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On the Distribution of Soil and Rhizosphere Actinomycetes of a Poplar Plantation*

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Abstract

Four out of nine genera of actinomycetes genera were found to be intimately associated with the poplar rhizosphere. The importance of the actinomycetes in the rhizosphere is emphasized.

Introduction

There is a growing interest in the possibility of using microorganisms in the rhizosphere in favour of plant growth and productivity. The search for such microorganisms has been focused on rhizosphere bacteria and mycorrhizal fungi; actinomycetes being the most neglected component of the rhizosphere populations. Some studies on the rhizosphere actinomycetes have revealed that there is a positive rhizosphere effect (high R:S ratio) and that they have an antagonistic effect on fungal root pathogens (cited in Williams et al., 1983). Our knowledge on the natural flora of actinomycetes in the rhizosphere is, however, very poor; this is especially true for tree species. This led us to study the rhizosphere actinomycetes of poplar as a representative of woody plants. In this study, a comparison was made between actinomycetes of the rhizospheres of 2 poplar clones and fallow soil from the same field. Isolation was performed on several different culture media, in order to obtain as many different types as possible.

Materials and Methods

Culture media

Four types of media were employed for isolation: Soil Extract Agar (Dahm, 1984);

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Nitrogen Free Glucose Medium (Krieg and Holt, 1984); Actinomycete Isolation Agar (Difco) and Root Extract Agar (prepared by replacing root exudate of medium of Kipe-Nolt et al. (1985) by root extract). Nutrient agar (NA) (Difco) was used for purification and storage of isolates.

Isolation procedure

Roots of 2 poplar clones (*Populus* sp.), "Ghoy" and "Beaupré" from the experimental plantation in Geraadsbergen, Belgium, were exposed at the depth of 10–15 cm. Small roots (diameter less than 3 mm) were excised and collected with the adhering soil. Root free soil was taken from the same field at the same depth. Large soil clumps were removed from roots, cut into ca. 2 cm pieces and mixed. A subsample of 1 g containing both mature and young root pieces and pieces of mycorrhizal roots was shaken in 10 ml of sterile distilled water (SDW) in a petri dish with sterile forceps. The root pieces were then removed to another petri dish. The resulting suspension was considered to represent the ectorhizosphere. Endorhizosphere samples were prepared according to Kleeberger et al. (1983). Those suspensions were serially diluted in SDW. One gram of root free soil was added to 100 ml of SDW in 250 erlenmeyer flasks and agitated vigorously. This suspension was also diluted serially with SDW. The 4 isolation media were inoculated with appropriate dilutions using pour the plate method. The plates were incubated at 25°C and observed daily for 15 days for macroscopic characters of individual colonies. They were also observed under the stereo microscope for more details. One colony of each different type was streaked on NA for purification. The isolates were kindly identified by Dr. A. MacCarthy, University of Liverpool, England.

Results and Discussion

Our collection of actinomycetes contains 40 isolates belonging to 9 different genera. Their distribution in soil and rhizosphere of poplar is illustrated in Fig. 1.

Out of 9 genera of actinomycetes, only 3, namely, *Streptomyces* spp., Unidentified 1 and Unidentified 4, were present in root zone as well as root free soil. *Micromonospora* sp. and Unidentified 3 were found only in soil whereas the remaining 4 types, *Nocardioform* sp., *Sporangia* sp. and 2 unidentified genera, were present only in root association (Fig. 1). There was no difference in actinomycete population between 2 clones. Interestingly, the root specific actinomycetes were confined to the endorhizosphere. De Lavel and Remacle (1969) did not study endo- and ectorhizosphere separately, however, they observed that the poplar rhizosphere was very selective to actinomycetes. Our results indicate that poplar roots not only select actinomycetes but also enter into very intimate association with those selected.

Actinomycetes generally do not receive much attention in rhizosphere studies,

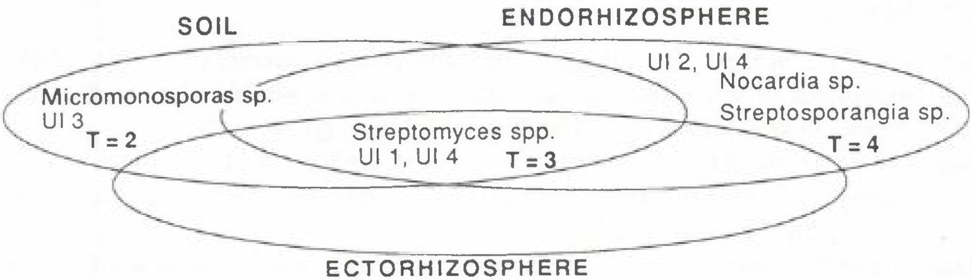

Figure 1. Distribution of actinomycetes in soil and rhizosphere of poplar (UI = unidentified, T = total).

probably because they are isolated less frequently than bacteria and also because of their slow growth. *In vitro* production of antibiotics is the most familiar property of actinomycetes. This may be operating *in vivo* too, helping them to be established in the highly competitive rhizosphere environment despite their slow growth and to exhibit antagonism against bacteria and fungi (see reviews by Weller, 1988 and Fravel, 1988). Strezelczek and Szpotanski (1989) discussed the possible importance of the production of cellulolytic and pectolytic enzymes by streptomycetes in the establishment of ectomycorrhizae. Such enzyme activities may also help the producer strains themselves to enter into the inner rhizosphere where there is a priority access to the root exudates, thus avoiding the intense competition at the outer rhizosphere. This would allow good colonization in the rhizosphere despite the slow growth. As mentioned by Weller (1988) an organism having cellulolytic and pectolytic activities has an advantage to be more competitive in colonization of root cap, thus to be carried by elongating root providing the inoculum for root colonization. Production by actinomycetes associated with pine mycorrhizae of auxins and gibberellin-like substances (Strezelczek and Pokojska-Burdzeij, 1987), which can affect the plant growth, and sugars (Rozycki, 1987), which may play a role in nutrition of other rhizosphere microbes including mycorrhizal fungi, has also been reported. They are capable of producing free amino acids and organic acids too (cited in Rozycki, 1987).

Therefore, actinomycetes also possess several characteristics beneficial to plant growth. These characteristics, together with an intimate association with plant roots might prove actinomycetes species to be good candidates for the use in the enhancement of plant growth. Another possible major advantage of using actinomycetes would be the production of spores which may persist in soil for long periods in the absence of roots providing inoculum for newly entering roots. Therefore, in our opinion, the search for plant growth promoting microorganisms should be extended to cover actinomycetes rather than be limited to bacteria and mycorrhizal fungi.

REFERENCES

Atkinson, T.G., Neal, J.L. Jr., and Larson, R.I. 1975. Genetic control of the rhizosphere microflora of wheat. In Biology and control of soil-borne plant pathogens. Bruehl, G.W. (ed.) St. Paul, *Am. Phytopathol. Soc.* pp 116–122.

Azad, H.R., Davis, J.R., Schnathorst, W.C., and Kado, C.I. 1985. Relationships between rhizoplane and rhizosphere bacteria and *Verticillium* wilt resistance in potato. Arch. Microbiol. **140:** 347–351.

Dahm, H. 1984. Generic composition and cultural properties of heterotrophic bacteria isolated from soil, rhizosphere and mycorrhizosphere of pine (*Pinus sylvestris* L.). *Acta Microbiologica Polonica.* **33**(2): 147–156.

DeLavel, J. and Remacle, J. 1969. A microbiological study of the rhizosphere of poplar. *Plant and Soil.* **31:** 31–42.

Fravel, R.D. 1988. Role of antibiosis in the biocontrol of plant diseases. *Ann. Rev. Phytopathol.* **26:** 75–91.

Kipe-Nolt, J.A., Avalokki, U.K., and Dart, P.J. 1985. Root exudation of Sorghum and utilization of exudates by nitrogen-fixing bacteria. *Soil Biol. Biochem.* **17(6):** 859–863.

Kleeberger, A., Castroph, H., and Klingmüller, W. 1983. The rhizosphere microflora of wheat and barley with special reference to gram negative bacteria. *Arch. Microbiol.* **136:** 306–311.

Kreig, N.R. and Holt, J.G. (eds). 1984. Bergey's Manual of Systematic Bacteriology. vol. 1. Williams and Wilkins, Baltimore. pp. 322.

Rozycki, H. 1987. Sugars production by bacteria and actinomycetes from soil, rhizosphere and mycorrhizosphere of pine (Pinus sylvestris L.). *Acta Microbiologica Polonica.* **36**(1/2): 93–99.

Strzelczk, E. and Szpotanski, T. 1989. Cellulolytic and pectolytic activity of streptomycetes isolated from root free soil, rhizosphere and mycorrhizosphere of pine (*Pinus sylvestris* L.). *Biol. Fetil. soils.* **7:** 365–369.

Strzelczk, E. and Pokojska-Burdzeij, A. 1987. Production of auxins and gibberellin-like substances by actinomycetes isolated from soil and mycorrhizosphere of pine (*Pinus sylvestris* L.). *Plant Soil.* **81:** 185–194.

Weller, D.M. 1988. Biological control of soilborne plant pathogens in the rhizosphere with bacteria. *Ann. Rev. Phytopathol.* **26:** 379–407.

Williams, S.T., Lanning, S., and Wellington, E.M.H. 1983. Ecology of actinomycetes. In The biology of the actinomycetes. M. Goodfellow, M. Mordarski, and S.T. Williams. (eds). Academic Press, London. pp. 481–537.