Soil Nitrogen Fixing Bacteria of Genus Xanthobacter

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In order to control the activity of free-living diazotrophs in arable soils detailed information are necessary on their physiological and biochemical features.

At present a vast amount of data is available on the genera Azotobacter, Clostridium and Azospirillum in the literature, whereas other nitrogen fixing bacteria, including those which use non-traditional carbon sources, are poorly studied.

Hydrogen oxidizing nitrogen fixing bacteria were first found in a sod-podzolic soil of the USSR and described as Mycobacterium flavum /FEDOROV and KALININSKAYA, 1961/. Later these and related microorganisms were redescribed as a new genus under the name Xanthobacter /WIEGEL et al., 1978/.

Bacteria of the Xanthobacter genus attracted attention as promising objects in biotechnology, particularly in the microbial transformation of valuable chemicals.

Still their activity in natural habitats is insufficiently investigated. Previously we showed that bacteria of the genus Xanthobacter dominate among diazotrophs in the highly salinized takyr-like soil of Kazakhstan and, possibly, greatly contribute to the nitrogen balance of these soils.

This paper presents results of the study of Xanthobacter distribution in soils of the USSR as well as the physiological and biochemical characteristics of strains, isolated from different soils.

The number of methylotrophic nitrogen fixing bacteria was determined in fresh soil samples by means of a method we had elaborated earlier /CHISTIAKOVA and KALININSKAYA, 1982/. Mineral nutrient medium without nitrogen and with methanol was used. Nitrogenase activity was determined by acetylene reduction test.

In most studied soils the number of methylotrophic diazotrophs does not exceed 10^3-10^4 cells per g of soil, except the takyr-like soil, where this value reaches $4.5 \cdot 10^6$ cells per g soil.

Bacteria of the genus Xanthobacter were found in all studied soils /except saline and brown forest soil/. In the takyr-like soil their number was $2.0 \cdot 10^6$ cells per 1 g soil on the average. Fraction of Xanthobacter did not exceed 10% of the total amount of methylotrophic diazotrophs except in the takyr-like soil, where it was as high as 40-70%.

All the bacteria in our collection correspond to the diagnosis of genus Xanthobacter. These are pleomorphic rods of a size of 0.5-1.2, 2.0-4.5 μm non-motile, gram-negative or gram-variable. They contain yellow carotinoid pigment and often form a considerable amount of slime. They grow chemo-autotrophically at the expense of hydrogen oxidation and CO_2 utilization and chemo-organotrophically, utilizing a large number of organic acids, alcohols, amino-acids. Sugar utilization is limited.

Nitrogenase /acetylene reduction/ activity on nutrient media with organic acids /succinic, lactic, malonic and propionic/ was 19.6-32.0 nmole C_2H_4/ml h, with alcohols /ethanol, propanol, butanol/ 10.6-17.1 nmole, with methanol utilization - 14.3 nmole, at hydrogen oxidation 16.7 nmole/ml/h.

Earlier we showed that Xanthobacter strains isolated from highly salinized soils, are salt resistant. This manifests in their ability for growth and nitrogenase activity on media with higher salt content. All tested strains of Xanthobacter /11 isolates/ were able to grow on media, containing 2.5-10% NaCl. They also had nitrogenase activity at 1-4% NaCl. Salt resistance was determined as a property, characteristic of all studied strains, including those which were isolated from non-salinized soils, as well as strains maintained on media without NaCl.

We propose to add salt resistance as a characteristic to the diagnosis of genus Xanthobacter; they show nitrogenase activity on media, containing 1.0-2.5% NaCl. It is promising to use media without nitrogen with methanol and 2% NaCl to isolate bacteria of genus Xanthobacter from natural substrates.

High NaCl concentrations were noted to depress nitrogenase activity to a greater extent than growth. Growth was observed on media containing 0-10% NaCl, whereas acetylene reductase activity on media with no more than 2.0-2.5% NaCl only.

Table 1

Numbers of methylotrophic diazotrophs and Xanthobacter spp. in soils of the USSR

Type of soil	Numbers per g of soil fresh weight	
	Methylotrophic diazotrophs	Xanthobacter spp.
Takyr-like	$4.5\ 10^6$	$2.0\ 10^6$
Dark-chestnut	$4.5\ 10^5$	$9.5\ 10^4$
Southern chernozem	$2.5\ 10^4$	$2.5\ 10^3$
Meadow	$2.5\ 10^4$	$2.5\ 10^3$
Brown forest	$7.5\ 10^4$	0
Solod	$3.5\ 10^4$	0
Saline	$2.5\ 10^3$	$2.0\ 10^3$
Light siercem	$2.0\ 10^4$	$9.5\ 10^2$
Alkali	$9.5\ 10^2$	0
Humic gley	$2.5\ 10^4$	$2.5\ 10^3$

Chemo-autotrophically grown isolates were shown to be more sensitive to NaCl than those under chemo-organotrophic conditions. On hydrogen as the only energy source bacteria grew under no more than 4-6% NaCl and showed nitrogenase activity only at 0.5-1.0% NaCl.

In order to estimate the effect of soil conditions on properties of strains we studied the physiological-biochemical characteristics of bacteria isolated from two different soils contrasting by their properties.

Strain 10 was isolated from a takyr-like soil with low /0.6%/ humus content, alkali reaction /pH 8.2-8.3/, high content of soluble salts /1.5-4.0/. Strain 661 was isolated from a southern chernozem, characterized by high humus content /3.6%/ and neutral medium reaction. Strain 10 appeared to be more salt resistant. There are differences in the ability to form slime and value of temperature interval, where growth and nitrogenase activity were observed, as well as ability to utilise carbon sources /xilose, sucrose, citric acid/.

References

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