SHORT COMMUNICATION

FLUORESCENT SIDEROPHORE-MEDIATED IRON DEPRIVATION—A CONTINGENT BIOLOGICAL CONTROL MECHANISM

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The surge of interest in fluorescent pseudomonads was spurred by discoveries that members of this group are capable of increasing plant growth and suppressing disease upon introduction into the soil or rhizosphere (Burr et al., 1978; Kloepper and Schrot, 1981; Olsen and Misaghi, 1982, 1984; Weller and Cook, 1983). These bacteria produce iron chelators (siderophores) in vitro with high stability constants (about 1027) (Meyer and Abdallah, 1978) which bind available Fe, depriving competing microorganisms of this vital element (Elad and Misaghi, 1985; Emery, 1980; Kloepper et al., 1980; Misaghi et al., 1982; Scher and Baker, 1982). Thus the growth of sensitive fungi and bacteria is inhibited in the presence of siderophores (Kloepper et al., 1980; Misaghi et al., 1982; Scher and Baker, 1982). The possible involvement of fluorescent siderophores (FS) in plant growth promotion and disease suppression has been inferred by observations that siderophore-producing pseudomonads (Bakker et al., 1986; Burr et al., 1978; Olsen and Misaghi, 1984; Weller and Cook, 1983), their siderophores (Kloepper et al., 1980), as well as Fe chelators [Fe (III) EDDHA] (Scher and Baker, 1982) can promote plant growth and reduce the incidence of some soil-borne diseases when they are introduced into the soil. Hydroxamate siderophores also may be important in the economy or metabolism of iron in microorganisms and probably in plants (Waid, 1975).

The hypothesis that FS may play a role in growth promotion and disease suppression in the field is based on assumptions that production of FS and their Fe-chelation are not modulated by variable soil and rhizosphere conditions. Although hydroxamate siderophores (Waid, 1975) have been isolated from different soils (Powell et al., 1980), there is as yet no direct evidence that FS consistently are produced in the soil or in the rhizosphere under field conditions. Moreover, since FS production is totally shut-off in the presence of low concentrations of Fe in vitro (Kloepper et al., 1980; Misaghi et al., 1982), it is likely that FS either are not formed or are only produced in small quantities in those soils and rhizospheres where Fe concentrations are above the threshold levels. Additionally, soil and rhizosphere conditions favoring FS production may not necessarily be conducive to siderophore activity (i.e. to induce Fe deprivation by chelating available Fe). For example, soil and rhizosphere pH should modulate siderophore chelation by directly affecting Fe availability. Thus in turn could influence the extent to which siderophores affect pathogens.

Our purpose was to examine the effect of pH, a highly variable soil factor, on FS-mediated iron deprivation.

The effect of pH on the fungistatic activity of the FS was determined by a bioassay procedure (Misaghi et al., 1982) using Pseudomonas fluorescens, recovered from field soil in Arizona, was streaked across the center of plates containing King's medium B (King et al., 1954) and held at 27°C for 36 h. Fluorescent siderophores which diffused into the agar were extracted by soaking several agar discs (aseptically removed distal to the bacterial streaks) in deionized sterile water for 1 h. Subsequently, the discs were removed and the siderophore solution was stored at 5°C until used for bioassay. The amount of the siderophore solution needed to cause a 50% inhibition of P. aphanidermatum at pH 6.0 (Misaghi et al., 1982) was added to 50-ml flasks containing 5.0 ml of filter-sterilized liquid King's medium B in a 50 mM phosphate buffer pH 6.0 or 8.0. Flasks containing 5 ml of buffered medium without the siderophores served as controls. Cultures were inoculated with 8-mm dia discs taken from 3-day-old cultures of P. aphanidermatum growing on potato dextrose agar and were grown in a shaker bath at 32°C for 48 h. Fungal mycelia were collected on oven-dried, tared weighing papers, dried at 60°C for 3 h and weighed. King's medium B, a bacteriological medium, was used because unlike other media, it contains low concentrations of Fe and is thus suitable for siderophore-mediated iron deprivation studies. The medium supported profuse growth of P. aphanidermatum. The tests were repeated five times, each with five to six replications. Differences among treatments were determined by analysis of variance.

The reduction of fungal growth in the presence of siderophores was significantly greater at the 1% level at pH 8.0 than 6.0 (Table 1) in all five repetitions. The inhibition could be counteracted by adding iron to the medium in excess of the chelating capacity of the added siderophores, indicating that the observed inhibition was not due to impurities in siderophore preparations. The shifts in pH value of the media after 48 h of incubation were between 0.1 and 0.3.

The contingency of FS-mediated Fe deprivation as a mechanism of disease suppression is further highlighted by the fact that FS production also is highly influenced by a number of factors which vary from soil to soil. Of all the variables which influence siderophore production, soil pH is probably the most important because FS are not produced in the presence of moderate amounts of Fe (Kloepper et al., 1980; Misaghi et al., 1982) and Fe availability in soil is inversely correlated with the pH (Brady, 1974). The quantity of the FS produced by P. fluorescens in soil extracts amended with sodium succinate, ammonium sulfate, magnesium sulfate and potassium phosphate was reduced precipitously with decreases in soil pH (Olsen and Misaghi, 1982). Thus, under field conditions, FS may be produced only in alkaline soils and not in acid soils where available Fe concentrations may be sufficient (Brady, 1974) to prevent production. Production and biological activity of siderophores are expected to be affected by soil factors such as...
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Table 1. Reduction of the growth (dry wt) of P. aphanidermatum by fluorescent siderophores produced by a strain of P. fluorescens at pH 6.0 and 8.0

<table>
<thead>
<tr>
<th>Experiment No.</th>
<th>pH 6 (%)</th>
<th>pH 8 (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>56.2 ± 10</td>
<td>99.2 ± 1</td>
</tr>
<tr>
<td>2</td>
<td>26.5 ± 15</td>
<td>84.5 ± 4</td>
</tr>
<tr>
<td>3</td>
<td>65.2 ± 10</td>
<td>96.5 ± 8</td>
</tr>
<tr>
<td>4</td>
<td>73.7 ± 7</td>
<td>94.0 ± 7</td>
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<tr>
<td>5</td>
<td>43.4 ± 13</td>
<td>70.8 ± 14</td>
</tr>
</tbody>
</table>

In each experiment growth reduction at pH 6 was significantly different from that at pH 8 by analysis of variance (P = 0.01).

References


moisture, aeration, type and quantities of fertilizers and soil amendments which influence soil pH.

Our data show that contrary to the original assumption (Emeroy, 1980; Kloepper et al., 1980) FS-mediated Fe deprivation may not be a consistent mechanism of disease suppression. Since production and biological activity (Fe competition) of FS are influenced by pH value which varies from soil to soil, FS-mediated Fe competition in the field may be limited to a few cases where pH and probably other factors are conducive not only to production of siderophores but also to their activity. Disease suppression or plant growth promotion mediated by fluorescent pseudomonads may also be due to antibiosis (Howell and Stipanovic, 1980), to competition for essential elements by microbially-produced non-fluorescent siderophores, and possibly to other unknown mechanisms.

The purity of the siderophores extracted by our simple procedure was as high as that obtained by Misaghi et al. (1982) through acetone precipitation and Sephadex chromatography separation, as judged by their absorption characteristics and fungistatic activity. The agar medium seems to serve as a chromatographic sieve which separates interfering substances with high molecular weights from siderophores which have low molecular weights and move quite readily in the agar.