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Contribution of *Cytophaga*-like Bacteria to the Potential of Turnover of Carbon, Nitrogen, and Phosphorus by Bacteria in the Rhizosphere of Barley (*Hordeum vulgare* L.)

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ABSTRACT

The functional potential of bacteria isolated from the rhizosphere of barley (*Hordeum vulgare* L.) in May, July, and August and cultivated on nutrient-rich substrate (1/10 TSBA) and nutrient-poor substrate (cold soil extract agar) was determined. There was no significant difference in numbers of CFU when counted on nutrient rich or poor substrate. Bacterial numbers increased approximately 3-fold in the rhizosphere soil from May to August but was unchanged in bulk soil over the same period. A total of 4474 randomly isolated bacteria were screened for enzymatic activities involved in carbon turnover (amylase, cellulase, mannanase, xylanase, and chitinase), nitrogen turnover (protease, nitrate and nitrite reductase), and phosphate turnover (phosphatase). In the rhizosphere soil, bacteria carrying C and P turnover enzymes were not stimulated by the growing plant whereas protease and nitrate and nitrite reductase were stimulated by the growing plant. No changes were observed in the bulk soil. Two taxonomic groups were followed: *Cytophaga*-like bacteria (CLB) and fluorescent pseudomonads, the latter being abundant in the rhizosphere and important contributors to the cycling of organic matter in soil. Unexpectedly in the spring samples, CLB were around 25% of all bacteria isolated, whereas fluorescent pseudomonads made up less than 10%. The relative proportion of these bacterial groups then decreased during the plant growth season but at all times showing a clear rhizosphere effect. Furthermore, up to 70% of the isolates carrying enzymes involved in the turnover of carbon, in the May sample, were identified as CLB, indicating the importance of this group in early colonization of the rhizosphere. The fluorescent pseudomonad group contributed less than 3%.

Introduction

Bacteria and fungi producing extracellular degradative enzymes are the primary decomposers of organic matter from plant roots [5]. Microbial transformations of organic matter are the basis of plant decomposition, soil aggregation, and nutrient availability [33]. In the rhizosphere, bacteria benefit from the diffusion of a wide variety of soluble materials, especially sugars and amino acids

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leaching from the roots [5, 24, 32], but also from the mucilage produced by the root cap and from sloughed-off plant cell materials [7, 19]. Carbon and nutrients support a milieu of high microbial activity [3], thus stimulating carbon turnover [5] and mineralization of inorganic nitrogen [15]. The number of bacteria are furthermore found at levels 2 to 20 times higher in the rhizosphere than in the surrounding bulk soil [5, 10]. Among bacteria in the rhizosphere, *Bacillus, Pseudomonas, Streptomycetes, Enterobacter, Arthrobacter,* and *Cytophaga*-like bacteria have been reported to be common [17, 19–21, 23, 26] and many studies have a higher number of species in the rhizosphere compared to bulk soil [e.g., 17, 20].

Taxonomic or molecular characterization of the bacteria isolated from the rhizosphere does not per se elucidate the functional potential with respect to enzymatic degradation of plant root material and mineralization of nutrients. In general, production of extracellular enzymes such as amylase, cellulase, chitinase, and protease is essential for degradation of high-molecular-weight compounds such as starch, cellulose, chitin, and proteins [1, 6]. Mineralization of nitrogen by proteases and oxidation of ammonium by nitrification followed by denitrification result in a loss of nitrogen from the rhizosphere [15, 27]. Further, mineralization of phosphate by bacteria can lead to phosphorus depletion along plant roots [16].

The aim of the present study was to describe the seasonal dynamics of the culturable bacterial population present in the bulk and rhizosphere soil during the growth season of barley. This was done by random isolation of culturable bacteria. Culturable bacteria have previously been suggested to dominate the turnover of carbon and nutrients in soils [25]. Functional dynamics was elucidated by characterizing the isolates with respect to the presence of enzymes having activity on carbon, nitrogen, and phosphorus compounds. Also the numbers of *Cytophaga*-like bacteria (CLB) and fluorescent pseudomonads were quantified as they have been reported to be abundant in the rhizosphere [7, 26] and important contributors to the cycling of organic matter in soil [8, 28, 31].

Materials and Methods

Study Site and Sample Collection

Samples of bulk and rhizosphere soil were collected three times during a growth season from a field with spring barley (*Hordeum vulgare* L.) located at the experimental station Højbakkegaard (Taastrup, Denmark). Barley mixed with clover (*Trifolium* sp.) was sowed at the end of April 1998. Bulk soil and barley plants were collected 14 May, 14 July, and 14 August 1998. About 15 plants with undisturbed soil around the roots were removed and placed in a plastic box. Bulk soil samples (about 3 L) were taken between plant rows and stored in single-use plastic bags. Rhizosphere soil was defined as the soil adhering to the roots after gentle shaking of the roots. Roots were cut and placed in Winogradsky salt solution (0.4 g K_2HPO_4; 0.13 g $MgSO_4 \cdot 7H_2O$; 0.13 g NaCl; 0.0025 g $MnSO_4 \cdot 7H_2O$; 0.5 g NH_4NO_3; 1000 mL Milli-Q water; pH 7.2). The suspended bulk and rhizosphere soil samples were diluted 10 times in Winogradsky salt solution and shaken for 15 min. Before further dilution, suspensions were briefly shaken and left for 1 min to allow coarse particles to settle. All sampling was done in triplicate and processed within the same day.

Soil Characterization

Soil temperature was measured at 15 cm below ground. The gravimetric water content was measured as the weight loss after 24 h at 105°C. Total carbon was measured as weight loss after 24 h at 550°C. pH of the soil samples was measured in 0.01 M $CaCl_2$. NO_3^- and NH_4^+ were determined after extraction of 4 g of bulk soil for 1 h in 20 mL of 2 M KCl. NO_3^- was reduced to NO_2^- and NO_2^- determined after addition of Griess–Illoways reagent (sulfanilamide, 10.0 g/L; concentrated phosphoric acid, 100.0 mL/L; *N*-1-naphthylethylenediamine dihydrochloride, 0.5 g/L; Milli-Q water, 900 mL) by using an Alpkem flowsolution IV autoanalyzer (OI Analytical, USA). NH_4^+ was measured photometrically by reaction with salicylate and free chlorine in the presence of sodium nitrocyanide [39]. Inorganic P was measured by extraction with 0.5 M $NaHCO_3$ and spectrophotometric analysis by Miljøkemi (Danish Environmental Center, Viborg, Denmark).

Isolation, Cultivation, and Enumeration of Bacteria

Dilution series of each bulk and rhizosphere soil sample were spread in triplicate on two different agar substrates. As a standard for isolation of soil bacteria, one-tenth strength of Tryptic Soy Broth (3.0 g/L (Difco)) with 15.0 g/L agar (Difco) (TSBA) was chosen. pH was adjusted to 7.0 by HCl/NaOH. For a diluted substrate, cold soil extract agar (CSEA) was chosen. CSEA substrate was prepared according to Olsen and Bakken [25] with several modifications. Soil from the barley field was sieved through a 6 mm mesh air-dried for 24 h. Dried soil (250 g) was extracted with 750 mL Milli-Q water by shaking vigorously for 15 min and allowed to settle for 10 min. The supernatant was decanted and centrifuged for 20 min at 15,000 rpm and finally filter sterilized with 0.2 μm Minisart celluloseacetate filter (Sartorius, Germany). The CSE was diluted 3 times in autoclaved Milli-Q water and Noble Agar (Difco) (25.0 g/L) added. pH was adjusted to 7.0. Both substrates were poured in

14 cm diam. petri dishes. The petri dishes were kept at 15°C in dark.

After 3, 5, 6, 11, 16, 25, and 58 days of incubation, colony-forming units (CFU) were counted, and each, time up to 32 colonies were selected randomly and purified. The isolates were stored in 96-well microtiter plates (Costar, Denmark) in 20% glycerol at −80°C. Total numbers of bacteria were determined by epifluorescence microscopy after dilution of the soil samples, filtration, and acridine orange staining as described by [4]. Between 450 and 1200 bacteria were counted in each sample.

Characterization of Enzymatic Activity

Bacteria isolated on 1/10 strength TSBA were maintained on this substrate during further characterizations. However, bacteria obtained from CSEA were maintained on 1/20 strength TSBA to obtain pure culture and for further characterization. The isolates were tested for a range of enzymatic activities of different carbon, nitrogen, and phosphate compounds. A 96-pin replicator (Life Technology, Denmark) was used to transfer approx. 1 μL of the bacterial suspension from each well of the storage microtiter plate to the different test substrates. Hydrolytic enzyme activity of α-amylase, α-cellulase, β-mannanase, and β-xylanase was tested by using chromogenic azurine-crosslinked-substrates (AZCL) (MegaZyme, Dublin, Ireland). Each well in an Omni-well microplate (Life Technology, Denmark) received agar substrate as a base and a topagar containing 0.5 g/L of the pulverized AZCL substrates. β-Chitinase activity was tested using 1.0 g L^{-1} remazol brilliant violet-linked chitin (Loewe, Germany) in the topagar. Protease activity was tested by addition of sterilized skimmed milk (Difco) (50.0 g/L) to substrate. Urease activity was tested on the Christensen Urea agar (1.0 g peptone; 1.0 g glucose; 5.0 g NaCl; 2.0 g KH_2PO_4; 0.012 g phenol red; 20.0 g agar; 1000 mL Milli-Q water). After autoclaving, urea (Difco) was added to a final conc. of 2%. Acid and alkaline phosphatase activity was tested by adding a 1% solution of filter-sterilized sodium salt of phenolphthalein diphosphate (Sigma) to a final conc. of 0.01% to molten agar. After growth, colonies were exposed to 25% ammonia solution (Merck) and a positive colony appeared as red/pink colored. Enzymatic reactions were followed during 7 days of incubation. Nitrate and nitrite reductase activity was tested in 1/10 or 1/20 strength TSB medium supplemented with 100 μM $NaNO_2^-$ and 2 mM $NaNO_3^-$. To each well in a 96-well microtiter plate, 200 μL medium and approx. 2 μL of the bacterial suspension was added. In each well paraffin oil was added on top of the medium to promote anaerobic conditions. During 7 days of incubation the presence/absence of NO_2^- was measured by addition of 40 μL Griess–Illoways reagent. A deep red color in the medium indicated nitrate reduction to nitrite (nitrate reductase activity), whereas no color development indicated a nitrite reduction (nitrite reductase activity). The number of bacteria with each enzymatic activity was calculated by computing the proportion of bacterial isolates positive for an enzyme and then multiplied by the total number of CFU for the specific sample.

Taxonomic Identification

Two groups of bacteria were identified by phenotypic criteria: *Cytophaga*-like bacteria and fluorescent pseudomonads. To avoid confusion concerning the systematics of the *Cytophaga–Flavobacterium–Bacteroides* group, we use the broader term *Cytophaga*-like bacteria (CLB) as suggested by Reichenbach [31]. CLB were identified by the following characteristics: yellow/orange colony pigmentation, flexirubin positive (tested by adding 10% KOH to the colony and observing a red coloring of the colony), and typical CLB colony morphology. Fluorescent pseudomonads were identified by siderophore production which was determined by colony fluorescence when exposed to UV light after growth on King's B substrate (protease peptone no. 3 (Oxoid) 20.0 g; glycerol 10.0 g; K_2HPO_4 1.5 g; $MgSO_4 \cdot 7H_2O$ 1.5 g; agar 15.0 g; Milli-Q water 1000 mL; pH was 7.2). All incubations were done at 15°C in darkness for 3–5 days.

Statistical Analysis

Direct bacterial counting, CFU numbers of bacteria, and CFU numbers of bacteria carrying the enzymes involved in the turnover of C, N, and P were analyzed. Variation between soil type (bulk or rhizosphere), sample time (May, July, or August), and substrate (CSEA or 1/10 TSBA) was evaluated by using a one- or two way ANOVA. Bacterial numbers were $\log_{10}$ or LOG_{10} $(x+1)$ transformed before statistical analysis. Results were considered significantly different when $P < 0.05$.

Results

Soil Physical and Chemical Parameters

Physical and chemical characteristics of the soils over the three sampling times were 14.9 ± 0.2°C, pH 6.4 ± 0.2, and soil water content 15.8 ± 1.47%. The nitrate pool decreased from 12.8 to 0.2 μg N g/soil (ww) during the growth season whereas the pools of nitrite (0.05 ± 0.03 μg N g/soil (ww)), ammonium (4.0 ± 0.3 μg N g/soil (ww)), and phosphate (3.5 ± 0.4 μg P g/soil (ww)), as well as total carbon by loss of ignition (4.8 ± 0.1%), were similar at the three sampling times.

Quantitative Changes of the Microbial Population

The numbers of colony-forming units obtained after 58 days of incubation on the nutrient-rich substrate (1/10 TSBA) and the nutrient-poor substrate (CSEA) are shown in Fig. 1. In bulk soil, colony-forming units were between 4.3×10^7 and 5.7×10^7/g of soil on 1/10 TSBA and between 3.5×10^7 and 4.6×10^7/g of soil on CSEA. In the rhizo-

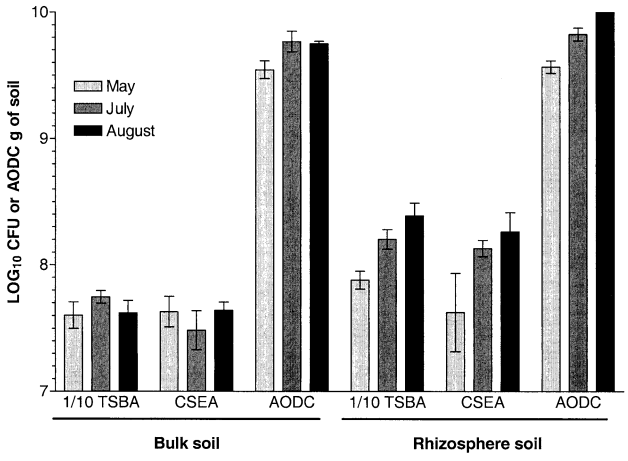

Fig. 1. Total number of bacteria in bulk soil and rhizosphere soil expressed as colony-forming units (CFU) on 1/10 TSBA and CSEA and direct counts (AODC). Mean ± standard error of mean; $n = 3$.

sphere, a significant increase in CFU, from 7.8×10^7 to 2.6×10^8/g of soil, was observed in bacterial numbers counted on 1/10 TSBA. No significant increase was found when counted on CSEA (6.1×10^7 to 2.0×10^8 CFU/g of soil). The total numbers of bacteria (Fig. 1) determined microscopically after acridine orange staining (AODC) were between 3.6×10^9 and 6.0×10^9/g of soil for bulk soil whereas a significant increase, from 3.7×10^9 to 1.0×10^{10}/g of soil, occurred in the rhizosphere soil during the growth season. The percentage of bulk soil bacteria able to form colonies was on average 0.9% for CSEA and 1% for 1/10 TSBA of the AODC numbers. In the rhizosphere, 1.9% of the bacteria formed colonies on CSEA and 2.4% on 1/10 TSBA of the AODC numbers.

Enzyme Characteristics of Bulk Soil and Rhizosphere Bacteria

A total of 5761 bacteria were randomly isolated from bulk and rhizosphere soil during the growth season of barley. The majority of the isolates could be purified (84%) and 78% survived preservation in 20% glycerol at −80°C. The loss of bacteria did not appear related to the samples and substrate used for isolation (data not shown).

All isolates were tested for the range of enzymes involved in the turnover of carbon, nitrogen, and phosphate (Fig. 2A–D). The number of bulk and rhizosphere soil bacteria positive for either amylase, cellulase, mannanase, xylanase, and chitinase activity were stable during the three samplings when counted on the nutrient rich substrate (1/10 TSBA). Depending on the specific enzyme, bacterial numbers varied between 5.6×10^5 and 1.1×10^7 CFU/g for bulk soil and between 2.8×10^6 and 2.5×10^7 CFU/g for rhizosphere soil (Fig. 2A). In general, numbers of bacteria isolated on CSEA that tested positive for enzymes were at the same level as bacteria isolated on 1/10 TSBA. In contrast to the constant number of isolates from 1/10 TSBA, the number of rhizosphere bacteria isolated on CSEA increased, depending on the enzyme, from 4.3×10^6 to 1.7×10^7 CFU/g of soil in May and to 8.2×10^6 to 2.8×10^7 CFU/g of soil in August (Fig. 2C). A rhizosphere effect

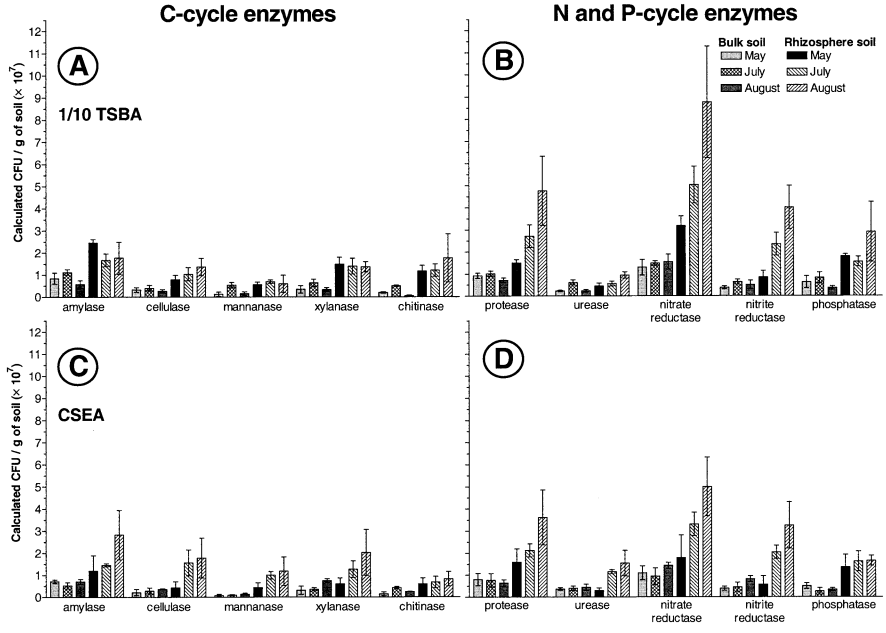

Fig. 2. Number of CFU that were positive for amylase, cellulase, mannanase, xylanase, and chitinase (A and C) and protease, urease, nitrate and nitrite reductase, and phosphatase (B and D). Bacteria isolated on 1/10 TSBA and on CSEA are shown at top and bottom of figures, respectively. When no bar is present, positive bacteria were not found at the specific sampling date. Mean ± standard error of mean; $n = 3$.

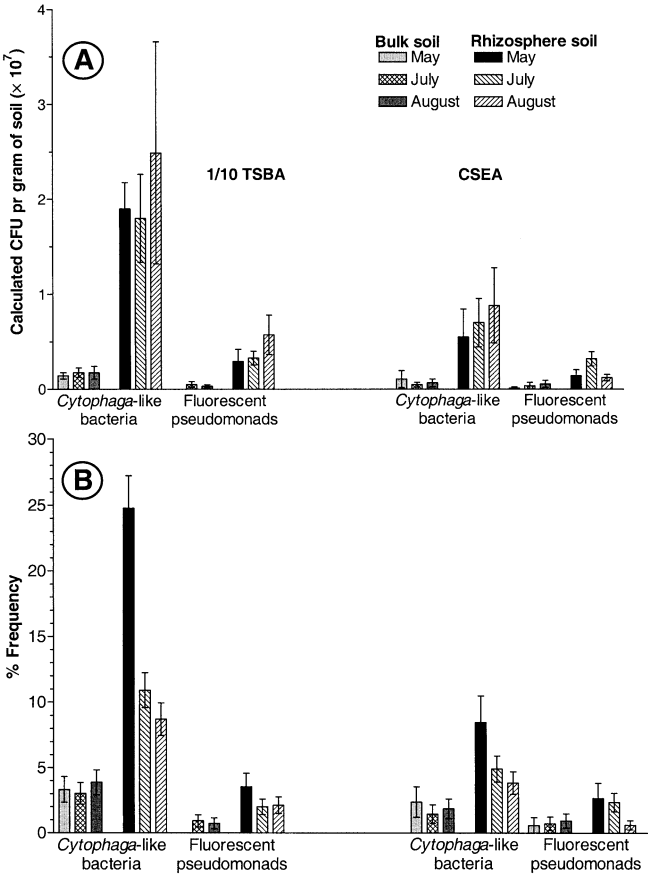

Fig. 3. Number of CFU (A) and frequency (B) of phenotypically identified *Cytophaga*-like bacteria and fluorescent pseudomonads. When no bar is present, positive bacteria were not found at the specific sampling date. Mean ± standard error of mean; $n = 3$.

was also observed among isolates from CSEA that resulted in 1.7 to 11.0 times more bacteria in the rhizosphere than found in bulk soil (Fig. 2C).

The number of isolates with protease, nitrate and nitrite reductase, and phosphatase were 2.0 to 8.1 times higher in the rhizosphere than in the bulk soil, whereas the numbers of urease-carrying bacteria were not different from those in bulk soil (Fig. 2B and 2D). Bulk soil isolates from 1/10 TSBA remained constant during the growth season (2.3×10^6 to 1.5×10^7 CFU/g of soil), depending on the enzyme. In the rhizosphere, however, an increasing number of bacteria positive for the enzymes were found during the growth season. Thus between 4.5×10^6 and 3.1×10^7 CFU/g of soil was found in May increasing to 9.4×10^6 to 8.8×10^7 CFU/g of soil in August corresponding to 1.6 to 4.7 times more bacteria in August carrying the tested enzymes. The number of bulk soil bacteria carrying one of the enzymes was not different when isolation was done on CSEA compared to 1/10 TSBA (Fig. 2B and 2D). CSEA also supported growth of an increasing number of bacteria in the rhizosphere during the growth season. Depending on the enzyme, numbers varied from 3.0×10^6 to 1.8×10^7 CFU/g of soil in May and were between 1.5×10^7 to 5.0×10^7 CFU/g of soil in August (Fig. 2D). The rhizosphere effect generally resulted in 1.5 to 6.1 times more bacteria than in bulk soil. Only the number of urease-positive bacteria in the May sample was not significantly different from that in the bulk soil (Fig. 2D).

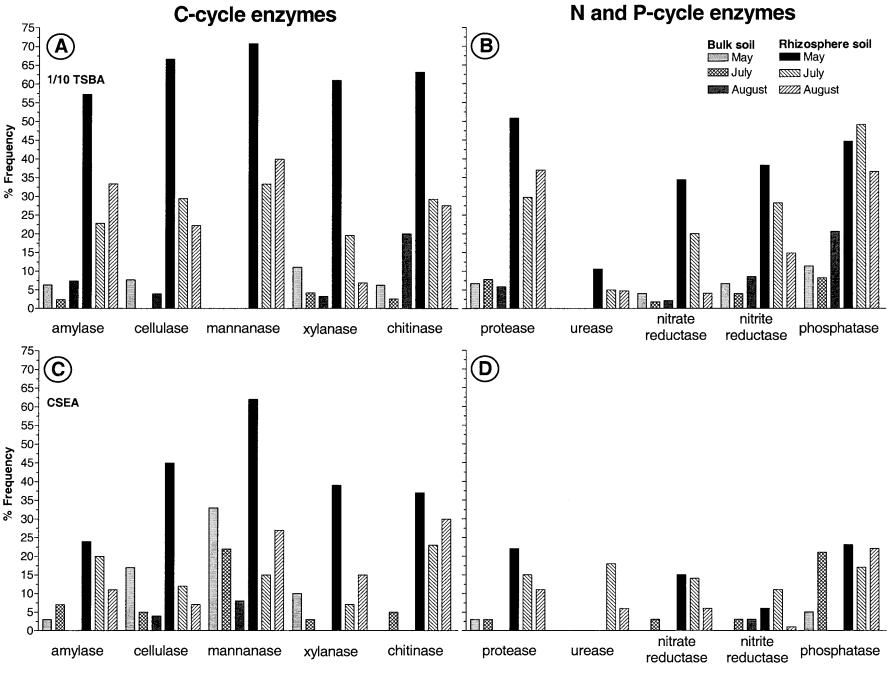

Fig. 4. Contribution by *Cytophaga*-like bacteria to the total number of bacteria positive for the 10 enzymes. Bacteria isolated on 1/10 TSBA and on CSEA are shown in top and bottom figures, respectively. Bacteria from the 3 replicates were pooled. When bar is not present, positive bacteria were not found for the specific enzyme.

Fluorescent Pseudomonads and Cytophaga*-like Bacteria*

Little seasonal variation was observed in the numbers of the two taxonomic groups of bacteria *Cytophaga*-like bacteria (CLB) and fluorescent pseudomonads obtained from bulk or rhizosphere soil samples (Fig. 3A). The number of bacteria in bulk soil was constant through out the sample time on both substrates ranging from 4.8×10^5 to 1.8×10^6 CFU/g of soil for CLB and from 1.2×10^5 to 5.0×10^5 CFU/g of soil for fluorescent pseudomonads (Fig. 3A). In the rhizosphere, CFU numbers of CLB and fluorescent pseudomonads ranged 1.9×10^7 to 2.5×10^7 CFU/g of soil and 2.9×10^6 to 5.8×10^6 CFU/g of soil, respectively, when isolated on 1/10 TSBA, while the numbers obtained on CSEA ranged from 5.5×10^5 to 8.8×10^6 and 1.2×10^6 to 3.2×10^6 CFU/g of soil, respectively. The rhizosphere thus contained about 12 times more CLB and 8 times more fluorescent pseudomonads than the bulk soil. The CLB seem to dominate the young rhizosphere of barley. That is, on average 9 to 25% of the isolates from 1/10 TSBA and 3 to 8% of the isolates from CSEA were identified as CLB (Fig. 3B). In contrast, less than 5% were identified as fluorescent pseudomonads, regardless of the growth substrate. In bulk soil, less than 5% of the bacteria were identified as CLB or fluorescent pseudomonads. The high number of CLB present in the rhizosphere in May was also reflected in the percentage of isolates from 1/10 TSBA that were positive for amylase, cellulase, mannanase, xylanase, chitinase, and protease (Fig. 4). CLB were thus responsible for up to 71% of the bacteria positive for these enzymes (Fig. 4A–D). During the plant growth season the CLB contribution was reduced successively as their numbers were stable while the total CFU increased by a factor of 3. The fluorescent pseudomonads contributed less than 3% of all bacteria positive for the 10 enzymes (data not shown).

Discussion

In our study we observed a stable bacterial community in the bulk soil and a more dynamic community in the rhizosphere soil during a growth season of barley plants. The numbers of bacteria determined by isolation from nutrient-rich (1/10 TSBA) and nutrient-poor (CSEA) substrates were in good agreement with published values [11, 17, 25, 40]. Up to 2.6% of bacteria from the rhizosphere were culturable and about 1% from the bulk soil were culturable, which is also in the same range previously reported [17, 25]. Even though culturable bacteria comprise a minor fraction of the total counts, Olsen and Bakken [25] found the culturable fraction represented 80–90% of the total bacterial biovolume. They were also important in turnover of carbon and nutrients in soil, although it can not be excluded that the nonculturable fraction also contribute to some extent to the turnover of carbon and nutrients. Thus, the higher culturability of rhizosphere bacteria could very likely reflect a higher nutritional status of the rhizosphere bacteria. Indeed, Söderberg and Bååth [35] found higher ^{3}H-thymidine and ^{14}C-leucine incorporation into bacteria from the rhizosphere as compared to bulk soil bacteria.

For growth of soil bacteria we tested two substrates: a standard substrate for cultivation of soil bacteria (1/10 TSBA) and the nutrient-poor substrate cold soil extract agar (CSEA). The CSEA substrate has been reported to support growth of a higher number of bacteria by mimicking the low nutrient content of the soil environment [17, 25]. However, our results showed no significant difference in CFU numbers when compared to 1/10 TSBA which can be caused by differences in the characteristics of the soil used or the degree of stress the bacteria were under at the sampling time. A rhizosphere effect was also seen in the number of bacteria carrying enzymes involved in C, N, or P turnover, in that they were higher than bulk soil. This agrees with earlier observations that the rhizosphere stimulated a higher enzyme activity than the bulk soil [3]. By direct measurements of enzyme activities involved in carbohydrate turnover, Mawdsley and Burns [22] found a higher activity in the rhizosphere soil compared to bulk soil. Bulk soil enzyme activities did not change during the 35 day long plant experiment. It was characteristic in our study that the number of bacteria carrying one of the C-turnover enzymes, as well urease and phosphatase, remained constant during the growth season although the CFU numbers increased. Bacteria carrying these enzymes are thus not favored by the rhizosphere or the growing plant. During plant growth a succession of different groups of organisms takes place in the rhizosphere [36]. Early colonization of roots is dominated by bacteria whereas during later plant growth fungi become important in the decomposition of cellulose and hemicellulose [38]. Thus competition for substrate with fungi might explain why bacteria carrying cellulase, mannanase, and chitinase do not increase in numbers during plant growth. Hu and van Bruggen [14] followed ^{14}C-labeled cellulose degradation by $^{14}CO_2$ evolution and found that when bacteria and/or fungi populations were

specifically inhibited by antibiotics, cellulose decomposition could be explained by a multiphasic event with bacteria dominating the initial phase and fungi the second phase of their 28-day experiment.

Among the other enzyme functions tested in our study only bacteria carrying protease and nitrate and nitrite reductase increased in numbers in the rhizosphere soil during plant growth. Since nitrate reductase and denitrification enzymes function only under low-oxygen conditions, the presence of these enzymatic activities suggest that the mature barley rhizosphere may become oxygen limited. Depletion of oxygen in the rhizosphere could be caused by consumption of root exudates and residues. Increasing numbers of bacteria with nitrate reductase and denitrification enzymes possibly reflect a widespread distribution of these enzymes among bacterial species [29]. Likewise, protease has also been reported to be an enzyme common to many species [2].

During the growth season with barley, CLB were found in much higher numbers than the fluorescent pseudomonads. This was surprising since it is well known that the bacterial community of young rhizosphere is dominated by fluorescent pseudomonads [10, 20, 21]. However, low numbers of fluorescent pseudomonads in the rhizosphere have been reported [12, 26, 30]. In our work they contributed less than 3% of the bacteria positive for the enzymes investigated and they were most frequently positive for protease and nitrate and nitrite reductase. The latter two enzymes are common traits of the fluorescent pseudomonads [37].

Dominance of CLB in the rhizosphere is seldom reported, although CLB are known to be common members of the soil bacterial community [17, 20, 21]. Recently, analyses of fatty acid profiles of bacteria present in the barley rhizosphere have shown an increasing frequency of CLB when approaching the root surface, suggesting that CLB were a dominant part of the bacterial community [26]. In a study of bacteria carrying peptidases [2], CLB were found to be important contributors and therefore should be considered important in N-mineralization in soils. In our study, CLB from bulk soil contributed less than 10% of all bacteria positive for protease, whereas in the rhizosphere, CLB contributed up to 55%. The CLB contribution to the other N-turnover enzymes (urease and nitrate and nitrite reductase) was also high (40%), as was their contribution of P-turnover enzymes (45%). Furthermore, CLB accounted for 71% of all bacteria carrying the C-turnover enzymes. The early and comprehensive colonization of the plant roots by CLB may be a consequence of the high output of organic compounds from the growing plant. Thus CLB could be favored in such habitats [31].

Our current knowledge about the ecology of CLB in the rhizosphere is limited. Gliding motility makes it possible for CLB to translocate to new sources of carbon and nutrients and thus perhaps gives it an advantage in the competition with other bacteria. Many CLB produce exopolysaccharides, which are considered important in stabilization of soil aggregates in the rhizosphere [8]. In addition, CLB are known to produce different kinds of antibiotics and extracellular enzymes [8, 31]. Consequently, it seems, that CLB are well suited for the rhizosphere where nutrients and energy sources can range from starch to recalcitrant biopolymers such as cellulose or chitin. The importance of CLB in habitats such as lakes, ocean, sediments, and active sludge has recently been established using oligonucleotide probes and *in situ* hybridization [9, 13, 18, 34]. Combining these results with ours makes CLB an interesting group of bacteria that are more widespread in nature and important in degradation of organic matter than previously known.

Our results demonstrate that enzymatic potentials of bacterial communities in the rhizosphere of barley are more dynamic than those in bulk soil. This occurred despite a reduction in numbers of CLB and fluorescent pseudomonads over time. Only the number of rhizosphere bacteria carrying the three nitrogen turnover enzymes, protease and nitrate and nitrite reductase, increased in response to the changing environment associated with barley growth. In contrast, the numbers of bacteria carrying the other enzymes were not stimulated by the growing plant. The numerical role of CLB as a contributor of around 25% of bacteria in spring rhizosphere samples and the very important contribution to bacteria carrying enzymes involved in the turnover of C, N, and P suggest that more studies on the presence and enzymatic activity of CLB in rhizosphere are needed.

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