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SPECIFICITY IN THE RELATIONSHIP BETWEEN CELLULOLYTIC BACTERIA AND AZOTOBACTER

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Introduction

The highly diverse soil microbial community includes, inter alia, azotobacter and cellulolytic bacteria which, like other microorganisms, synthesize a wide spectrum of substances and release them into the environment, making them a vehicle for establishing specific interrelationships.

Relationships between azotobacter and cellulolytic bacteria have been studied by Jensen (1940, 1941) and Fedorov (1940), who defined them as being metabiotic, whereas Štucer (1945), Voznjakovskaja (1954) and Rubenčik (1960) determined the existence of symbiotic relationships between these bacteria, as later confirmed by the research results of numerous authors (Holsall and Gibson, 1985, 1986$_a$; Holsall and Goodchild, 1986$_b$; Chunzhi et al., 2002; Haiying; Xiuhong, 2012; Ahmad et al.,2013;).

The objective of this study was to conduct further research into the relationship between cellulolytic bacteria and azotobacter.

Material and methods

Two methods were used in this study. In the disk method, two layers of Fedorov's medium containing 1.5% and 0.8% agar, respectively, were poured into Petri dishes. The upper layer of the medium was inoculated with a thick suspension of azotobacter spread over the surface of the Petri dish. Agar disks impregnated with cellulolytic bacterial cultures were placed on

the surface of the inoculated medium. Thereafter, the Petri dishes were incubated for 48 hours at a temperature of 27^0C.

The effect of cellulolytic bacteria on azotobacter was estimated based on heavier growth or absence of growth in the zone around the disk, and the intensity of the effect of some cellulolytic bacterial cultures was determined by the size of the zone around the disk.

The second method involved placement of Fedorov's medium inoculated with an azotobacter suspension on the surface of 15-day-old cultures of cellulolytic bacteria. Upon incubation for 48 hours at 27^0C, the growth of azotobacter was monitored.

Results and discussion

The soil hosts many cellulolytic bacteria which are involved in the decomposition of harvest residues containing more than 50% of cellulose. In addition, the azotobacter present in the soil plays an important role in maintaining its nitrogen regime. Azotobacter utilizes cellulose decomposition products, while cellulolytic bacteria uptake azotobacter-producing nitrogen compounds. Therefore, importance was given to this research on the relationship between these highly beneficial microorganisms (Tabs. 1, 2).

Tab. 1. Effect of some cellulolytic bacteria on *Azotobacter chroococcum*

Cellulolytic bacterial cultures	Strain designation	Azotobacter stimulators	Azotobacter inhibitors
Sporocytophaga myxococcoides	1	+	-
’’	4	+	-
’’	17	+	-
’’	23	+	-
’’	115	+	-
’’	118	-	+
’’	120	+	-
’’	122	-	+
’’	128	-	+

Sporocyophaga ellipsospora	21	-	+
’’	127	-	+
’’	716	-	+
’’	771	+	-
’’	780	-	*
Cytophaga sp.	176	+	-
’’	176*a*	+	-
Cellfalcicula sp.	101	-	+
Sorangium sp.	796	-	+

Tab. 2. Cellulolytic bacterial cultures as stimulators and inhibitors of *Azotobacter chroococcum*

Cellulolytic bacterial cultures	Total number of strains	Stimulators	Inhibitors
Sporocytophaga myxococcoides	9	6	3
Sporocyophaga ellipsospora	5	1	4
Cytophaga sp.	2	2	-
Cellfalcicula sp.	1	-	1
Sorangium sp.	1	-	1

The tables show that 18 cellulolytic bacterial cultures, including 9 belonging to the species *Sporocytophaga myxococcoides*, 5 to *Sporocyophaga ellipsospora*, 2 to *Cytophaga sp.*, 1 to *Cellfalcicula sp.* and 1 to *Sorangium sp.*, and a culture of *Azotobacter chroococcum* were used in the study. The results revealed that some strains of cellulolytic bacteria stimulated azotobacter growth, as azotobacter utilized cellulose decomposition products for its source of carbon and energy. Similar findings were reported by Voznjakovskaja (1954), Rubenčik (1960), Ocampo et al. (1985), Holsall and Gibson (1985, 1986$_a$, 1986$_b$), Chunzhi et al. (2002), Alyl

et al. (2012), Haiyin and Xiuhong (2012) and Ahmad et al. (2013), who found physiological synergism between cellulolytic bacteria and free-living nitrogen-fixing bacteria.

Moreover, some strains of the tested cellulolytic bacteria were inhibitory to azotobacter growth. Of the 9 tested strains of *Sporocytophaga myxococcoides,* the number of strains (6) stimulating azotobacter growth was twice as high as that of inhibitory strains of this species. Almost all strains of *Sporocyophaga ellipsospora* had an inhibitory effect (4 out of 5 strains) on azotobacter growth. Both strains of *Cytophaga sp.* were found to be stimulators, whereas *Cellfalcicula sp.* and *Sorangium sp.* strains acted as inhibitors.

The intensity of the effect of cellulolytic bacteria on azotobacter varied across strains. The strongest stimulators were strains of *Sporocytophaga myxococcoides* designated by the numbers 4, 17, 23 and 120. The *Cytophaga sp.* strains designated as 176 and 176*a* exhibited a marked stimulatory effect on azotobacter. As for the inhibitory strains, the size of the inhibition zone ranged from 3.3 to 4.1 cm in *Sporocytophaga myxococcoides,* and from 3.7 to 4.2 cm in *Sporocyophaga ellipsospora*.

In most antagonistic cultures, next to the inhibition zone, there was a stimulation zone, with antibiotic substances released by the tested strains stimulating azotobacter growth (Jemcev, Đukić, 2000). The morphology of azotobacter was changed by almost all tested cultures of cellulolytic bacteria. For example, *Azotobacter chroococcum* was of doughy dry consistency and formed wrinkled colonies, but when affected by antagonistic strains or stimulators its consistency became slimy or even drier with colony surface increasingly wrinkling. Microscopic examination showed that azotobacter cells were somewhat smaller or even deformed within the inhibition zone range. In cases when the growth of the test cultures was stimulated, azotobacter cells were found to be larger in control Petri dishes.

Conclusion

The following conclusions can be drawn from this study:

- cellulolytic bacteria include both stimulators and inhibitors of azotobacter growth;
- representatives of *Cytophaga sp.* have a positive effect on azotobacter growth;
- a number of representatives of *Sporocytophaga myxococcoides* species act as stimulators of azotobacter growth, whereas *Sporocyophaga*

ellipsospora is dominated by strains which are inhibitory to azotobacter growth;

- the tested strains of *Cellfalcicula sp.* and *Sorangium sp.* have an adverse effect on azotobacter.

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