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# Resistance of various strains of azotobacter chroococcum to antibiotics

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**Abstract:** The subject of the study was resistance of 12 strains of *Azotobacter chroococcum* to varying concentrations of 4 antibiotics (streptomycin, neomycin, tetracycline and ampicillin). The largest zones of inhibition were achieved with the highest concentrations of ampicillin. Streptomycin, neomycin and ampicillin were shown to be the best for the identification of strains, whereas the sensitivity of different strains to tetracycline proved to be very similar.

**Key words:** antibiotic, *Azotobacter chroococcum,* resistance, strain.

## Introduction

The nonsymbiotic nitrogen fixer *Azotobacter* is an obligate aerobe living in the soil, the rhizosphere and the plant root. In a chernozem soil, it is the rhizosphere that provides *Azotobacter* with the best living conditions (Sari 1968). The ability of various strains to fix atmospheric N positively affects plant growth and yields (Jagnow 1987. Once they are introduced into the rhizosphere, active strains of nitrogen fixers increase $N_2$-fixing activity and the productivity of rye by as much as 12 to 18% (Maltseva 1995).

Soil is the habitat of many microorganisms, including those that produce antibiotics which have an inhibitory effect on other microorganisms. Antibiotic properties of *Azotobacter* strains can, therefore, serve as an important criterion in the selection of this nitrogen fixer for the preparation of microbiological fertilizer for sugar beet.

The objective of this study was to investigate antibiotic properties of *Azotobacter chroococcum* strains isolated from the rhizosphere of sugar beet.

## Materials and methods

In this study, 12 strains of *Azotobacter chroococcum* (1, 2, 3, 5, 7, 8, 9, 11, 12, 13, 15 and 16) isolated from the rhizosphere soil of sugar beet were subjected to investigation. The antibiotics streptomycin, neomycin, tetracycline and ampicillin were used in 5 different concentrations (0.2, 2, 20, 200 and 400 µg/ml). The antibiotic solutions were prepared in sterile $H_2O$ just before the use.

The susceptibility determination was conducted using the standard agar diffusion test. The procedure involves applying the antibiotic to the disc, whereupon it diffuses through the agar bringing or not bringing about inhibition of bacterial growth and the formation of zones of inhibition (Piddock 1990).

After having been previously grown at 3°C in the liquid medium of Fjodor placed in a shaker incubator for 48 hours, a liquid culture of the studied *Azotobacter chroococcum* strain was added to a melted solid medium of Fjodor and poured into Petri dishes. This medium was then cooled and paper discs placed onto it. No antibiotic was added to the central disc, whereas 0.1 ml of certain concentration of antibiotic was applied to all the others. Finally, the Petri dishes were incubated at 3°C in a thermostat for 48 hours, after which zones of inhibition were measured in mm, in relation to the edge of the disc.

## Results

The sensitivity of the strains varied according to the antibiotic used and its concentration. The results of the study are shown in the graph 1.

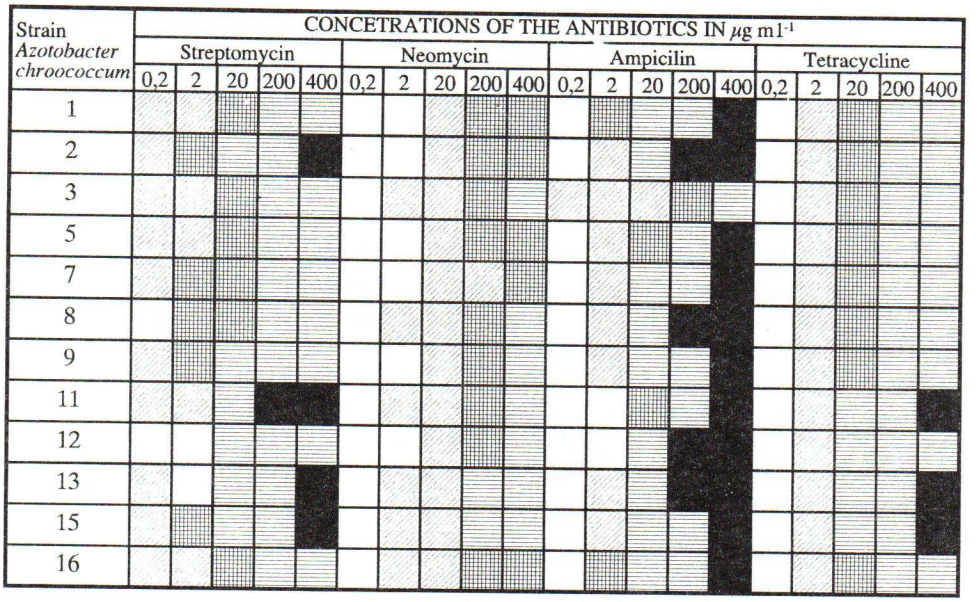
*Sensitivity to streptomycin*

According to Bergey (1984), strains from genus *Azotobacter* are sensitive to streptomycin concentrations of 0.2 µg/ml, and it is this concentration that all the strains, with the exception of strains 8 and 12, were also shown to be sensitive to. In terms of the degree of sensitivity, i.e. growth inhibition zones, strains 1, 3, 5, 12 and 16 proved to be less sensitive to the concentration of 2.0 µg/ml than the other strains. The greatest sensitivity to 400 µg/ml was exhibited by strains 2, 11, 13 and 15 (20-36 mm).

*Sensitivity to neomycin*

All the strains were shown to be resistant to 0.2 µg/ml. Strains 3, 8, 11, 13, 15 and 16 proved sensitive to the next concentration (2.0 µg/ml), with the inhibition zone of 1-2 mm. The highest concentration of this antibiotic produced smaller growth inhibition zones than that of streptomycin (8-16 mm).

The effect of the antibiotics on the *Azotobacter chroococcum* strains




*Sensitivity to ampicillin*

This antibiotic was the one that produced the largest growth inhibition in the strains. The concentration of 0.2 μg/ml managed to inhibit growth only in strain 3, whereas 2.0 μg/ml did so in all the strains except 11 and 12. The greatest growth inhibition at the concentration of 200 μg/ml was in strains 2, 8, 12 and 13 (25-28 mm) and at 400 μg/ml in all the strains except strain 3.

*Sensitivity to tetracycline*

All the strains except strains 11, 13 and 15 exhibited great similarity in their sensitivity to this antibiotic, showing no sensitivity to 0.2 μg/ml and only slight inhibition of growth at 2.0 μg/ml. At 400 μg/ml, it was in strains 11, 13 and 15 that the largest inhibition zones were observed (21-25 mm).

## Discussion

In this study, the largest growth inhibition zones were produced by the highest concentrations of ampicillin (200 and 400 μg/ml), which contrasts a previous study on the sensitivity of the symbiotic diazotroph *B. japonicum* to this antibiotic (Mrkovaki 1990).

Studies on antibiotic properties of *Azotobacter chroococcum* strains similar to this one have shown the bacteria's sensitivity to streptomycin, hystrecin, plivacillin, crystalcillin and geomycine (Prša 1964).

Results by Fedorov and Szegia (1961) show that small amounts of streptomycin, clortetracyclin, oxitetracyclin and viomycin have a bacteriostatic effect on *Azotobacter chroococcum*. This is explained by the authors as a result of the fact that large doses bring cell respiration to a halt, i.e. deactivate enzymatic respiration systems. Such doses of antibiotics in the cytoplasm disrupt the structure of the lipid-protein complex and RNA.

The strains investigated in this study exhibited varying degrees of sensitivity to streptomycin and neomycin, which are specific inhibitors of protein synthesis, as well as to ampicillin, which inhibits synthesis of the cell wall. Sensitivity to varying concentrations of tetracycline was similar in all twelve strains. Since resistance to this antibiotic is linked to a change in the cell's permeability to tetracycline, it can be concluded that the studied strains have cell walls which are permeable to a similar degree and that they also have similar ways of blocking the functioning of ribosomes to bring about inhibition of growth.

In conclusion, it can be said that streptomycin, neomycin and ampicillin are antibiotics suitable for identification of *Azotobacter chroococcum* strains.

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# REZISTENTNOST SOJEVA *Azotobacter chroomococcum* PREMA ANTIBIOTICIMA

- Originalni naučni rad -

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## Rezime

Cilj ovog rada bio je da se ispitaju antibiotske osobine sojeva *Azotobacter chroomococcum* izolovanih iz rizosfere šeerne repe. Antibiotske karakteristike sojeva pored kriterijuma u klasifikaciji sojeva bi mogle posluiti i u selekciji sojeva *Azotobacter chroomococcum* za pripremu mikrobiološkog djubriva za šeernu repu.

U ovom radu ispitana je osetljivost 12 sojeva *Azotobacter chroococcum* (1,2,3,4,5,7,8,9,11,12,13,15, i 16) izolovanih iz rizosfernog zemljišta šeerne repe. Korišeni su antibiotici streptomycin, neomycin, tatracycline i ampicillin sa pet razliitih koncentracija (0.2, 2, 20, 200 i 400 µg/ml). Rastvori antibiotika su pripremljeni neposredno pre upotrebe u sterilnoj $H_2O$. Osetljivost sojeva ispitana je standarnom metodom agar difuzionog testa.

Posle 48 asova inkubacije Petri kutija u termostatu na 30°C, merene su zone inhibicije u mm od ivice diska.

Dobijeni rezultati pokazuju da je osetljivost sojeva zavisila kako od vrste antibiotika, tako i od njihove koncentracije.

Za identifikaciju sojeva *Azotobacter chroococcum* najpogodniji su antibiotici streptomycin, neomycin i ampicillin.