl. However, if the zoospores were incubated in DS rather than distilled water and these zoospores were mixed with HCl, only a final concentration of 10 mM-HCl brought about a loss of cell viability (Table 1). At all lower concentrations of HCl the zoospores continued to swim normally for the 20 min duration of the experiment. Other cations, even if the zoospores were resuspended in distilled water, did not affect motility (Table 1).

Sites of interaction

As a result of the above observations, further experiments were designed to examine if there were sites of cation saturation and if such sites were the same for all the repellents studied. Zoospores of A. macrogynus were incubated in various dilutions of 10 mM-KCl and then challenged in swim-out assays with 10 mM-KCl. If there are sites of saturation, incubation in a lower concentration of KCl should at least partially titrate the sites and challenging with 10 mM-KCl should bring about less or no repulsion in swim-out assays. The results demonstrated that this was the case (Fig. 3). As the initial cation concentration was increased, zoospore repulsion decreased to the point where no repulsion was noted. Similar results were observed using NH₄Cl or CaCl₂ (data not shown).
Negative chemotaxis of *Allomyces*

Fig. 4. Similarity of cationic sites on *A. macrogynus* zoospores. Zoospores were incubated in either 10 mM NH₄Cl or CaCl₂ (△), or distilled water (○) and then challenged in swim-out assays with 10 mM-KCl.

Fig. 5. Effects of a 10 mM-HCl challenge on *A. macrogynus* zoospores incubated in either 10 mM-KCl (□) or distilled water (○). Zoospores in 10 mM-KCl and challenged in a swim-out assay with 10 mM-HCl were not repulsed but rather lysed at a critical HCl concentration. Thus the KCl curve represents the distance from the mouth of the microslide that the living/lysed cell boundary has moved with time.

The similarity of cationic sites was determined by incubating zoospores in a 10 mM concentration of one repellent and then challenging them in swim-out assays with a 10 mM concentration of another repellent. If the sites are the same, the zoospores should be insensitive to the second repellent, while if they are different, the zoospores should be fully sensitive and repelled. The results pointed to the former, that is the sites being the same (Fig. 4). Zoospores incubated in microslides with 10 mM-NH₄Cl were challenged with 10 mM-KCl; no repulsion was observed and the cells swam out of the microslide (Fig. 4). Similar observations were noted when zoospores in 10 mM-CaCl₂ were challenged with 10 mM-KCl (Fig. 4).

When 10 mM HCl was used as the challenging repellent, cells were repelled if they had been incubated in distilled water (Fig. 5). However, if the cells had been incubated in another cationic solution (i.e. 10 mM-KCl), no repulsion was seen and the cells lysed. Diffusion of the HCl through the microslide was monitored by the position of the interface between lysed and living (motile) cells (Fig. 5).

Repulsion versus attraction

It was of interest to examine the response of male gametes to a simultaneous encounter with repellent and attractant. As indicated in Table 2, male gametes were as effectively repulsed from the cationic solutions as they were attracted by the female-produced attractant sirenin. Control assays demonstrated that male gametes were strongly attracted to sirenin within 5 min while another bioassay showed that the male cells were repulsed within the same time by 10 mM-KCl (Table 2). Therefore, a chemotaxis' bioassay was constructed such that one well contained 10 mM-KCl plus sirenin, while the adjacent well contained a mixture of male gametes and zoospores. Within 10 min all the male gametes remained motile and were attracted to the well wall closest to the diffusing KCl/sirenin, while the motile zoospores were repulsed to the far side of the well (Table 2).

Male gametes also responded to cationic interactions in a manner similar to that described in Figs 3 and 4 for the zoospores. In these assays, the male gamete population was purposely 'contaminated' with a few female gametes. If the gametic cells were in distilled water and challenged with 10 mM-KCl, the majority of male gametes were repulsed as a band moving up
Table 2. Effects of KCl and sirenin on the chemotactic behaviour of A. macrogynus gametes and zoospores

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Conc (mM)</th>
<th>Effect on motility*</th>
</tr>
</thead>
<tbody>
<tr>
<td>KCl</td>
<td>10</td>
<td>-</td>
</tr>
<tr>
<td>Sirenin</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>KCl + sirenin</td>
<td>10</td>
<td>+</td>
</tr>
</tbody>
</table>

*-, Negative chemotaxis; +, attraction; 0, random motility.

the microslide. However, those male gametes near to the female gametes remained motile and attracted to the female cells (data not shown). Thus both the chemotactic bioassay and the microslide swim-out assay demonstrated that at these concentrations, the overriding behaviour for male gametes is attraction, not repulsion.

**DISCUSSION**

The observations reported here demonstrate that posterior uniflagellate cells respond to mono-, di-, and trivalent cations in a manner very similar to that described for the oomycetous, biflagellate fungus *Phytophthora* (Allen & Harvey, 1974; Cameron & Carlile, 1980). In addition, our observations extend those of Cameron & Carlile (1980) in that we have observed negative chemotaxis to non-chlorine salts, demonstrating that the cell behaviour is a cation-induced effect.

Allen & Harvey (1974) demonstrated that *Phytophthora* zoospores lost motility and often encysted in 5 mM-HCl. All cations examined in the present study with *Allomyces* had no effect on motility except for H⁺ (as HCl), which brought about the loss of zoospore viability without the cells retracting their flagella or encysting. This is quite different from *Phytophthora* (Allen & Harvey, 1974; Byrt et al., 1982) and from Blastocladiella emersonii, a close relative of *Allomyces*, where divalent cations can lead to encystment (Soll & Sonneborn, 1972). In swim-out assays with *Allomyces* zoospores, high concentrations of HCl caused cell lysis without repulsion when zoospores had been incubated in another cation. This is probably due to the cationic sites being occupied by the other cations, so that repulsion does not occur and the resulting decrease in pH eventually kills the cells. In the case of incubation in DS (Table 1), the mild buffering action of DS probably delayed zoospore lysis until excess H⁺ accumulated.

As mentioned by Carlile (1983), negative chemotaxis may be of ecological significance in that cells behave in such a way as to avoid highly acidic or cationic environments. However, the mechanism responsible for repulsion in these fungi is less clear. Negative chemotaxis of *Phytophthora* zoospores is thought to be the result of repellents affecting flagellar membrane properties rather than by binding to some type of sensory receptor (Cameron & Carlile, 1980). Our results, which are similar to those described for *Phytophthora*, provide additional information with regard to repulsion. We have shown that (1) cells incubated in higher concentrations of cation exhibit less or no repulsion by a 10 mM cation challenge and (2) the sites of interaction are the same for all cations tested. It remains to be shown if this behaviour actually involves the flagellar membrane specifically or if it is a general membrane response.

Similar to that of *Phytophthora*, zoospore motility in *Allomyces* abruptly changed when the cells encountered a critical repellent concentration. In our hands, all the cations were equally effective as repellents, except that H⁺ caused a higher rate of repulsion of zoospores in distilled water. From all the experiments carried out in this study, it appears that the critical threshold concentration is similar to that for *Phytophthora* zoospores (i.e. 50–100 μM).

Negative chemotaxis of zoospores of *Phytophthora* was used to propose a mechanism for repulsion. In this model, Cameron & Carlile (1980) suggested that negative chemotaxis is dependent on cations interacting with a negatively-charged flagellar membrane. They further state that providing there is no internal ionic change, a change in the surface charge could cause
a transmembrane potential (hyperpolarization) which in turn influences flagellar activity and turning behaviour. Through such turning movements, Cameron & Carlile (1980) suggest that the zoospores will move to lower concentrations of cations and smoother swimming.

The mechanism of chemotaxis to sirenin in *A. macrogynus* male gametes has been proposed as being, in part, regulated by Ca$^{2+}$ influx (Pommerville, 1981). This, through an incompletely characterized transduction mechanism, biases the swimming direction of the male gamete so that turning movements direct the cell up the concentration gradient. If the mechanism of repulsion of male gametes by cations is similar to that suggested by Cameron & Carlile (1980) for *Phytophthora* zoospores, then there must be some overriding mechanism resulting from the sirenin communication system that does not allow the hyperpolarization attributable to negative chemotaxis.

The results of male gamete behaviour to simultaneous interaction with attractant (sirenin) and repellent (10 mM-KCl) perhaps provide one explanation for negative chemotaxis. As mentioned by Carlile (1983), cations interacting with the negatively-charged flagellar membrane will tend to negate this surface charge, causing the inside of the cell to become relatively more negative (hyperpolarized). If, at the same time, sirenin is causing an influx of Ca$^{2+}$, this would tend to counteract the hyperpolarization by providing positively-charged ions intracellularly and may even bring about a depolarization. Thus the overriding mechanism may be the inability to hyperpolarize causing the male gametes not to be repulsed. Obviously, much more work is required to test this hypothesis and to understand this very interesting cellular behaviour.

REFERENCES


