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Preliminary Studies on Bacteria of Soil and of the Root Zone of Black [*Alnus glutinosa* (L.) Gaertn.] and Grey [*A. incana* (L.) Moench.] Alder Seedlings

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Abstract

Studies on the bacterial numbers and morphological, nutritional and physiological properties of bacteria isolated from root-free soil and from the root zone of black (*Alnus glutinosa*) and grey (*A. incana*) alder seedlings were carried out. Bacteria were at least by 2–3 orders of magnitude more numerous on the root surface of alder seedlings (both species) growing in hydroponic and agar cultures than in the root-free and rhizosphere soil. Bacteria giving slowly growing colonies were more numerous on the root surface of seedlings growing in hydroponic and agar cultures than in the rhizosphere of both alder species. In the root-distant soil, the sporeforming bacilli predominated (43.8%), while in other sources of isolation either gram-negative rods (rhizosphere of both species of *Alnus* and the root surface of *A. incana* seedlings growing in agar) or Coryneform bacteria (root surfaces of *A. glutinosa* seedlings growing in agar and those of both alder species growing in hydroponics). In most cases bacteria of the nutritional group „AG" (requiring both amino acids and growth factors) were predominated in several sources of isolation, relatively frequent occurence of bacteria of simplest nutritional requirements (group „B") was stated. The differences in frequency of occurence (depending on the source of isolation) of bacterial strains having the following physiological properties: hydrolytic activity, capability of growing in nitrogen-free medium and in the media with 4 and 6% NaCl were observed.

Introduction

It is known that bacteria (including actinomycetes) are very numerous in alder forest soils – much more than fungi, reaching many millions c.f.u. per gram of dry soil (Acero *et al.*, 1993; Gonzales *et al.*, 1995). A high microbial activity of soils under alders is undoubtedly implicated by nutrient-rich (especially nitrogen-rich) easily de-

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composable leaf litter produced by trees of the genus *Alnus* (Tarrant, 1968; Bormann *et al.*, 1994).

Alder trees are known to form two kinds of root-microbe symbioses: a) with N_2-fixing actinomycete (endophyte): *Frankia* (actinorhiza) and b) with ectomycorrhizal fungi (ectomycorrhiza) (Molina *et al.*, 1994; Li *et al.*, 1996). Importance of this tree for the forest ecosystem is ascribed mainly to highly efficient N_2-fixation by symbiotic *Frankia* in alder root nodules, thus enriching the soil in organic nitrogen (Tarrant, 1968; Bormann *et al.*, 1994; Molina *et al.*, 1994). It is known that both root symbioses of alder can be affected by the adjacent soil microflora; in particular, the root hair infection by *Frankia*, nodule formation and actinorhiza functioning can be stimulated by associated soil bacteria and actinomycetes, thus named „helper microorganisms" (Knowlton *et al.*, 1980; Knowlton and Dawson, 1983; Rojas *et al.*, 1992; Molina *et al.*, 1994). Although a positive influence of such microorganisms on *Frankia* and on actinorhiza was proved in many cases (Knowlton and Dawson, 1983; Molina *et al.*, 1994), the microbial colonization of the roots of alder seedlings and basic properties of bacteria colonizing these roots (morphology, nutritional requirements and physiological properties) still remain unexplored. Therefore this study was undertaken.

Experimental

Materials and Methods

Culturing of alder seedlings. Black [*Alnus glutinosa* (L.) Gaertn.] and grey [*A. incana* (L.) Moench.] alder seeds of uniform size were surface sterilized with 30% H_2O_2, followed by several washing with sterile distilled water. The seeds were germinated on peptone containing water agar (0.1% peptone). Uncontaminated seeds of an uniform stage of root emergence were transferred aseptically either to plastics containers (125 cm^3) with soil or to large test tubes (350 x 35 mm) with Hoagland's medium without nitrogen (Hoagland and Arnon, 1950). To each container (test tube) 5 germinated seeds of a given tree were planted. There were two kinds of cultures in Hoagland's medium: a) medium solidified with 0.7% agar; b) hydroponics – a liquid medium between glass beads. For each species of tree the following experimental combinations were set up: a) seedlings growing in sterilized (autoclaved) soil; b) seedlings growing in non-sterile soil; c) non-inoculated seedlings, growing in Hoagland's medium solidified agar (0.7%); d) seedlings, growing in Hoagland's medium solidified with agar (0.7%), inoculated with 1 cm^3 of 100-fold diluted soil suspension; e) non- inoculated seedlings growing in liquid Hoagland's medium (hydroponics); f) seedlings growing in liquid Hoagland's medium (hydroponics), inoculated with 1 cm^3 of 100-fold diluted soil suspension.

The seedlings were grown for 6 weeks in a plant-growth chamber, which gave a 16 hours light period of 10,000 lx (sodium vapour lamps) with a temperature of 25°C ± 2°C and 8 hours dark period with a temperature of 25°C ± 2°C. Seedlings cultures in soil were watered daily with sterile, distilled water and with 10-fold diluted sterile Hoagland's medium – once per week. Alder forest soil used in this study, taken from the forest reservation „Las Piwnicki" had pH_{H_2O} = 5.82, and losses on ignition = 11%. Upon completion of the experiment, seedlings were harvested and their roots were used for enumeration and isolation of bacteria.

Enumeration and isolation of bacteria. Bacteria were enumerated and isolated from root-free soil, rhizosphere of alder seedlings growing in soil, as well as from the roots of plants growing in agar and hydroponic cultures inoculated with the soil suspension. Soil Extract Agar according to Lochhead and Chase (1943) was used as a plating medium (seven replicates). To evaluate the time-course of bacterial colonies development, colonies were counted after 1, 2, 3, 4, 5, 6 and 10 days of incubation at temperature of 26°C (De Leij *et al.*, 1993).

Bacteria were subcultured and stored in „R2A/4" semisolid medium (modified R2A medium (Difco)] of the following composition (g x L^{-1}: glucose 1.0, Yeast Extract (Difco) 0.5, Bacto R2A Agar (Difco) 4.55, soil extract 100 cm^3, distilled water 900 cm^3; pH 7.0–7.2. Bacterial strains were purified on R2A Agar (Difco) plates (enriched with 10% of soil extract, v/v); 50 strains from each source of isolation were isolated. As some strains (1–12 per source of isolation) died during studies – their final number was 320 instead of 350.

Morphology and nutritional requirements of bacteria. Morphology of bacterial cells was studied (Gram strains, according to Rodina, 1968) after 1, 3 and 5 days of culturing (26°C) in the liquid „YS" medium (Lochhead and Chase, 1943).

Nutritional requirements were studied using the modified method of Lochhead and Chase (1943), where the liquid media were replaced with the solidified ones. Bacteria were multiplied on the rich agar medium of the following composition (g x L^{-1}): Tryptic Soy Agar (Difco) 4, Yeast Extract (Difco) 2, Bacto Agar (Difco) 15, distilled water 1000 cm^3 (pH 6.8–7.2; 26°C, 5 days) according to Hadegorn and Holt (1975). Subsequently the grown bacteria were replicated on the following seven agar media: medium „B" – basal medium (mineral salts + glucose), medium „A" – basal medium + amino acids, medium „G" – basal medium + growth factors, medium „AG" – basal medium + amino acids + growth factors, medium „Y" – basal medium + yeast extract, medium „S" – basal medium + soil extract, medium „YS" – basal medium + yeast extract + soil extract. After 6 days of incubation at 26°C, the growth response of each isolate was determined by assigning a value of 4 to the heaviest growth intensity (colony size) and rating the others relatively. A difference of not less than two points was considered significant.

Physiological properties of bacteria. 18 physiological tests were carried out; 4 of them were performed in the liquid media (hydrolysis of arginine, reduction of nitrate to nitrite growth in nitrogen-free and carbon-free media). All the remaining ones were done using the media solidified with 1.5% (w/v) of Bacto Agar (Difco). Hydrolysis of carboxymethylcellulose (CMC) was tested using the Wood's (1980) method. Hydrolysis of pectin was studied using the plate method given by Strzelczyk and Szpotański (1989). Starch hydrolysis was examined in the same basal medium as CMC and pectin hydrolysis, but containing 0.5% of soluble starch as a substrate and Lugol's solution as the developing reagent. Gelatine and casein hydrolysis was tested, using the plate methods described by Strzelczyk *et al.* (1990). Lipolysis was examined on Gibson's and Gordon's (1974) basal medium, containing 0.5% of tributyrin as a substrate. Hydrolysis of urea was studied in the Kutzners (1981) agar medium with urea (3%) and cresol red (0.000125%). Aesculin hydrolysis was tested in the solid Kutzner's (1981) medium, containing 0.1% of aesculin and 0.05% of ferric-ammonium citarte. Hydrolysis of arginine was examined in the liquid medium according to Shewan *et al.* (1960). Reduction of nitrate to nitrite was studied in the liquid, synthetic medium according to Allen (1951). Salinity tolerance was tested on the rich Hadegorn's and Holt's (1975) agar medium, supplemented with: 0 (control), 4%, 6% and 10% of NaCl (w/v). The growth reaction was noted after 7 days of incubation at 26°C.

Ability of the bacteria to grow oligotrophically was examined in the liquid basal „B" medium (Lochhead and Chese, 1943), without the carbon or nitrogen source. After 14 days of culturing at 26°C, the turbidity of the cultures was checked visually.

Effect of temperature on bacterial growth was studied, using TSA – Yeast Extract, rich agar medium (Hagedorn and Holt, 1975). After the multipoint inoculation, the plates were incubated: a) at 5°C – for 3 weeks, b) at 42°C – for 5 days, c) at 50°C – for 5 days and the growth reaction was noted.

Statistical evaluation of the results. The results of determination of the total number of bacteria were statistically evaluated, using 1-way analysis of variance (AVOVA) and Newman-Keuls multiple range test ($p \leq 0.05$; for comparison of averages) – after the previous log-transformation of the c.f.u. data [$y = \log_{10}(X + 1)$]. Frequencies of occurence of strains having a given feature in sources of isolation were tested for departures from the random distribution,

using χ^2 test. All statistical calculations were performed using: Statistica for Windows, release 5.1 (1996; StatSoft, Tulsa, Oklahoma, USA).

Results

Results concerning estimation of bacterial numbers in root-free soil (soil), rhizosphere of *Alnus glutinosa* seedlings growing in soil (rhizosph), on the roots of *A. glutinosa* growing in hydroponic (hydro) and in agar (agar) cultures as well as in the rhizosphere (rhizos_i) and on the roots of hydroponic (hydro_i) and agar (agar_i) cultures of *A. incana*, calculated per 1 gram of dry mass (c.f.u. x g^{-1}) are shown in Table I. These numbers ranged from 5.17 x 10^6 (soil) to 1.42 x 10^{10} (agar) c.f.u. x g^{-1} of dry mass and were in the following order: soil < rhizosph < rhizos_i < hydro < hydro_i < agar_i < agar; differences between c.f.u.'s for any pair of sources of isolation were significant ($p \leq 0.05$) – except hydro_i and agar_i (Table I).

Table I

Bacterial numbers [expressed as $\log_{10}$ of c.f.u.'s; mean values (n = 7) ± 95% confidence limits] in soil and the root zone of black [*Alnus glutinosa* (L.) Gaertn.] and grey [*A. incana* (L.) Moench.] alder seedlings.

	Source of isolation		Mean values ± 95% confidence limits ($\log_{10}$ of c.f.u. x g^{-1} of dry mass)
No.	Abbreviation	Full name	
1.	Soil	Root-free soil	6.704 a ± 0.088
2.	Rhizosph	Rhizosphere of *A. glutinosa* growing in soil	7.250 b ± 0.042
3.	Hydro	Roots of *A. glutinosa* growing in hydroponic cultures	9.769 d ± 0.046
4.	Agar	Roots of *A. glutinosa* growing in agar cultures	10.149 f ± 0.049
5.	Rhizos_i	Rhizosphere of *A. incana* growing in soil	7.857 c ± 0.050
6.	Hydro_i	Roots of *A. incana* growing in hydroponic cultures	9.968 e ± 0.027
7.	Agar_i	Roots of *A. incana* growing in agar cultures	10.007 e ± 0.044

Mean values marked with the same letter do not differ significantly ($p \leq 0.05$).

It appears (Fig. 1) that the growth curves for colonies of bacteria and actinomycetes isolated from soil and from the rhizosphere of *A. glutinosa* were close to their saturation level (the asymptote) between days 6 – 10. By contrast, numbers of bacterial colonies from the roots of the same tree, growing in hydroponic and in agar cultures, continued to grow even beyond the time of termination of the experiment – on 10^{th} day. For *A. incana*, the time-course of colony number was similar for all the sources of isolation (both soil and the root zone) – with apparent slowing-down of its increase between days 6 – 10 (Fig. 1, 2 left graphs).

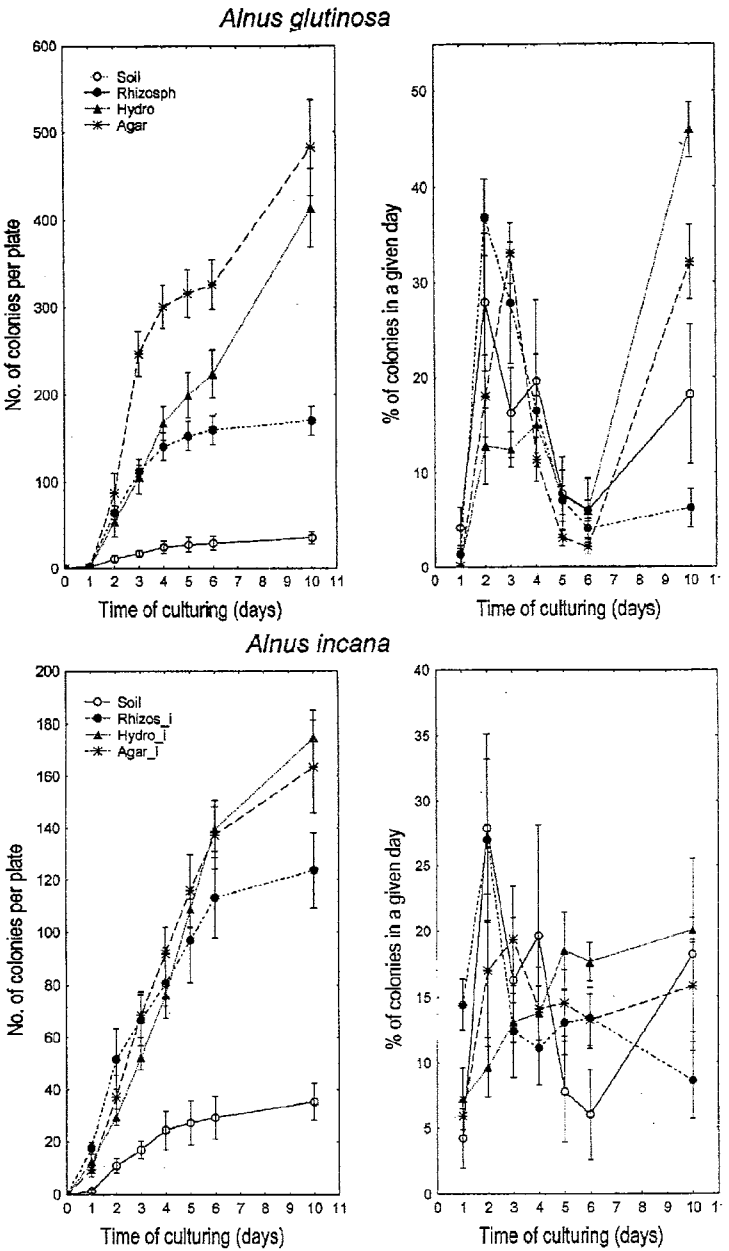

Fig. 1. Time-course of the colony number on plates (with soil extract agar medium), inoculated with suspensions made from soil and the root zone of alder seedlings (left 2 graphs), and comparison of population structure of bacteria and actinomycetes based on time-course of colony number (right 2 graphs).

Mean values (n=7) ± 95% confidence limits. See Table I for explanations of abbreviations for sources of isolation. Dilutions: soil and rhizosphere (both *A. glutinosa* and *A. incana*) – 10^{-5}; all the remaining sources of isolation – 10^{-6}.

Two right graphs on Fig. 1 indicate that the structure of bacterial population (based on the colony appearance kinetics) was not homogenous for organisms of root-free soil as well as for those associated with the roots of *A. glutinosa*. More bacterial colonies appeared on 2nd and 3rd day of culturing than during the remaining days of counting; minimal numbers of colonies appeared on days 5th and 6th. By contrast, the structure of bacterial population in the root zone of *A. incana* was rather relatively homogenous (Fig. 1, 2 right graphs).

Fig. 2 shows that in the root-distant soil the sporeforming bacilli predominated (43.8%); in other sources of isolation either gram-negative rods (rhizosphere of both species of *Alnus* and the roots of *A. incana* seedlings growing in agar) or Coryneform bacteria of the *Arthrobacter* type, *i.e.* strongly pleomorphic (the remaining sources of isolation) were predominating. In all sources of isolation (except „hydro_i") bacteria of the nutritional group „AG" (*i.e.* requiring both amino acids and growth factors) were predominating; among strains isolated from the roots of both alder species growing in agar cultures, as well as – from the roots of *A. incana* growing in hydroponic cultures – organisms of the simplest nutritional requirements (group „B") were also numerous. Bacteria of the complex nutritional demands [requiring yeast – and/or soil extract(s)] were relatively frequent in root-free soil as well as in the rhizosphere of *A. glutinosa* and other roots on the same tree growing in hydroponic cultures. It is noteworthy that most of morphological and nutritional group of bacteria studied was unevenly distributed between the sources of isolation (significant test χ^2 at $p \leq 0.05$), except the Coryneforms of *Corynebacterium* type (weak pleomorphism), cocci and group „AG" (Fig. 2).

Fig. 3 points out that the total frequencies of bacterial strains having the respective physiological properties were in the following order: hydrolysis of tributyrin (208 of 320 strains, 65%) > oligonitrophilic growth > growth at 5°C > nitrate reduction > hydrolysis of arginine > oligocarbophilic growth > hydrolysis of aesculin > hydrolysis of CMC starch hydrolysis > gelatine hydrolysis > growth at 4% NaCl > casein hydrolysis > pectin hydrolysis > urea hydrolysis > growth at 6% NaCl > growth at 42°C > growth at 50°C > growth at 10% NaCl (4 of 320 strains, 1.25%). The physiological tests, which discriminated strains the most (depending on their source of isolation) were those, that revealed hydrolytic activity (towards: CMC, starch, gelatine, casein, urea, tributyrin and aesculin) and nitrate reduction. In the last one the differences were due to the predominance of nitrate reducing strains in soil and rhizosphere of both alders (58.3–89.6%), as opposed the root zone of seedlings growing in hydroponic and agar cultures (21.7–39.5%). There were significant ($p \leq 0.05$) differences in the frequency of oligonitrophilic growth, depending on the source of isolation [mostly more frequent feature among strains of the root zone of both trees (up to 52.6%) than in those of the root-free soil (27.1%)] and in media containing 4 and 6% NaCl (strains isolated from the rhizosphere of *A. glutinosa*aa and partially from the root-free soil were more salt-tolerant than those from the remaining sources of isolation). Among bacteria of the root-distant soil the ability to hydrolyse starch, gelatine, casein, urea and aesculin was significantly ($p \leq 0.05$) more frequent than among organisms isolated from remaining sources. Strains derived from the roots of *A. incana* growing in hydroponic cultures hydolysed tributyrin more rarely (46.7%)

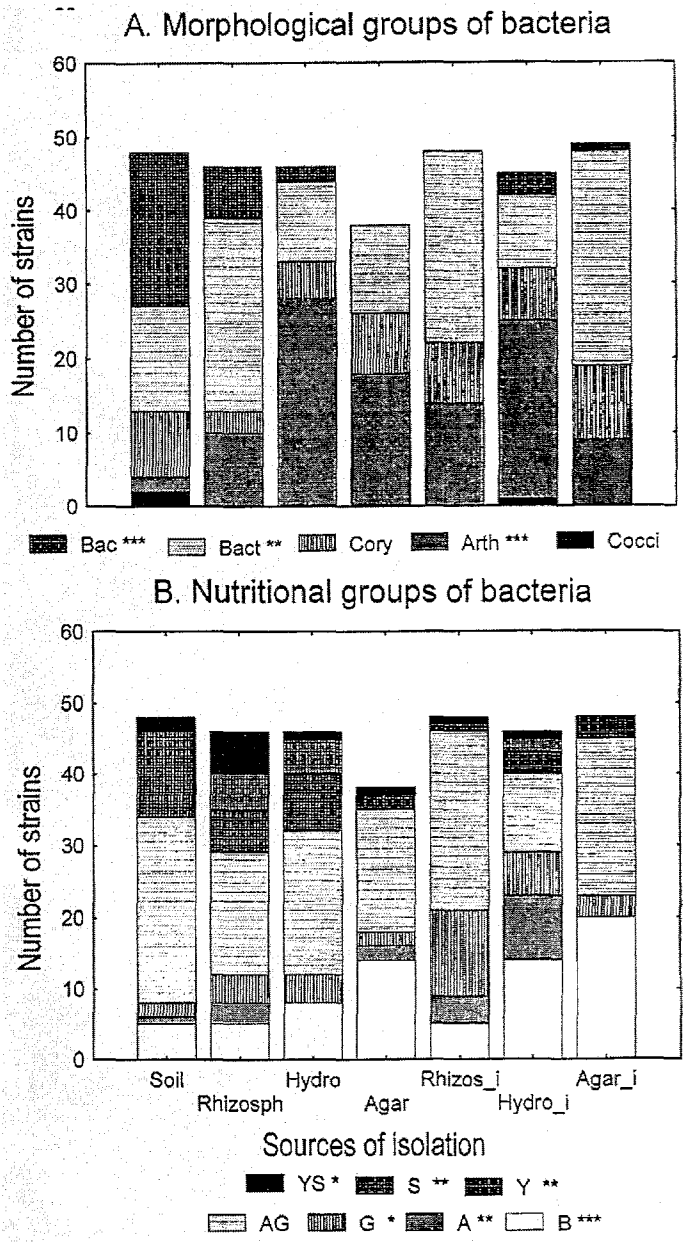

Fig. 2. Morphological and nutritional groups of bacteria of soil and of the root zone of *A. glutinosa* and *A. incana*.

See Table I for explanations of abbreviations for sources of isolation. Abbreviations for morphological groups: Cocci – Gram(+) cocci, Arth – Gram-variable, strongly pleomorphic Coryneforms, Cory – Gram-variable, weakly pleomorphic Coryneforms, Bact – Gram(-) rods, Bac – Gram(+) sporulating bacilli abbreviations of media used – see Materials and Methods. In figure legends the appropriate morphological/nutritional groups, which have shown significant departures from the random frequency distribution between the respective sources of isolation (test χ^2) are marked: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

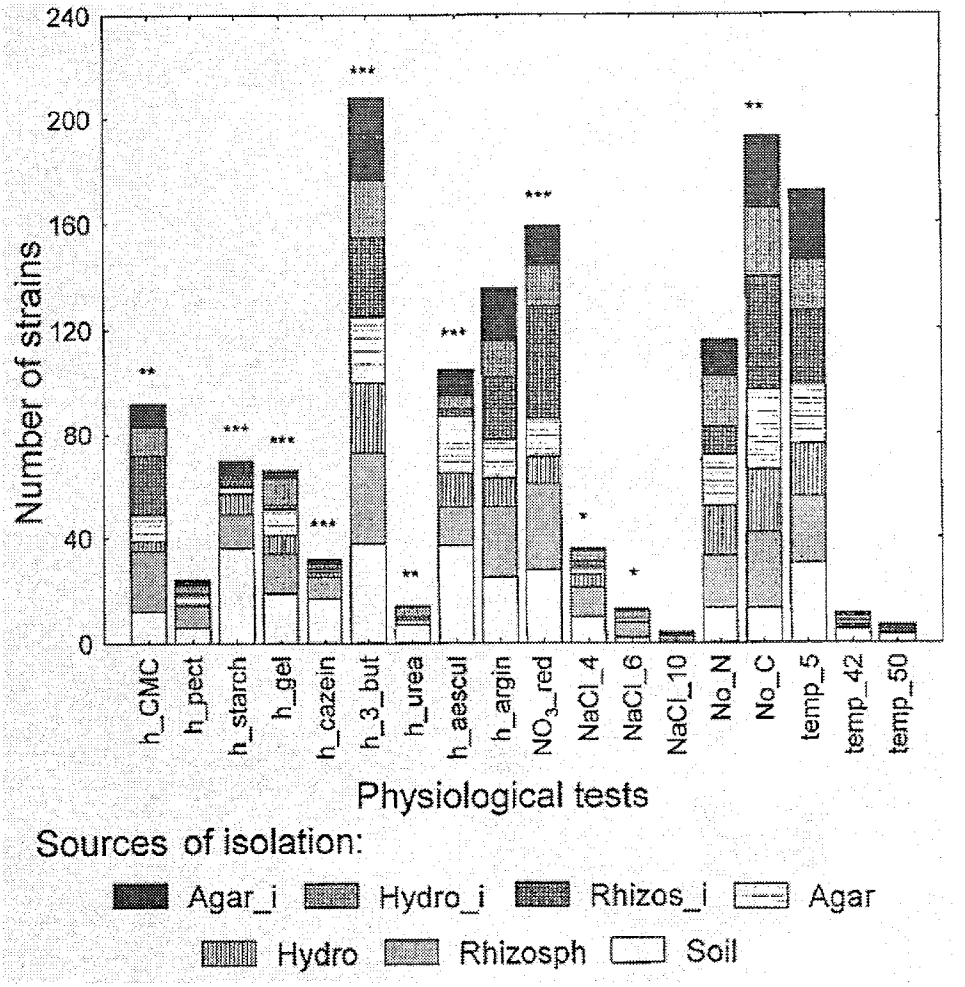

Fig. 3. Physiological properties of bacteria of soil and of the root zone of *A. glutinosa* and *A. incana*.

See Table I for explanations of abbreviations for sources of isolation. Abbreaviations for physiological properties: h_CMC – hydrolysis of CMC, h_pect – pectin hydrolysis, h_starch – starch hydrolysis, h_gel – gelatine hydrolysis, h_casein – casein hydrolysis, h_3_but – tributyrin hydrolysis, h_urea – urea hydrolysis, h_aescul – aesculin hydrolysis, h_argin – arginin hydrolysis, NO_3_red – nitrate reduction, NaCl_4 – growth at 4% NaCl, NaCl_6 – growth at 6% NaCl, NaCl_10 – growth at 10% NaCl, No_N – growth in basal medium without nitrogen (oligonitrophilic growth), No_C – growth in basal medium without carbon (oligocarbophilic growth), temp_5 – growth at 5°C, temp_42 – growth at 42°C, temp_50 – growth at 50°C. Bars representing the physiological properties, which have shown significant departures from the random frequency distribution between the respective sources of isolation (test χ^2) are marked: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

than all remaining ones (58.7–79.2%). Occurence of bacteria degrading CMC was relatively the most frequent in the rhizosphere of both trees (up to 50%; remaining sources – up to 26.3%). There were no significant differences in occurence (depending

on the isolation source) of the following features: ability to hydrolyse pectin and arginine, growth in the presence of 10% NaCl, oligocarbophilic growth, and growth in different temperatures (Fig. 3).

Discussion

The results presented in this paper indicate that the numbers of bacteria were different depending on the source of isolation; in particular their numbers were much higher in the root zone of alder seedlings than in the root-distant soil. Similar differences betweeen the bacterial numbers in root-free soil and in the root zone were stated by Lu *et al.* (1968), who compared microbial populations under alders and under coniferous trees, and by Gonzales *et al.* (1995) in their studies on the microflora of soil and of the root zone of alder. The enhanced microbial activity in the root zone can be ascribed to the stimulation exerted by plant root exudates as well as by chemical compounds released during the decay and autolysis of dying epidermal cells (Curl and Truelove, 1986; Grayston *et al.*, 1997).

The differences in bacterial numbers (expressed as c.f.u. per dry mass unit) noted in this work between a) root-free soil and rhizosphere and b) root zone of seedlings growing in hydroponic and agar cultures were significant [≥ 1000-fold higher c.f.u.'s for b) than for a)]. It can be explained not only by the fact of very strong proliferation of bacteria on the roots surface in seedlings growing in hydroponics and in agar (probably due to specific plant growth conditions – different than in soil), but also by the difference in the specific gravity of: a) dried soil (both root-free and rhizosphere) and b) dried seedling roots (from hydroponic and agar cultures).

Results of c.f.u. estimations obtained in our work were expressed as logarithms of the basis 10. Purposes of log-transformation were as follows: a) to make the distribution of replicate estimations closer to the normal distribution; b) to eliminate the positive correlation between averages and variances, which diminishes the reliability of the parametric statistical tests, like Students t-test and ANOVA (Fry, 1989). Log-transformation of c.f.u. values obtained for bacteria living on the roots can be additionally justified by their log-normal distribution on the root surface (Klopper and Beauchamp, 1992); it has been used by many researchers (De Leij *et al.*, 1993; Behrendt *et al.*, 1997).

The time course of bacterial colonies appearance, investigated in this work indicates that percentage of the most slowly growing colonies (appearing on the 6^{th} and 10^{th} day of culturing) was in the following order: hydro > hydro_i > agar > agar_i > soil > rhizosph > rhizos_i. According to ecological theory of r- and K-selection (De Leij *et al.*, 1993) in bacterial communities inhabiting the roots in hydroponic cultures, K-selection predominated (communities of high population density) and in communities inhabiting the rhizosphere of both trees, r-selection was predominant (communities of low population density). According to the above mentioned theory, the microbial communities in the rhizosphere of *A. glutinosa* and *A. incana* had the lowest ecological stability; stability of those on the roots of plants grown hydroponically was the highest one. In case of the remaining sources of isolation (root surface of seedlings of both *Alnus* species in agar cultures, root-free soil) microbial communities were in the intermediate stability stages (equilibrium between the r- and K-selection). The pre-

dominance of K-strategists on the roots of seedlings grown in hydroponics can be explained by the fact that in this habitat concentrations of nutrients were low. The only nutrient source here seemed to be the seedling root exudates (and sloughing off and/or dying the root epidermal cells), which were quickly diluted in the liquid plant growth medium.

We have stated the predominance of spore-forming bacilli in the root-free soil. This observation is in agreement with the results obtained earlier in our laboratory (Różycki *et al.*, 1986; Różycki, 1987). In other sources of isolation studied presently, gram-negative rods (rhizosphere of the both *Alnus* species, the root surface of *A. incana* seedlings grown in agar cultures) and Coryneform bacteria of the „arthrobacter" type (the root surface of the seedlings of both species growing in hydroponics and of those of *A. glutinosa* in agar cultures) predominated. The predominance of Coryneform bacteria in the root zone of Scots pine was noted by Różycki *et al.* (1986) and Różycki (1987). It is accepted that the plant root exudates of forest trees (like: Scots pine) are particularly good carbon source for the Coryneform bacteria inhabiting the rhizosphere of these trees (Różycki, 1987; Grayston *et al.*, 1997). The occurence of gram-negative rods in the root zone of forest trees seems to be less common than Coryneform bacteria (Strzelczyk *et al.*, 1978). However the gram-negative rods were numerous in the soil and in the root zone of the grown-up trees of *Alnus glutinosa* (Acero *et al.*, 1993), similarly as we noted for alder seedlings.

According to the results of our work, both in the soil and in the root zone of alder seedlings the bacteria requiring both amino acids and growth factors were predominating (except the root zone of *A. incana* seedlings growing in hydroponic and agar cultures). This observation contradicts with the results of some earlier studies on the soil bacteria and root zone of the grown-up Scots pine trees bacteria, where the organisms of the simplest nutritional requirements (groups „B" and „A") were most frequent (Różycki *et al.*, 1986). It can be assumed that the bacteria occurring in the root zone of alder seedlings (whose properties and nutritional requirements were dependent on the composition of the root exudates), studied in the present work were different from those occurring in the root zone of Scots pine trees. The importance of higher fertility and higher pH of the soil used in the present study than in case of soil used by Różycki *et al.* (1986) also cannot be excluded.

The results of our work have shown that bacteria hydrolysing urea, casein, gelatine, aesculin and starch were relatively more frequent in the root-free soil than in the root zone of the both alder species. This observation is in agreement with the results of earlier studies on bacterial microflora of soil and the root zone of Scots pine (Dahm, 1984; Różycki *et al.*, 1986; Różycki, 1987). The above mentioned differences in the composition of physiological groups can be explained by the differences in the sources of nutrients: plant and animals residues in soil and plant root exudates in the rhizosphere (Dahm, 1984). According to Gonzales *et al.* (1995) the physiological groups of bacteria, active in nitrogen cycling are of special importance in the root zone of black alder trees, as in this habitat microorganisms active in proteolysis and ammonification (but not in nitrification) were the most numerous ones.

In the present work (similarly as in the earlier studies, performed on the bacteria of the soil and the root zone of pine by Dahm, 1984) the occurrence of bacteria

showing the ability to oligocarbophilic and oligonitrophilic growth in soil and the root zone of alder seedlings was stated. There were differences in the frequency of occurrence of these bacteria stated in the present work (soil and root zone of alder seedlings) as compared to cited studies (soil and root zone of Scots pine trees). These differences could be explained by the different properties of soils, differences in the composition of plant root exudates (between grown-up pines and alder seedlings) and by different taxonomic composition of bacteria in both cases, which could react in different manner to the carbon or nitrogen deficiency in the medium. It has to be stressed, that bacteria studied here were not obligatory oligocarbophiles and oligonitrophiles (very slowly growing and non-tolerating high concentrations of nutrients in the media), as they grew relatively quickly and were grown and stored in the media containing high concentrations of nutrients (Hattori, 1985).

The results of our work indicate that bacteria tolerating the moderate salinity (4–6% NaCl) were more numerous in the root-free soil and in the rhizosphere of *A. glutinosa* than in all the remaining sources of isolation. This observation can indicate the more frequent occurrence of physiologically gram-positive bacteria (more tolerant to salinity than the gram-negative ones, Buchanan and Gibbons, 1974) in soil and the rhizosphere of black alder than in the remaining sources of isolation.

In the present studies we have not stated any formation of actinorhizal nodules on the roots of alder seedlings inoculated with soil suspension. It could be caused either by too low number of active *Frankia* propagules in the soil inoculum used or by too short duration of the experiment. However the imporatnce of „helper soil microorganisms" in the process of actinorhiza formation has to be also stressed here. The knowledge about such „helper bacteria" is rather scarce, *e.g.*: it is known that they can belong to the following genera: *Pseudomonas*, *Chromobacterium*, *Bacillus* and *Streptomyces* and they can produce some plant growth hormones, *e.g.* IAA (Knowlton *et al.*, 1980; Knowlton and Dawson, 1983; Rojas *et al.*, 1992; Probanza *et al.*, 1996; Manero *et al.*, 1996). Both basic (taxonomy, physiology and ecology) and applied (uses a component of actinorhizal, artificial inocula) studies on the potential „helper bacteria" should be continued in the future, as their results may lead to the improvement of nitrogen fixation by *Frankia* in symbhiosis with alders and other actinorhizal plants.

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