[SIV-1]

Advances in Soil Microbial Ecology and the Ecocollections

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Abstract

Oligotrophic bacteria isolated from forest soil showed a specific community consisting of various taxonomic groups compared with those in other soil or aquatic habitats. Based on the cell shape, the isolates were divided into four groups: regular rod, curved/spiral rod, irregular rod, and prosthecate bacteria. The cellular fatty acids 60 oligotrophic isolates were analyzed. At the dendrogram based on cellular fatty acid composition, four clusters(I-IV) were separated at a euclidian distance of about 50. Based on the 16S rDNA sequence analysis, the two representative strains(MH256 and MA828) of cluster 3 showed the close relation to genera, *Xathomonas/Stenotrophomonas,* but were not included in these genera. The isolates with Q-10 were also studied. They are corresponded to the two large groups in Proteobacteria alpha subdivision. One was incorporated in the genus *Bradyrhizobium* cluster, which also includes *Agromonas*, a genus for oligotrophic bacteria. The strains of the other group showed high similarity to the genus *Agrobacterium.*

We attempted to screening of bioactive compounds from oligotrophs which was isolated from forest soil. The active compounds were analyzed by mass and NMR spectrum, one of them identified as crisamicin A. Another one designated as SAPH is a new compound. The results indicate that there were possibilities for finding new compounds from the rare microorganisms such as oligotrophs.

Introduction

In soil microbial ecology one of the most important questions is why there is a large discrepancy between the viable and the direct microscopic counts of soil bacteria. Many soil microbiologists have used the plate method to study bacteria both qualitatively and quantitatively. It is often considered that the plate count method is useless in understanding bacterial life in soil or gives false information about soil bacteria.

Cultivation of isolated bacteria supplies us with a great amount of information useful for both the taxonomy and the ecology of the organisms. The isolation of soil bacteria, however, has been mostly confined to either those participating in the biogeochemical cycling process or those with easily recognized traits. Therefore the list of isolated organisms is largely biased and narrowly limited. For the analysis of the bacterial community of th soil, extensive studies should be made to isolate and characterize the 'viable' cells of soil bacteria.

In natural environments such as soil, river water the concentrations of nutrients for microbial growth are

usually very low compared with those of laboratory media; the concentration of C source in soil solution is usually less than several ppm, although that of laboratory media is about 1%. Under such a poor nutrient condition there may be found unknown types of bacteria physiologically different from those used routinely in laboratory experiments (5, 6, 9). Since Hattori & Hattori (3) used a 100-fold dilution of the full strength nutrient broth(DNB) to isolate soil bacteria, and found many isolates which were unable to grow on the full strength medium: such organisms were called DNB organisms. These bacteria may be a part of oligotrophic bacteria, although the term has not received any strict definition. We observed that oligotrophic bacteria, which could develop on the medium containing less then 1 mg of organic carbon per liter.

Oligotrophic bacteria are found in aquatic and terrestrial environments, and they may play an important role in decomposing organic matter and recycling nutrients in such low-nutrient environment as ocean, lakes or soil (1, 6). However, information on their physiology, biochemistry and taxonomy is still so far very limited (7, 8).

In this study, We examined 203 strains of oligotrophic bacteria from forest soil at different layers(L, F, H and A layers) on the basis of chemotaxonomic and phenotypic characters, such as cellular fatty acids, quinone system, and DNA base composition. The 16S rDNA sequence (about 1500 bp) was determined for the isolates from each DNA-DNA hybridization groups. We shall outline our ecocollections hitherto established and described some unique features of soil viable bacteria (10, 11).

Results and Discussion

1. A quantitative evaluation of VBNC soil bacteria using epifluorescence microscopy

The direct viable count (DVC) and plate count (PC) methods was used to measure the number of viable bacteria in forest soil samples. As a result, the number of living bacteria by DVC was comprised 25% of the total direct count (TDC), whereas the number of living bacteria by PC was less then 1% of DVC. Such results show that viable but non-culturable (VBNC) bacteria exist in the soil.

2. Dilution effect of nutrient broth on the plate count

The average number of bacteria on the DNB medium was larger than that from the NB medium with all samples taken at different depth throughout the year. Such results suggest that DNB-organisms exist abundantly in bacterial population of rendzina forest soil throughout the year. The number of colonies on NB plates became almost maximal within in a few days, but the number of colonies on DNB plates increased with incubation time following double or triple colony formation curves (5), and thus the final counts on the DNB medium were about 10-fold higher than those on the NB medium.

3. Isolation of oligotrophs

Isolates from DNB plates were tested for their capability of growing on the NB medium and divided into two groups: NB and DNB organisms. About half of the isolates from the upper layers of the soil profile were DNB organisms. Among 393 DNB organisms, 203 isolates were selected as oligotrophs. A large percentage of

such oligotrophs was isolated from the colonies which appeared after 600 h incubation time, whereas, only a few oligotrophic bacteria were isolated from the colonies formed before 600 h.

4. Grouping of 203 oligotrophic isolates

Based on the cell shape, isolates were divided into four groups: 1. Regular rods shaped organisms (53 isolates); 2. Curved and spiral shape organisms (30 isolates); 3. Irregular rods shaped organisms (66 isolates); and 4. Prosthecate organisms (54 isolates). I made further subdivision of these groups .

5. Physiological characteristics of the oligotrophic isolates

None of the oligotrophic isolates decomposed cellulose. Only very few isolates hydrolyzed macromolecular compounds, like starch (6% of the total number of isolates), gelatin(12% of the total of the isolates), and casein(10% of the total isolates). Sixty two percent of the prosthecate bacteria (Group 4) utilized methanol; whereas small amounts of methanol were utilized by the other group of isolates. Only 10% of the oligotrophic isolates reduced nitrate to nitrite.

It is notable that the growth of organisms is remarkably affected by 0.5% each NaCl and peptone-meat extract mixture.

6. Grouping of oligotrophic isolates using cellular fatty acid profiles

Analysis of the cellular fatty acid composition is useful for the classification and identification of various bacterial genera. It is considered that cellular fatty acid analysis is suitable for differentiation and grouping of bacterial isolates. Sixty bacterial strains used are selected from the oligotrophic gram-negative bacteria. The cellular fatty acids 60 oligotrophc isolates were analyzed. The 30 fatty acids which were identified or characterized are classified.

At the dendrogram based on cellular fatty acid composition, four clusters(I - IV) were separated at a euclidian distance of about 50; 15 strains belonged to group I, 17 strains to group II, 4 strains to group III, and group IV included 24 strains. Group IV showed further subdivision 2.

7. Phylogenetic analysis

The strains representing cluster 4a contained C16:0 and C18:0 acids predominantly. 3OH-C14:0 and 3OH-C16:0 acids were also found as the characteristic components, Their quinone system is Q-8. Seven strains of this cluster were divided into three DNA homology groups. They are accommodated in the cluster of the genus *Burkholderia* in the Proteobacteria gamma subdivision. The chemotaxonomic profiles of the isolates showed good agreement with those of the genus *Burkholderia*.

Cluster 3 was characterized by the presence of branched-chain fatty acids, iso-C15:0, iso-C17:1, and iso-C17:0 as the major components. The strains also possessed Q-8 These chemotaxonomy suggested the close relationship of the isolates with *Xathomonas/Stenotrophomonas* group. Based on the 16S rDNA sequence analysis, the two representative strains(MH256 and MA828) of cluster 3 showed the close relation to genera,

Xathomonas/Stenotrophomonas, but were not included in these genera. These strains were even further away from core *Xanthomonas*, and clearly were seen to branch outside the cluster formed by the *Stenotrophomonas maltophilia*. MH256 and MA828 16S rDNA sequence was different enough to put new genus on a separate branch.

In addition to these Q-8 containing strains, the isolates with Q-10 were also studied. They are corresponded to the two large groups in Proteobacteria alpha subdivision. One was incorporated in the genus *Bradyrhizobium* cluster, which also includes *Agromonas*, a genus for oligotrophic bacteria. The strains of the other group showed high similarity to the genus Agrobacterium.

The oligotrophic bacteria studied here showed diversity and indicated the close relationship to established genera of non-oligotrophic bacteria. The results of this study will be a great help for elucidation of oligotrophy to compare the two types of nutrition-utilization.

8. The Ecocollections

By this time, screening of antibiotics was set limits to copiotrophs which needed a high-concentration of organic matter for growing, and oligotrophs which grow only in low nutrients were left untouched. Accordingly screening of bioactive compounds from oligotrophs has a high possibility as compare with copiotrophs in the development of new or leading compounds. So, we attempted to screening of bioactive compounds from oligotrophs which was isolated from forest soil. The active compounds were analyzed by mass and NMR spectrum, one of them identified as crisamicin A. Another one designated as SAPH is a new compound. The results indicate that there were possibilities for finding new compounds from the rare microorganisms such as oligotrophs.

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