Characteristics of Newly Isolated Nitrifying Bacteria from Rhizoplane of Paddy Rice

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An ammonia-oxidizing bacterium, strain NRS527 and nitrite-oxidizing bacterium, strain NRB 5220 were newly isolated from rhizoplane of paddy rice. The cells of strain NRS527 are spiral (0.2–0.3 μm wide, with 3–6 turns), gram negative, obligately aerobic, and chemolithotrophic. Intracytoplasmic membranes, a characteristic of ammonia-oxidizing bacteria are absent. The optimum concentration of ammonium sulfate (substrate) in the medium is 38 mM. The G+C content of the total DNA is 59.18 mol%. The similarity of 16S rRNA (%) to Nitrosospira briersis C-128 is 94.71. The supplemental effect of organic compounds could not be recognized. The cells of strain NRB5220 are rod shaped (0.5–0.8×1.0–2.0 μm), gram negative, and obligately aerobic. The G+C content is 60.37 mol%. The similarity of 16S rRNA (%) to Nitrobacter agilis ATCC14123 is 97.38. The optimum concentration of sodium nitrite (substrate) in the medium is 22 mM. Growth of strain NRB5220 is hastened when peptone and yeast extract are added to the medium as nutrients. Both strains were resistant to tetracycline at 33 μg/ml. The new nitrifying bacteria isolated from rhizoplane of paddy rice were identified as Nitrosospira sp. NRS527 and Nitrobacter sp. NRB5220.

Key words: nitrifying bacteria, Nitrosospira, Nitrobacter, rhizoplane, paddy rice

In recent years, preservation of the environment has been advocated and maintenance of the global ecosystem is being taken seriously. Moreover, how to secure resources in connection with the supply of food has become an important research subject. As a result, research on nitrifying bacteria that live universally in nature is becoming more and more popular. It has become clear that nitrifying bacteria are microorganisms deeply involved with environment protection and maintenance of resources.

Being chemooautotrophic, nitrifying bacteria are difficult to isolate and culture. Due to this, there are few studies in which a pure bacterium has been used in symbiosis with plant roots though many investigations have been carried out on various respects of these bacteria. We are very interested in the relationship between rice, the staple food in Japan, and the nitrifying bacteria living symbiotically with the rice root15. In contrast with most crops that use nitrogen

from nitrate as a nitrogen source, rice uses ammonical nitrogen20. It is rather interesting that ammonical nitrogen is the energy source of ammonia-oxidizing bacteria living on the rice roots. In nitrifying bacteria which are strict aerobic bacteria, there is much interest in the reason for inhabiting the rhizoplane of paddy rice which stretches the root in the reduced layer of paddy fields where little oxygen exists. There is a need to clarify the method for acquiring oxygen and its mechanism, since oxygen is inhibited in the rhizosphere, especially in the rhizoplane.

To carry out a biochemical study on the nitrifying bacteria living symbiotically on the rice root, it is necessary to isolate pure nitrifying bacteria. Therefore, we have tried to isolate ammonia- and nitrite-oxidizing bacteria from the rice root according to a pure isolation procedure we have designed. Here we report the results on the acquisition of pure strains and the properties of these strains.

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Materials and Methods

Strains and cultivation

The organisms examined were ammonia-oxidizing bacterium, Nitrosomonas europaea ATCC25978T; the type strain of genus Nitrosomonas\textsuperscript{20}, Nitrosomonas sp. TK794\textsuperscript{10}, Nitrobacter agilis ATCC 14123T (Nitrobacter winogradskyi IFO14297T); the type strain of genus Nitrobacter\textsuperscript{20}, strain NRS527 (ammonia oxidizer) and strain NRB5220 (nitrite oxidizer) isolated in this study. The cultivation medium used for ammonia-oxidizing bacteria was HEPES-medium containing 2.5 g (NH$_4$)$_2$SO$_4$, 0.5 g KH$_2$PO$_4$, 11.92 g HEPES (N-2-hydroxyethyl-piperazine-N'-2-ethane sulfonic acid), 0.5 g NaHCO$_3$, 100 mg MgSO$_4$·7H$_2$O, 5 mg CaCl$_2$·2H$_2$O and 75 mg Fe-EDTA per liter of distilled water. The medium pH was adjusted to 7.8–8.0. Since nitrification lowers the medium pH, the growth of nitrifiers can be detected by the addition of cresol red. Since the above media act as buffers against a reduction in pH owing to the presence of HEPES, the long-term culture of ammonia-oxidizing bacteria is possible. The cultivation medium used for nitrite-oxidizing bacteria was BE-medium containing 2.0 g NaNO$_3$, 0.5 g NaCl, 0.05 g MgSO$_4$·7H$_2$O, 0.15 g KH$_2$PO$_4$, 7 mg CaCO$_3$, 50 μg (NH$_4$)$_2$Mo$_7$O$_{24}$·4H$_2$O and 150 μg FeSO$_4$·7H$_2$O per liter of distilled water. Gelann gum (Phytage; Sigma, MO, USA)\textsuperscript{9} was mixed with the media to a final concentration of 1% (w/v) for gelification of the plate culture. Nutrient agar, malt extract agar and YM agar (Difco, MI, USA) were used to detect contaminating bacteria. Nitrous acid was determined using the Griess-Ilosvey reagent\textsuperscript{6} and a spectrophotometer (160A; Shimadzu, Kyoto).

Pure isolation

The nitrifying bacterium was isolated in pure form, as described previously\textsuperscript{17}. In each of the ten samples, the paddy rice was collected from a paddy field in Tokyo Machida City in mid-July and mid-August, 1997. The soil adhered to the root of the rice was removed, and subsequently the root was washed several times. The root was then placed in a 500-ml shoulder-type shaken flask containing 100 ml of ammonium sulfate-free HEPES-medium, and shaken for 30 min. It was next transferred to an identical flask containing fresh HEPES-medium, and then shaken at room temperature overnight before 10 ml of a supernatant was inoculated to each flask containing HEPES- or BE-medium. Both cultures were prepared from rhizoplane of the same root of paddy rice. The shaking was followed by culturing at 30°C for 2 weeks.

Each culture that produced or consumed nitrous acid at more than 100 μg/ml was inoculated at 10% into a 300-ml Erlenmeyer flask containing 100 ml HEPES- and BE-medium and subcultured for 5 days. This subculture was repeated 5 times. Following the second subculture, the culture medium was filtered through a polycarbonate membrane (pore size, 2.0 μm; PCMB) to eliminate large contaminating microorganisms and the like. Three samples that produced or consumed more than 100 μg/ml nitrous acid were used for further isolation. Culture No. 527 (ammonia oxidizer) and No. 5220 (nitrite oxidizer) were produced and each consumed more than 60 μg/ml nitrous acid during 7 days at 30°C. The final subculture of both cultures was diluted and plated on gellan gum plates and incubated at 30°C for 15–20 days. On plates at dilutions of 10$^{-2}$ and 10$^{-4}$, colony formation was noted after 14-days incubation. Its extent was assessed in terms of the number of colonies divided by the number of cells spread over the plate. Thirty large colonies (about 1.0 mm in diameter) from each plate were inoculated into a test tube containing 5 ml of medium and shaken at 300 strokes/min. At 10–15 days, five cultures produced and consumed more than 50 μg/ml nitrous acid. The nitrous acid formation capacity and the presence of contaminating bacteria were examined using the three media for heterotrophs. Culture purity was confirmed by microscopic observation.

Electron Microscopy

Both strains were characterized taxonomically by morphological observation. After critical point drying, all specimens were sputter-coated with gold palladium and observed with a scanning electron microscope (S-3500N; Hitachi, Tokyo). Ultra-thin sections of the cells were post-stained in uranium acetate and lead citrate by the standard method and viewed with a transmission electron microscope (JEM1200E; JEOL, Tokyo).

DNA isolation, PCR amplification and sequencing of amplified 16S ribosomal RNA genes and data analysis

DNA was extracted from cells and purified according to Marmur\textsuperscript{22} with some modification. Purified DNA was hydrolyzed and analyzed by high performance liquid chromatography (HPLC) as follows\textsuperscript{10}. Purified DNA was hydrolyzed with nuclease P1 (Yamasu Shoyu, Tokyo) and digested with alkaline phosphate (Sigma). The molar ratio of deoxyribonucleosides in the reaction mixture was determined by liquid chromatography (JASCO, Tokyo) with a shim-pack GLCOS(M) column. A standard mixture of the four deoxyribonucleotides was prepared using a DNA-GC Kit (Yamasu Shoyu) with alkaline phosphatase. The DNA
Fig. 1. Scanning electron micrograph (A) and transmission electron micrograph (B and C) of strain NRS527 cells grown in HEPES-medium at 30°C for 7 d. The bars represent 0.5 μm.

Fig. 2. Scanning electron micrograph (A) and transmission electron micrograph (B) of strain NRB5220 cells grown in BE-medium at 30°C for 5 d. The bars represent 1.0 μm.

preparation was also used as a source of DNA for PCR amplification. The oligonucleotide primers U1 and U2 originally designed by Weisburg et al. as the latter half (20b) of fD1 and fD223 were used. Bacterial chromosomal DNA (1 μg) was subjected to PCR in a total volume of 100 μl of a reaction mixture containing 1.0 unit of Taq DNA polymerase (Takara Shuzo, Kyoto), 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM MgCl2, 20 pmol of each primer, and 0.2 mM of each deoxyribonucleotide (Takara Shuzo). The reaction mixture in a 0.5-ml tube was overlaid with 50 μl of mineral oil to prevent evaporation. The thermal profile consisted of an initial denaturation at 94°C for 5 min, followed by 30 continuous cycles of denaturation at 94°C for 1 min, primer annealing at 60°C for 1.5 min, and extension at 72°C for 1 min. In the final cycle, extension was done for 10 min to ensure full extension of the products. The PCR products
were extracted with chloroform and analyzed by agarose gel electrophoresis. Amplified 16S rRNA gene (16S rDNA) sequences were determined by direct PCR sequencing by the dideoxy chain terminating method. DNA sequences were obtained from the PCR products using the same primers and an automatic sequencer (ABI377). Sequences were aligned using the Clustal program. For distance matrix analysis the Jukes and Cantor correction and the UPGMA method were used. In all cases, analyses was performed by GENETYX-WIN Ver. 4.04 (SDC, Tokyo).

Results and Discussion

Morphology

The isolates that showed the most growth were designated as NRS527 (ammonia oxidizer) and NRB5220 (nitrite oxidizer). When cells of strain NRS527 and NRB5220 were smeared and cultured on gellan gum plates, circular, reddish (NRS527) and creamy white (NRB5220) colonies 1.0–2.0 mm in diameter appeared at 4–5 weeks. Under optimal conditions, from a 10-L mass culture, nearly 1.0–1.5 grams (wet weight) of cells were obtained.

The cells of strain NRS527 were observed to be spiral, 0.2–0.3 μm in width, with between 3 and 6 turns, and lacking flagella (Fig. 1A). Short spirals have the appearance of short rods cells. The cells were non-motile in wet mounts. In most Nitrosomonas strains, the cytomembranes are arranged as peripheral lamellae. As shown in Fig. 1B and 1C, the cells of strain NRS527 lack any of the extensive intracytoplasmic membrane systems typical of nitrifying bacteria; however, they do have plasma membranes. The cells of strain NRB5220 were rod-shaped, 1.0–2.0 μm long and 0.5–0.8 μm wide, lacking flagella (Fig. 2(A)). The cells were non-motile in wet mounts. In most Nitrobacter strains, the cytomembranes are arranged as peripheral lamellae. As shown in Fig. 2(B), the cells of strain NRB5220 were observed in the intracytoplasmic membrane. Carboxysomes were typical inclusion bodies. Other cell features of both strains are summarized in Table 1.

Physiology

Strain NRS527 was an obligate aerobe. Urea is used as the ammonia source by most isolates but not by strain NRS527. Strain NRS527 sufficiently grew unlike the strains at pH 7.0, and growth at other pH was inferior. The optimum temperature for growth of strain NRS527 and NRB5220 was essentially the same as for strain ATCC25978T, TK794 and ATCC14123T. Strain NRB5220 was an obligate aerobe. The effects of the various media and their ammonium salt and nitrite concentration on the growth of strain NRS527 and NRB5220 were examined and compared with those for strain ATCC 25978T and ATCC 14123T. Nitrous acid formation (indicating the degree of growth) by strain NRS527 was significant in the HEPES-

<table>
<thead>
<tr>
<th></th>
<th>NRS527a</th>
<th>NRB5220b</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell morphology</td>
<td>Spiral, 0.2–0.3 μm width, 3–6 turn</td>
<td>Rod-shaped, 0.5–0.8×1.0–2.0 μm</td>
</tr>
<tr>
<td>Reproduction</td>
<td>Binary fission</td>
<td>Budding</td>
</tr>
<tr>
<td>Gram stain</td>
<td>Negative</td>
<td>Negative</td>
</tr>
<tr>
<td>Size of colonies</td>
<td>1–1.5 mm</td>
<td>1–2.0 mm</td>
</tr>
<tr>
<td>Motility</td>
<td>Nonmotile</td>
<td>Nonmotile</td>
</tr>
<tr>
<td>Growth</td>
<td>Chemolithotroph</td>
<td>Chemolithotroph</td>
</tr>
<tr>
<td></td>
<td>Obligate aerobic</td>
<td>Obligate aerobic</td>
</tr>
<tr>
<td>Cytomembranes</td>
<td>Lacking</td>
<td>Lacking</td>
</tr>
<tr>
<td>G+C (mol%)</td>
<td>59.18</td>
<td>60.37</td>
</tr>
<tr>
<td>Similarity of 16S rRNA (%)</td>
<td>94.71c 94.02d 84.24c</td>
<td>97.45f</td>
</tr>
<tr>
<td>Optimum temp. for growth</td>
<td>30°C</td>
<td>30°C</td>
</tr>
<tr>
<td>Optimum pH for growth</td>
<td>7.5</td>
<td>7.0</td>
</tr>
<tr>
<td>Optimum conc. of substrate</td>
<td>38 mM [NH₄]₂SO₄</td>
<td>22 mM [NaNO₃]</td>
</tr>
<tr>
<td>Use of organic compounds</td>
<td>Negative</td>
<td>Positive (peptone, yeast extract)</td>
</tr>
<tr>
<td>Resistance for antibiotics (33 μg/ml)</td>
<td>Tetracycline</td>
<td>Tetracycline, Chloramphenicol</td>
</tr>
<tr>
<td>Habitat</td>
<td>Rhizoplano</td>
<td>Rhizoplano</td>
</tr>
</tbody>
</table>

Ammonia oxidizer, b Nitrite oxidizer, c Nitrosospira briensis C-128, d Nitrosovibrio tenuis Nv12, e Nitrosolobus multififormis C-71, f Nitrobacter agilis ATCC14123.
Table 2. Effect of organic compounds on nitrite consumption by strain NRB5220.

<table>
<thead>
<tr>
<th>Addition to HEPES-medium</th>
<th>Final conc. of medium</th>
<th>Consumed NO$_2$-N (µg/ml)</th>
</tr>
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<tbody>
<tr>
<td>None</td>
<td>—</td>
<td>40</td>
</tr>
<tr>
<td>Yeast extract</td>
<td>100 µg/ml</td>
<td>100</td>
</tr>
<tr>
<td>Peptone</td>
<td>200 µg/ml</td>
<td>105</td>
</tr>
<tr>
<td>Casamino acid</td>
<td>200 µg/ml</td>
<td>50</td>
</tr>
<tr>
<td>Casamino acid (Vitamin-free)</td>
<td>200 µg/ml</td>
<td>60</td>
</tr>
<tr>
<td>Soil extract</td>
<td>10% W/V</td>
<td>80</td>
</tr>
</tbody>
</table>

Organic compounds were sterilized by membrane filtration and added to give final concentrations as shown above. Incubation with shaking was carried out at 30°C for 5 days. Values, expressed in micromolars per milliliter of culture, represent averages for duplicate flasks.

medium, but poor in P- or PM-medium$^9$ due to inhibition by the high phosphate concentration in the latter. Strain NRS527 produced the greatest amount of nitrous acid when the basic ammonium sulfate concentration in the medium was 38 mM. The BE-medium was suitable for strain NRB5220, and the optimum sodium nitrite concentration in this medium was 22 mM. Strain NRS527 and NRB5220 also required a nitrogen source (energy source) of standard concentration, and this tended to be the case for non-photosphere nitrifying bacteria. The effect of several organic compounds in culture medium was examined in order to investigate the nutritional requirement of microorganisms of the rhizoplane. The following were examined: yeast extract, casamino acid, peptone of 0.01-0.1 µg/ml concentration and soil extract$^9$ of 10% (w/v) concentration. No supplemental effect of either compound could be recognized in the ammonia-oxidizing bacterium, strain NRS527. The growth of nitrifying bacteria is inhibited by most amino-compounds$^{10}$. The nitrate-oxidizing bacteria studied differed in their ability to use organic compounds as a carbon and energy source. About half of the strains studied could be grown heterotrophically on acetate, while the remaining strains appeared to be obligate chemolithotrophs$^{14}$. As shown in Table 2, an effect of all compounds except for casamino acid was recognized in strain NRB5220. Notably, there was a supplemental effect following the addition of peptone on the additive-free case.

The manifold microorganisms in the soil are affected by the secretions in which they are formed. Therefore, it is anticipated that the kinds of materials (for example antibiotics, etc.) affected differ considerably. The effect of the eight antibiotics (conc.: 3.3-330 µg/ml) on the growth of strain NRS527 and NRB5220 was compared. Both strains were easily affected in comparison with the type strains (ATCC 25978T and ATCC 141237T), and there was growth inhibition even at low concentrations. There was resistance only to 3.3-33 µg/ml tetracycline (NRS527, NRB5220) and chloramphenicol (NRS527). Many antibiotics had resistance at between 3.3 and 33 µg/ml to ATCC25978T, which tends to be different from NRS527. However, in ATCC 141237T, there was similar resistance to NRB5220 for tetracycline and chloramphenicol, and there was no such resistance between both strains on occasion.

**Taxonomy**

Since Koops's work, four other species besides *Nitrosospira briensis* have been identified by DNA homology analyses, and divided into two groups (53-54 and 55 mole%) based on GC content$^{11}$. The G+C content of the DNA of strain NRS527 was 59.18 mol%, which is relatively high compared to 54.1 mol% for the genus *Nitrosospira*$^{22}$. The distinguishing phenotypic characteristics of these new species are not known. An UPGMA tree showing the relationships between ammonia-oxidizing bacteria based on

<table>
<thead>
<tr>
<th>Ammonia-oxidizing bacteria</th>
<th>Homology (%)</th>
<th>Nitrite-oxidizing bacteria</th>
<th>Homology (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Nitrosospira</em> sp. NRS527</td>
<td>—</td>
<td><em>Nitrobacter</em> sp. NRB5220</td>
<td>—</td>
</tr>
<tr>
<td><em>Nitrosomonas europaea</em> ATCC2597</td>
<td>88.63</td>
<td><em>Nitrobacter agilis</em> ATCC14123</td>
<td>97.38</td>
</tr>
<tr>
<td><em>Nitrosospira</em> sp. GS833</td>
<td>93.89</td>
<td><em>Nitrobacter hamburgensis</em> X14</td>
<td>95.02</td>
</tr>
<tr>
<td><em>Nitrosospira</em> briensis C-128</td>
<td>94.71</td>
<td><em>Nitrobacter</em> sp. KB212</td>
<td>91.84</td>
</tr>
<tr>
<td><em>Nitrosobacter tenuis</em> C-141</td>
<td>93.37</td>
<td><em>Nitrobacter</em> sp. KB215</td>
<td>92.07</td>
</tr>
<tr>
<td><em>Nitrosobacter tenuis</em> Nv1</td>
<td>92.54</td>
<td><em>Nitrobacter</em> sp. TH21</td>
<td>98.57</td>
</tr>
<tr>
<td><em>Nitrosobacter tenuis</em> Nv12</td>
<td>94.04</td>
<td><em>Nitrobacter winogradskyi</em> IFO1429</td>
<td>97.39</td>
</tr>
<tr>
<td><em>Nitrosolobus multisulfuricon C-71</em></td>
<td>84.24</td>
<td><em>Nitrospina gracilis</em> Atlantic Nb-211</td>
<td>72.50</td>
</tr>
<tr>
<td><em>Nitrosolobus</em> sp. PJA1</td>
<td>92.62</td>
<td><em>Nitrospina gracilis</em> Pacific Nb-3</td>
<td>68.28</td>
</tr>
<tr>
<td><em>Nitrosolobus</em> sp. TCH716</td>
<td>95.81</td>
<td><em>Nitrospira marina</em> 295</td>
<td>67.50</td>
</tr>
</tbody>
</table>
Method: UPGMA

- Nitrosolobus sp. PJA1 (AF353163)
- Nitrosospira sp. GS833 (AF353162)
- Nitrosospira brieris C-128 (M96396)
- Nitrosovitriobrio tenuis Nv12 (M96405)
- Nitrosovitriobrio tenuis C-141 (M96397)
- Nitrosolobus sp. TCH716 (AF353156)
- Nitrosospira sp. NRSS27 (AF353158)
- Nitrosovitriobrio tenuis Nv1 (M96404)
- Nitrosovitriobrio sp. E12 (AF353157)
- Nitrosovitriobrio sp. TYM9 (AF080256)
- Nitrosolobus multiformis C-71 (M96401)
- Nitrosococcus mobilis M93 (AF037105)
- Nitrosomonas sp. TN0632 (AF353159)
- Nitrosomonas europaea ATCC25978 (AF353160)
- Nitrosomonas sp. DYS323 (AF353164)
- Nitrosomonas sp. CNS326 (AF353161)
- Nitrosomonas sp. G1 (AF353155)
- Nitrosomonas sp. TK794 (AB031960)
- Nitrosomonas europaea M103 (AF037106)
- Nitrosococcus mobilis Nc2 (M96403)
- Nitrosomonas marina C-56 (M96400)
- Nitrosomonas europaea C-31 (M96399)
- Nitrosomonas eutropha C-91 (M96402)
- Nitrosococcus oceanus C-107 (M96395)
- Nitrosococcus oceanus C-27 (M96398)
- Escherichia coli (AF233451)

Fig. 3. Phylogenetic tree inferred from comparison of 16S rRNA sequences. The tree was generated using the UPGMA method (13). The accession no. is shown in the parentheses.

The 16S rRNA sequences was constructed (Fig. 3). The newly isolated ammonia-oxidizing bacterium strain NRS527 was clustered with the three genera, Nitrosovitriobrio (strain Nv1, Nv12, C-141, TYM9 and E12), Nitrosospira (strain C-128 and GS833) and Nitrosolobus (strain TCH716 and PJA1). As shown in Table 3, 90% homology was generally noted for the strains of the three genera, Nitrosovitriobrio, Nitrosospira and Nitrosolobus for strain NRS527, and there was no clear distinction. Strain NRS527 showed homology in the 16S rRNA gene of Nitrosospira, N. briensis C-128 of 94.71%. Head et al. (19) recommended that the genera Nitrosovitriobrio, Nitrosospira, and Nitrosolobus be combined in a single genus based on the 16S rRNA sequence comparison. Though Nitrosovitriobrio and Nitrosospira were found to show resemblance by Teske et al. (18) with respect to the presence of a single genus, Nitrosolobus did not appear
present in any cases based on differences in ultrastructure of the cytmembranes\(^2\)). There should be no classification of these three genera based only on phylogenetic relationships of the 16S rRNA gene. In identification of the strain NRS527, morphological characteristics and the phylogenetic tree obtained using the 16S rRNA gene were important, and were based on the genus *Nitrosospira*. Thus, the strain was designated *Nitrosospira* sp. NRS527.

The G+C content of the DNA of strain NRBS220 was 60.37 mol\%, which is consistent with that of the genus *Nitrobacter*\(^3\). The general phylogenetic distribution along with the relation between the purified nitrite-oxidizing bacterium strain NRBS220 and the type strain *Nitrobacter agilis* ATCC 14123T is briefly as follows. Strain NRBS220 clustered with the type strains (data not shown), and exhibited a high level of similarity, its homology being 97.38\% (Table 3). It was placed in the genus *Nitrobacter*, and was designated *Nitrobacter* sp. NRBS220.

In conclusion, the nitrifying bacteria isolated from rhizoplane of paddy rice were identified as an ammonia-oxidizing bacterium, *Nitrosospira* sp. NRS527 and a nitrite-oxidizing bacterium, *Nitrobacter* sp. NRBS220. No supplemental effect of either organic compound (as shown in Table 2) could be recognized in strain NRS527. However, the effect of all organic compounds except for casamino acid was recognized in strain NRBS220. Although the strains living symbiotically on the rhizoplane of paddy rice have not yet been clarified, uptake experiments suggested that nitrifying bacteria are distributed widely at rhizoplane sites, and may function in the growth of paddy rice. Taxonomical study of newly isolated nitrifying bacteria from the rhizoplane of paddy rice should contribute to the ecological analysis of this symbiotic relationship.

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