Interaction of Humic Acid and Bacterial Exopolysaccharides

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Saccharides account for a substantial proportion up to 20% of the organic compounds in soils. The majority of them is present in polymerised form and the rest is absorbed into inorganic colloids. The polysaccharide macromolecules are polydisperse and heterogeneous /SWINCER, 1968/. For this reason the incorporation of ^{14}C glucose was necessary for distinguishing the possible carbon sources for polysaccharide synthesis. A relatively rapid incorporation of ^{14}C was observed in sugar containing organic soil components /OADES and WAGNER, 1970/. It was also found that humic acids contained polysaccharides in bound form or in the constitutive part of fulvic and hematomelanic acids /CLARK and TAN, 1969; TAN and CLARK, 1969/. The microbial origin of the substantial properties of polysaccharide complexes belonging to humified organic compounds has to be mentioned here /FULLER, 1947; FINCH et al., 1971/.

Exocellular polysaccharides are utilised by soil microorganisms as a carbon source. This is valid particularly for their own polysaccharides which serve as a reserve nutrition source. This phenomenon is an important feature of the ecological function of polysaccharides. On the other hand the utilization may be inhibited by various inorganic and organic components of the soil. This extends the influence of polysaccharides on the soil and modifies its physico-chemical properties /the other ecological role of polysaccharides in the soil/.

Humic acids can be utilised by microorganisms as carbon and nitrogen sources /ANDREYUK and GORDIENKO, 1973/, or can directly influence bacterial enzymes /ASO and TAKAI, 1965; ASO and TAKENAGE, 1968; LADD and BUTLER, 1969a,b, 1971/ to such an extent that the bacterial growth can be either stimulated or inhibited. This was observed in pathogenic strains of E.coli and B.subtilis /CHODAN and SOBIERAJ, 1966/.

In the present study we investigate the interactions of humic acids and bacterial exopolysaccharides and their combined influence on soil microorganisms, including ultrastructural changes as they can be observed after the ultra-cytochemical staining of exocellular polysaccharides.

Materials and methods

The employed strain, Achromobacter delicatulus, which was isolated from a wheat rhizosphere soil, exhibited substantial capacity of secreting exocellular polysaccharides.

Individual Ukraine low peat sample was extracted with 0,1 N NaOH and Na_2PO_7 respectively /KONONOVA, 1961/. The sample was gently shaken for 5 hours, allowed to stand overnight and the supernatant solution was separated from the peat by centrifugalization. The residual peat was similarly reextracted several times with an additional volume of alkali until an almost colorless solution was obtained. The combined supernatant solutions were acidified with HCl to pH 1.5 to precipitate the humic acid fraction which was resolved in alkali again. The resulting humic acid solution was dialysed to remove Cl^- ions and the fractions with a molecular weight, higher than 50,000 daltons, were obtained by means of Sephadex G 50 column /80x900 mm/. IR spectra were measured by means of UNICAM SP-200 registration spectrophotometer using the KBr pellet technique. 1.5-2 mg of dried humic-polysaccharide material was ground, mixed in agar with 120 mg KBr and pressed into a die for making pellets under vacuum. Cultivation medium WH was supplemented with humic acid in concentrations 0.005, 0.05 and 0.5 g/l. The medium was inoculated with Achromobacter delicatulus capable of polysaccharide excretion.

A modified version of ruthenium red staining described by SPRINCER and ROTH /1973/ was employed for exocellular polysaccharides of Arthrobacter. We found that the following order is necessary of pipetting the prescribed solutions to the pelleted bacteria or to highly viscous cultivation fluid containing bacteria: cacodylate buffer, glutaraldehyde, and the ruthenium red solution. A fresh solution of ruthenium red in buffered OsO_4 has to be used for the second step of staining. The cells were embedded in Vestonal W and their ultrathin sections were viewed under the electron microscope JEOL JEM LOOB /80 kV/ without counterstaining.

Results and discussion

Peat humic acids in concentrations of 0.0005 to 0.5 g/l did not inhibit the growth of and the polysaccharide excretion by Achromobacter delicatulus /Fig. 1/.

We observed that within the first four hours of incubation, in the presence of humic acids, the decomposition of humic acid - polysaccharide complexes was inhibited in bacterial cultures /Fig. 2/. Infrared spectroscopy: Broad bands were characteristic of the spectra of polysaccharides excreted by soil bacteria which therefore could not be well distinguished /Fig. 3/. This was valid particularly for the wavelengths between 400-700 nm and 3,100-3,600 cm^{-1} /wavelengths of stretching vibrations of hydroxyl groups included in hydrogen bonding/. Broad bands were distinct also at 3,400 cm^{-1} low ones between 2,800 and 3,000 cm^{-1} /wavelengths of stretching vibrations of hydroxyl group and CH, CH_2 groups/. The studied humic acid exhibited a sharp peak of stretching vibrations of methylene and methyl group at 2,890 cm^{-1} and at 1,720 cm^{-1} an observable absorption of C-O in COOH and at 1,625 cm^{-1} a band stretching vibrations of C=C and C=O, finally at 1,420 cm^{-1} a band of bending vibrations CH in $C\text{-}CH_3$.

It is obvious that the interaction of humic acids with the exocellular polysaccharides of the studied bacteria resulted in complex-formation /Fig. 4/. This was made visible by the spectroscopic demonstration of a polysaccharide component /peaks at 1,650 and 1,400 cm^{-1}/ in the complex molecule. The general character of the spectra resembles that of humic acid. As cytochemical experiments have shown the presence of humic acid accelerated the ageing of the culture and in this way may also have influenced the rate ot polysaccharide synthesis and excretion.

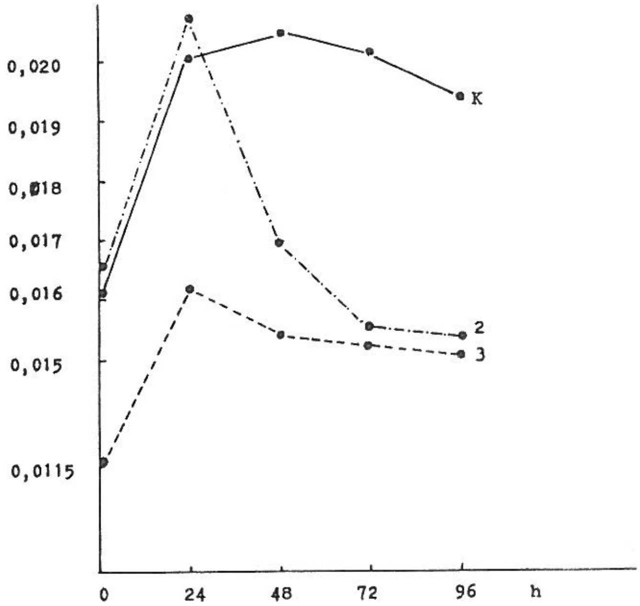

Fig. 1

Influence of humic acid on the excretion of polysaccharides of bacteria Achromobacter delicatulus in pure culture experiment

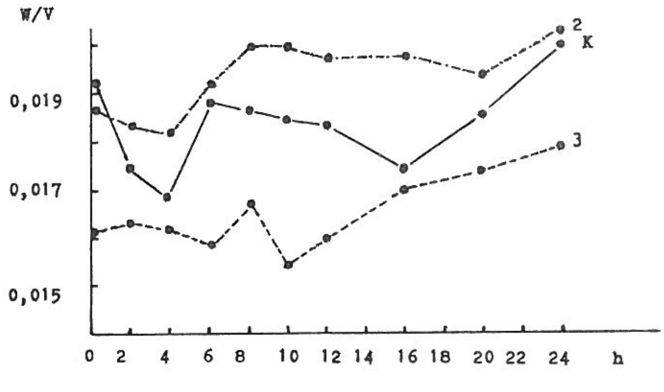

Fig. 2

Influence of various concentrations of humic acid on the decomposition of humic acid polysaccharide complex

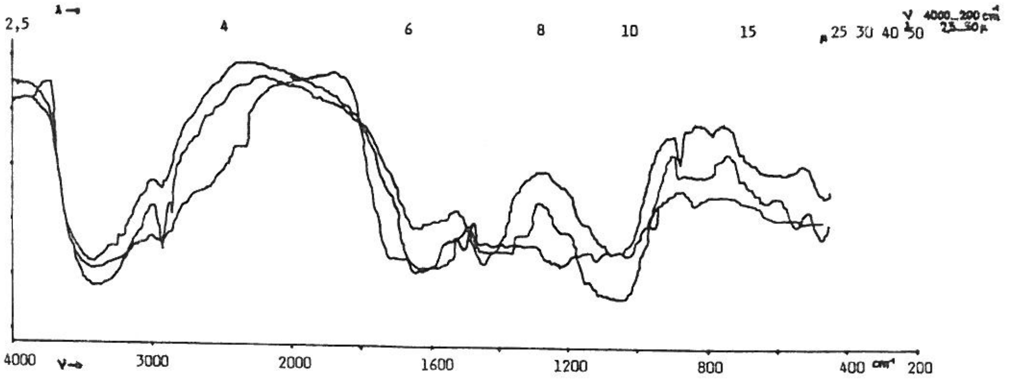

Fig. 3
Infra-red spectra of the humic acid - polysaccharide complex, humic acid and reference polysaccharide of A. delicatulus

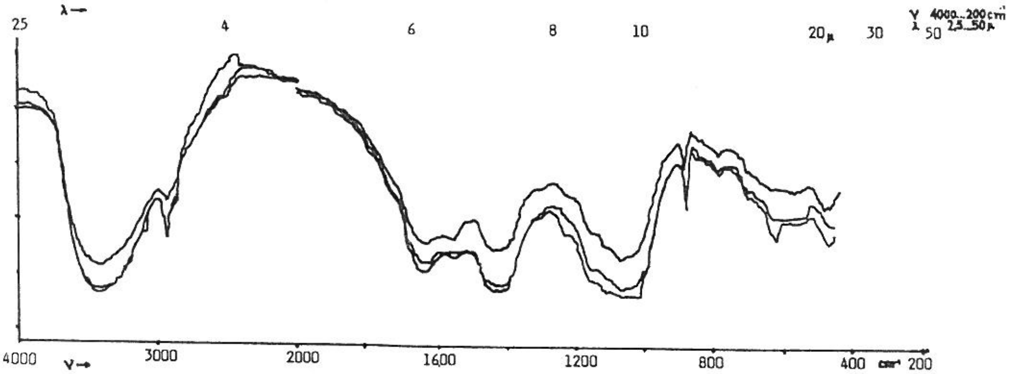

Fig. 4
Infra-red spectra of humic acid - polysaccharide complexes with different humic concentrations in cultivation broth

Electron microscopical observation of the control culture after staining with ruthenium red showed two types of cells. About half of the population was represented by cells with relatively osmiophilic cytoplasm and only few with polysaccharide microfibrils around the cells. In these cells few intracytoplasmic inclusions, probably polysaccharide granules, were present /LASIK and VORISEK, 1979/. The other half of the cells excreted substantial amounts of fine polysaccharide fibrils which condensed into fibrils about 20 nm thick. The number of intracellular inclusions was extremely high in some of these cells. The electrondense staining of the outer layer of the cell envelope was characteristic of the whole population.

The staining of exocellular polysaccharides in the culture treated with humic acid showed that the cells excreting polysaccharides were bigger in size when compared with the control and contained numerous intracytoplasmic inclusions. On the surface of all cells numerous thin polysaccharide fibrils condensed into an amorphous material similar to that observed to surround Xanthomonas fuscans /LASIK and VORISEK, 1976/ and Pseudomonas. The staining pattern of the cell envelope was not different when compared with the control. About 20% of the cells were autolysed. Here, too, the intracellular membraneous vesicles and the cytoplasmic membrane were stained.

During the cytochemical experiments it became obvious that the presence of humic acid accelerated the ageing of the culture and in this way possibly enhanced polysaccharide synthesis and excretion.

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