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# RHIZOSPHERE CYANOPHYCEAE OF SOYBEAN INOCULATED WITH RHIZOBIUM JAPONICUM

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*R I N G K A S A N*

*Penelitian terhadap species Cyanophyceae jang terdapat didaerah rhizosphere dan nonrhizosphere akar kedele, telah dilakukan dalam kondisi rumahkatja dengan menggunakan pot. Sebelas species Cyanophyceae telah didapatkan. Kehadiran Rh.japonicum ternjata tidak mempengaruhi diversitas species ganggang. Hanja empat species dalam djumlah jang sangat sedikit, didapatkan pada pot tanpa kedele.*

*Perhatian sangat ditudjukan karena kurangnja informasi mengenai ganggang didaerah rhizosphere, serta kepentingannja didalam populasi jang heterotropis.*

*A B S T R A C T*

*A study has been made of the species of Cyanophyceae occuring in the rhizosphere and nonrhizosphere regions of soybeans in pot culture under glasshouse conditions. Eleven species of Cyanophyceae were detected. The algal species were not influenced in diversity by the presence of Rh.japonicum. Very few cells of only four species occurred in unplanted pots.*

*Attention is drawn to the dearth of information available on rhizosphere algae and to their likely importance for the heterotrophic populations.*

## I. INTRODUCTION

The root systems of higher plants are associated not only with an inanimate environment composed of organic and inorganic substances, but also with a population of metabolically active microorganisms that are distinctly different from the characteristic soil population

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(Katznelson 1946, Katznelson et al. 1948, Starkey 1958, Timonin & Lochhead 1948). This unique environment under the influence of plant roots is called the rhizosphere (Hiltner cited by Katznelson 1948). Not only do the plant roots affect microbial development, but also the plant is affected by the increased activity of the microorganisms in the rhizosphere regions. The details of the causes and effects are still obscure (Cullimore & Woodbine 1963, Patel & Brown 1969, Paterson & Rovatt 1967, Rovira 1969, Starkey 1958).

Most rhizosphere microorganisms are saprophytes, but not all of their relations to plants are incidental, some live on the root surface whereas others penetrate the roots (Dart & Mercer 1964, Gonzalves & Yalavigi 1959, Katznelson et al. 1948).
The facts that the soil microbial population on plant roots is dense and occurs at all stages of plant growth, have been verified repeatedly (Harris 1953, Ivarson & Katznelson 1960, Leval & Remacle 1969).

Inoculation of leguminous plants with Rhizobium may stimulate their growth and cause greatly increased crop yields. But the reciprocal effects of nodulation on the rhizosphere flora have not been studied. Patel (1969) reports that inoculation of the rooting medium of non-leguminous plants species with Azotobacter stimulates rhizosphere organisms but algal species were not studied. Soil algae have been grossly neglected, particularly in rhizosphere studies, and in view of their potential contribution to soil productivity, they deserve close attention. Soil algae are prominent in Indonesia and include many species of nitrogen-fixing blue-green algal forms. Their importance in the nitrogen economy of the paddy fields has been shown by Suriawiria (1969, 1970 unpublished).

The present study was, therefore, undertaken to determine and to see whether inoculation with rhizobia affected this rhizosphere Cyanophyceae of soybean. Circumstances permitted that this study be made under glasshouse conditions in the School of Botany, University of New South Wales, Sydney, Australia, 1970.

## II. MATERIALS AND METHODS

Open pots containing 1,250 - 1,500 g of the U.C.Soil mix. C which includes no available nitrogen (Baker 1957) were steam sterilized and set out in a glasshouse with a day temperature of 18 - 26°C. and night temperature of 13 - 18°C. The soil mixture consisted of 50% fine sand and 50% peat moss with 8 oz potassium sulphate, 2½ lb single superphosphate, 7½ lb dolomite lime and 2½ lb calcium carbonate lime per cubic yard.

Lee and Semstar varieties of soybean were chosen and clean, undamaged seeds of a reasonably uniform size were selected by hand sorting followed by weighing. They were sterilized by the method of Vincent (1970): rinsed with 95% ethanol for 30 seconds and then immersed for 3 minutes in 0.2% mercuric chloride. The seeds were then washed thoroughly with at least five changes of sterile water.

Three inoculation treatments with commercial Rh.japonicum inoculant ("Nitrogerm" from Root Nodule Pty. Ltd., Sydney) were used:
(1) directly onto the seed surface followed by immediate planting.
(2) directly into the soil when the seedlings had emerged above the surface soil,
(3) directly onto the seedling roots before planting. These seeds

were germinated on moist sterile paper in petri dishes.

A control series of unplanted pots was also included and all pots were watered every day.

Samples of soil from each treatment were taken at the following stages of growth:

(1) when the first leaves opened,
(2) at intervals of two weeks from the date when the first leaves opened,
(3) at the flowering stage.

The soils were sampled as follows (Gonzalves & Yalavigi 1959 and Patel 1969):

(1) from the rhizosphere regions: the roots from soybeans were taken out carefully with a sterilized spatula, and were cut off with sterilized scissors. The extra soil particles attached to them were dislodged by gentle shaking, so that the roots held only the soil particles closely adhering to them. The roots from plants between 2-6 weeks were put into sterilized 250 ml conical flasks containing 100 ml of sterile distilled water. The roots from older plants up to the flowering stage were placed into sterilized 500 ml conical flasks containing 200 ml of sterile distilled water.

The flasks were shaken vigorously to obtain a uniform suspension of the rhizosphere samples. After the root pieces were taken out 50 ml of the soil suspension were transferred to a flask containing sterile liquid medium.

(2) from the nonrhizosphere region: the soil samples were taken a few cm away from roots and 10 g were added to 100 ml of sterile distilled water. 50 ml of this solution were introduced to the flasks containing sterile liquid medium. The unplanted pots also were sampled in this way.

The liquid medium used by Wieringa (1968) for the bacteria-free culture of Cyanophyceae was employed: 5 g $K_2HPO_4$, 5 g $MgSO_4.7H_2O$, 0.5 g $Na_2CO_3$, 10 ml Fe-EDTA containing 50 mg Fe, 10 ml microelements, and 1 l distilled water.

All cultures were set out in a glasshouse, and examined at approximately weekly intervals. The first examination was made when algal growth appeared. Only a qualitative record was kept of the species of Cyanophyceae which occured in the cultures.

## III. RESULTS AND DISCUSSION

Rhizobium inoculation markedly improved the growth of the soybeans. However, after approximately 6 weeks, many of the uninoculated plants developed nodules which were small, especially since these plants remained small and yellow.

The species of Cyanophyceae isolated from the washed root segments of inoculated and uninoculated plants were generally similar at all atages of plant growth. It appears that inoculation does not influence the rhizosphere algal flora (Table 1).

Eleven species of Cyanophyceae were isolated from these experiments. These belong to the genera Anabaena(1 species), Chroococcus (2 species), Lyngbya (2 species), Microcystis (1 species),Nostoc (3 species), Oscillatoria (1 species) and Phormidium (1 species) (Table 1).

The four species, Anabaena naviculoides, Chroococcus minutus, Microcystis pulverea and Nostoc ellipsosporum, were predominant in all of the samples where soybean was growing. Of these, Chroococcus minutus, Microcystis pulverea and Nostoc ellipsosporum, were also found in small numbers in the unplanted pots, as well as a few cells of Chroococcus turgidus.

The number of species was greater in the rhizosphere region than in the soil a few cm away from the roots until the plants were 4 weeks old. No difference was detected after this time, since the pots were filled with roots and watering would wash the cells or their spores throughout the soil.

It was noted that greater numbers of all species of Cyanophyceae occured in the rhizosphere and this suggests that the rhizosphere provides a favourable environment for these soil algae. As the plants developed larger root systems, the soil throughout the pot became a more favourable medium for algal growth. Exudates from roots would be penetrating throughout the whole pot (Katznelson 1946, Katznelson et al. 1948, Peterson 1932, Rovira 1965, 1969).

From studies to date, it appears that the majority of organisms present in the rhizosphere regions are those which are capable of decomposing fresh organic matter or utilizing inorganic and simple organic nutrients directly - the bacteria, fungi and actinomycetes (Katznelson et al. 1948, Leval & Remacle 1969).
Virtually nothing is known of algal rhizosphere populations, but it is likely that, being autotrophs, their physiological functions may contribute significantly to the support of the vast heterotrophic populations.

This study has revealed the variety of Cyanophyceae species that occurs in a typical glasshouse in the Sydney district and that can develop where higher plants are growing. The medium of the soybean and algal cultures contain available nitrogen at low concentration that the algae must either be oligonitrophiles, nitrogen-fixing forms or associated with nitrogen-fixing bacteria.

The presence of species of Cyanophyceae well below the soil surface is probably due to the washing down of algal forms from the surface layers (Peterson 1935). The light requirements of Cyanophyceae do not confine them to upper layers of the soil and the importance of Cyanophyceae in rhizosphere populations, even at considerable depths has been overlooked (Cullimore & Woodbine 1963).

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Table 1.: Cyanophyceae species occuring in the rhizosphere and nonrhizosphere regions of soybeans cultures inoculated with *Rhizobium japonicum*, of non-inoculated controls and in unplanted soil.

| Algal species | Soybeans cultures | | | | Unplanted soil |
|---|---|---|---|---|---|
| | Inoculated | | Un-inoculated | | |
| | A | B | A | B | |
| *Anabaena naviculoides* Fritsch. | +++ | ++ | ++ | ++ | - |
| *Chroococcus turgidus* (Kutz.) Naeg. | + | + | + | ++ | + |
| *Ch. Minutus* (Kutz.) Naeg. | +++ | ++ | ++ | ++ | + |
| *Lyngbya limnetica* Lemm. | + | + | + | + | - |
| *L. ceylanica* Wille. | + | + | + | + | - |
| *Microcystis pulverea* (Wood) Fort. | +++ | +++ | ++ | +++ | + |
| *Nostoc verrucosum* Vauch. ex Born. et Flah. | + | + | + | + | - |
| *N.spongiaeforme* Agardh. ex Born. et Flah. | + | + | + | + | - |
| *N.ellipsosporum* (Desm.) Rabenh. ex Born. et Flah. | ++ | +++ | +++ | +++ | + |
| *Oscillatoria princeps* Vauch. ex Gomont. | + | + | ++ | + | - |
| *Phormidium fragile* (Menegh.) Gomont. | + | + | + | + | - |

Note: A : rhizosphere region, B : soil region a few cm away from the roots.
- : none, + : rarely, ++ : many, +++ : abundant.

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