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# Notes on the Behaviour of Fluorescent Pseudomonads in Rhizosphere Studies*

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**Abstract**

Attention is drawn to a common fallacy that fluorescent pseudomonads constitute a major group in the bacterial flora of the soil and rhizosphere. It is argued that the large discrepancies occurring in rhizosphere studies result largely from the incorrect application of isolation media, incubation times and temperatures. As the abundance of fluorescent pseudomonads in the rhizosphere also decreases rapidly during plant development, the relevance of intensive research into this group of organisms for biological control must be questioned.

## Introduction

The influence of plant exudates on the indigenous microflora in the close proximity of root or root system has frequently been studied for short periods of time, usually during the early stages of plant development. This has enabled the enforcement of rigid environmental conditions in the laboratory not attainable under field conditions. Similarly, relationships involving organisms which stimulate plant growth or antagonistic activity in relation to plant disease have often only been examined during the early stages of a plant's life cycle. Field experiments are then usually reserved for rhizosphere studies during the whole plant growth period. This has the disadvantage that environmental conditions are not under control and intricate relationships are not easily studied or understood. Frequently the step from laboratory experimentation to field experimentation has resulted in gross disappointment.

Fluorescent pseudomonads are believed to be most typical of the rhizosphere (Curl and Truelove, 1986), and largely for this reason have become a hub of soil research.

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Bacterial populations in the rhizosphere, however, are known to vary with plant age (Rovira, 1965; Martin, 1971) and their composition can be greatly affected by the qualitative and quantitative changes of root exudation patterns (Matsumoto et al., 1979). Influences on pseudomonad abundance within the rhizosphere, therefore, should be investigated during longer periods of the plant's life cycle and under well defined and controlled environmental conditions.

The intensity with which the fluorescent pseudomonads is being studied, especially in the area of biological control (Schroth and Hancock, 1982; Geels et al., 1986), warrants a closer examination of fluorescent pseudomonad investigation. Results obtained from recent rhizoplane and rhizosphere studies (Miller et al., 1989, 1990) promoted the author to question its relevance.

**Methods, Results and Discussion**

Miller et al., (1989) reported that fluorescent pseudomonads rarely represented more than 1% of the total bacterial population in the rhizospheres of maize, wheat and grass. This result was derived with 1/10 strength TSB agar (Lawley et al., 1983), a low nutrient medium, for total bacterial counts (colony forming units) and two selective media for the direct counting of the fluorescent pseudomonads: King's B (King et al., 1954) and S1 (Gould et al., 1985). Using the combination of 1/10 TSB agar and S1 medium in experiments with two Canadian spring wheat lines, less than 0.5% fluorescent pseudomonads were found in the rhizosphere (Miller et al., 1990). After an initial increase during the early stages of root development in soil, the numbers of fluorescent pseudomonads declined rapidly. In the rhizoplane fluorescent pseudomonads were found to represent less than 0.1% of the total bacterial population after five weeks and in the endo-rhizosphere they could no longer be detected.

Sperber and Rovira (1959) stated that *Pseudomonas* only constitutes a minor group in the total bacterial flora of the rhizosphere although they did not distinguish between fluorescent and non-fluorescent species. Sands and Rovira (1971) estimated that fluorescent pseudomonads constituted 0.06% and 0.27% of the bacterial populations in soil and wheat rhizospheres respectively. In contrast, Vancura (1980) reported numbers of fluorescent pseudomonads for wheat, maize and barley, without reference to total numbers of bacteria, and stated: "Pseudomonads represent the most numerous group of bacteria in the rhizosphere". Many authors (Vágnerová et al., 1960; Kleeberger et al., 1983; Azad et al., 1985; Iswandi et al., 1987), not always distinguishing between fluorescent and non-fluorescent pseudomonads, have reported high percentages of pseudomonads in their rhizoplane and rhizosphere studies. Lalande et al., (1989) reported 63% pseudomonads in the rhizosphere of maize.

Although the S1 medium used by Miller et al. (1989) for counting fluorescent pseudomonads may have been too specific, they found that on average the counts

were four times lower on S1 medium as compared to the widely used King's B medium which is known for its non-selectivity of fluorescent pseudomonads (Sands and Rovira 1970). Gould et al. (1985) claimed a recovery from soil of 82.5% of fluorescent phenotypes using S1 medium. This indicates that the numbers of pseudomonads reported by Miller et al. (1989, 1990) would be at least close to actual population numbers. Compared to the percentages reported by Miller et al. (1989, 1990) and Sands and Rovira (1971), the percentages of pseudomonads reported by Vágnerová et al. (1960), Kleeberger et al. (1983), Azad et al. (1985), Iswandi et al. (1987) and Lalande et al. (1989) are 10–200 times higher.

The isolation of bacteria for total bacterial numbers and further identification was accomplished by the latter authors who all used media with relatively high nutrient values. This will favour the growth of bacterial groups such as the Pseudomonadaceae and Enterobacteriaceae which grow readily when high levels of nutrients are available as compared to the needs of other groups e.g. corynebacteria (Hattori, 1980, 1986). The influence of the nutrient status of different media on phenotypical divergences between populations of soil bacteria has recently been discussed (Sørheim et al. (1989). The present author (unpublished results) found that of nine different media tested, 1/10 strength TSB agar provided the highest total bacterial counts. Although no medium is ideal (Lambert et al., 1987), initial isolation media must be of the type that will give the most representative image of the bacterial composition of the soil being studied.

Two more important factors which must be considered when soil bacteria are being investigated, are incubation temperature and incubation time. In a recent study (unpublished results) a temperature of not more than 20°C appeared to give the most reliable results. Most Gram negative soil bacteria, especially groups allied to the Pseudomonadaceae have optimum temperatures between 25°C–30°C but will grow well at 20°C whereas many Gram positive bacteria, e.g. corynebacteria, have optimums well under 20°C but will still grow at 20°C. These bacteria generally have lower temperature maximums for growth than the former groups.

Incubation times, if reported at all, vary considerably and are frequently poorly defined. However, in the above study, the incubation time was also investigated: most bacterial colonies became visible between 8–14 days. After 14 days the number of visible colonies did not increase significantly and some of the colonies already present became more difficult to distinguish. Sufficient time is, therefore, necessary to allow the slower growing organisms to develop and be counted on a medium (Hattori, 1986). These factors, e.g. high level of nutrients in the agar, high incubation temperature, e.g. 27°C, and short incubation times (2 days), and especially combinations of them, will lead the scientist to believe that his soil contains a high percentage of fluorescent pseudomonads.

Therefore, the conclusions reached from these observations are: Fluorescent

pseudomonads are less abundant in the soil and plant rhizosphere than is generally accepted. Members of this group of organisms, which may be present in the rhizosphere in larger numbers only during initial root development due to a richer nutrient supply, develop poorly in the later stages of plant growth (Miller et al., 1990). As these stages constitute the longest part of the plant's growth period including flowering, an active role for fluorescent pseudomonads, e.g. biological control, will be greatly restricted. It would be advisable, therefore, to examine the rhizosphere microflora for those bacteria which are stimulated by the plant over a longer growth period.

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