Influence of Different Cultivars on Populations of Ammonia-Oxidizing Bacteria in the Root Environment of Rice

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Comparisons of the activities and diversities of ammonia-oxidizing bacteria (AOB) in the root environment of different cultivars of rice (Oryza sativa L.) indicated marked differences despite identical environmental conditions during growth. Gross nitrification rates obtained by the 15N dilution technique were significantly higher in a modern variety, IR63087-1-17, than in two traditional varieties. Phylogenetic analysis based on the ammonium monooxygenase gene (amoA) identified strains related to Nitrosospira multiformis and Nitrosomonas europaea as the predominant AOB in our experimental rice system. A method was developed to determine the abundance of AOB on root biofilm samples using fluorescently tagged oligonucleotide probes targeting 16S rRNA. The levels of abundance detected suggested an enrichment of AOB on rice roots. We identified 40 to 69% of AOB on roots of IR63087-1-17 as Nitrosomonas spp., while this subpopulation constituted 7 to 23% of AOB on roots of the other cultivars. These results were generally supported by denaturing gradient gel electrophoresis of the amoA gene and analysis of libraries of cloned amoA. In hydroponic culture, oxygen concentration profiles around secondary roots differed significantly among the tested rice varieties, of which IR63087-1-17 showed maximum leakage of oxygen. The results suggest that varietal differences in the composition and activity of root-associated AOB populations may result from microscale differences in O2 availability.

The supply of nitrogen in paddy fields is recognized as a major limitation toward increased productivity of rice (Oryza sativa L.) (17, 18). Since rice paddies are typically maintained under flooded conditions, ammonium constitutes the major form of mineral N in the bulk soil (14), which is a form to which rice is especially adapted (47, 56). However, considerable amounts of nitrate formed through nitrification do accumulate in the oxygen-rich surface layer of irrigated paddy soils as well as in well-drained upland rice fields and in rain-fed environments during the dry season (14). Compared to ammonium, which is usually bound to the soil matrix, nitrate is mobile within soils, making it susceptible to loss due to leaching and denitrification (21, 53). Nitrification is therefore critical to the supply of N in rice fields as well as to the balance of ammonium and nitrate.

The initial, rate-limiting step of nitrification is carried out by the chemolithoautotrophic ammonia-oxidizing bacteria (AOB) of the β subdivision of the class Proteobacteria (β-Proteobacteria) (38). All AOB are obligately aerobic and generally recalcitrant to cultivation in artificial culture (38). Therefore, molecular methods have been instrumental to our current understanding of their ecology. Based on 16S rRNA sequence analysis, all known AOB of the β-Proteobacteria occurring in terrestrial environments belong to a monophyletic assemblage represented by members of only two genera: Nitrosomonas spp. and Nitrosospira spp. (24, 52). Analysis of the functional ammonium monooxygenase gene (amoA) yields similar, although not identical, evolutionary relationships (39). Studies conducted in various systems suggest that in a variety of soils, Nitrosospira spp. are predominant (23, 27, 31, 43, 49), while environments with high ammonium loads, such as sewage sludge and wastewater, favor Nitrosomonas spp. (32, 35, 43). At present, very little is known about how the population structure of these bacteria affects nitrification in rice soils or how AOB populations are influenced by rice plants.

Under flooded conditions, rice soils become anoxic almost immediately beneath the soil-water interface (42). However, the immediate surface of rice roots offers a supply of oxygen through leakage from aerenchymatous tissue (7, 42). It is recognized that rice roots release oxygen at rates sufficient to support nonspecific aerobic microbial processes (9, 13). The possibility that rice roots may support significant rates of nitrification has also been suggested (10, 33). However, very little information is available regarding the microbiology of AOB on or near the root surface of rice plants. The purpose of this study was to study the abundance, composition, and activity of populations of AOB occurring in the rice root environment.

To detect and characterize the AOB from the roots and rhizosphere, we used PCR coupled with denaturing gradient gel electrophoresis (DGGE) targeting the amoA gene. This approach, combined with clone screening and sequencing (generally but not exclusively targeting the 16S rRNA gene), has been used successfully in various systems, including agronomic soils (16, 29, 31, 36, 43, 50). For quantification of AOB, we employed fluorescence in situ hybridization (FISH) using...
rRNA-targeted oligonucleotide probes. The method has been reliably used for identification and quantification of AOB (22, 32, 35, 55). Aside from allowing direct visualization of bacteria in the environment, the method also has the added advantage of detecting active cells, since the target molecule is the rRNA. However, the above studies have focused mainly on nutrient-rich environments where the abundance and ribosome content of cells are relatively high. The application of FISH in nutrient-poor environments such as soil and roots is more problematic due to lower cell numbers and the presence of interfering autofluorescence from roots and soil particles. In this study, however, we found that the biofilm coating the root surface can be detached by moderate sonication, concentrated, and then probed using FISH. Since the abundances detected were within the detection limits of FISH, we are able to present, for the first time, population estimates of AOB on the rice root surface.

In this study, all experiments were conducted under artificial conditions set to simulate rain-fed paddy fields that favor nitrification (alternating flood and dry periods). In order to account for the possible effect of genotypic variation, all experiments were performed using different rice cultivars. The varieties were chosen based on their drought tolerance and represent a modern hybrid rice variety (IR63087-1-17) and two improved traditional rice varieties (Kao Dawk Mali 105 [KDM1 105] and Mahsuri). To complement results of molecular analysis, gross nitrification rates associated with the three varieties and unplanted soil were estimated by the 15N isotopic dilution technique. Finally, oxygen concentration profiles from the roots of two contrast varieties grown in hydroponic culture were measured to determine the possible relationship between the composition and activity of AOB populations in the root environment and oxygen release from rice roots.

MATERIALS AND METHODS

Plant growth conditions. Three drought-tolerant rain-fed lowland rice cultivars (Oryza sativa L. cv. KDM1 105, Mahsuri, and IR63087-1-17) were pregerminated prior to transplanting into pots. Soil was collected from a rain-fed lowland area in Tarlac Province, the Philippines. The soil is a Luisita series, containing no plants were included as nonrhizosphere controls. Roots that had been scraped off the soil adhering to the root surface. Roots which were extended to 12 h in order to better resolve the more stable DNA duplexes within the detection limits of FISH, we are able to present, for the first time, population estimates of AOB on the rice root surface.

Root samples for FISH (also collected after the 3rd week of induced drought) were fixed in 4% paraformaldehyde pH 7.2 and then fixed in 4% paraformaldehyde in PBS. The roots were immersed in this solution for 3 to 4 days to allow the soil loosely adhering to the root surface to settle. Thereafter, roots were transferred to a 1:1 mixture of ethanol and PBS and then stored at −20°C for long-term storage.

DNA extraction, PCR, and DGGE. DNA from rhizosphere and roots was extracted using a FastDNA SPIN Kit for Soil (Bio 101, Inc. Vista, Calif.) following the manufacturer’s instructions. Prior to extraction, root samples were snap frozen in liquid nitrogen and then ground by mortar and pestle. Extracts from 400 mg of fresh soil and 100 mg of crushed root materials comprising rhizosphere and root DNA extractions, respectively, were finally resuspended in 50 μl of sterile DNase- and RNase-free water (Sigma, St. Louis, Mo.). Two microliters of a 1:10 dilution of the DNA extracts was used as the template for subsequent PCRs using amoA-1F and amoA-2R primers (43) with modifications suggested by Stepchenk et al. (50). PCRs were performed in a total volume of 50 μl using Ex Taq DNA polymerase (Takara Shuzo, Shiga, Japan) in the supplied buffer and a Robocycler 96 thermocycler (Stratagene, La Jolla, Calif.). Amplification proceeded for 35 cycles using previously published cycling parameters (45). PCR products were resolved by electrophoresis on 1.5 to 2% agarose gels and visualized by UV transillumination after staining with SYBR Green I (Molecular Probes, Rockland, Maine). The amount of PCR product was estimated from the gel by using image analysis software (ImageJ 1.26 [freely available at http://rsb.info.nih.gov/ij]) with appropriate DNA size and concentration standard (stable DNA ladder; Sigma-Genosys, Ishikari, Japan). For DGGE, a GC clamp (5′-CCCGGGCGGGGGCCGCGGGGGGGCGGGGG-3′) (34) was attached to the forward primer. PCR amplification for DGGE analysis of environmental samples used 1.5 to 2 ng of initial amoA PCR product as the template followed by 12 cycles using the primers with the GC clamp and employing the cycling parameters described above (43).

DGGE of amoA PCR products was performed as described by Muyzer et al. (34) using a D-Code system (Bio-Rad, Hercules, Calif.). Polycyclamidic gradient gels (6% acrylamide, 2% gelatin, and 37.5:1 acrylamide-bisacrylamide in 1× Tris-acetate-EDTA; 60 to 100% denaturant; 1 mm thick by 16 cm by 16 cm) were poured to achieve a parallel gradient with the aid of a model 475 gradient delivery system (Bio-Rad) according to the manufacturer’s instructions. A 100% denaturant was defined as 6% acrylamide containing 7 M urea and 40% deionized formamide. An estimated 50 ng of PCR product was loaded into each lane. Environmental samples were run at 120 V for 7.5 h at 30°C and were visualized by UV transillumination after staining with SYBR Green I.

Construction and analysis of amoA gene fragment libraries. Purified amoA PCR products retrieved from Mahsuri roots and IR63087-1-17 roots and rhizosphere were cloned using the pPCR-Script Amp cloning kit (Stratagene). Ligation and transformation steps were performed according to the manufacturer’s instructions. All plasmids bearing inserts of the correct size (491 bp) were used as template (approximately 0.1 ng of plasmid DNA) for PCR employing the same thermocycling conditions as described for environmental samples, except that the cycle number was altered per set of PCR. With 50 cycles, a total of 22 to 38 clones bearing an amoA insert were screened by DGGE under the same conditions as described for environmental samples, except that run times were extended to 12 h in order to better resolve the more stable DNA duplexes that tend to migrate to the bottom half of the gel. Under these conditions, certain less-stable DNA duplexes that migrate within the top half of the gel may become fully denatured. Therefore, clones representing these sequences were screened using a 7.5-run time. Fifteen unique amoA sequences were resolved based on differences in mobility in DGGE gels. Representative clones from each DGGE type were selected for sequencing and phylogenetic analysis.

Sequencing and phylogenetic analysis. Representative clones from each DGGE type were sequenced using the ABI-PRISM Dye Terminator Cycle Sequencing Core Kit (Applied Biosystems, Perkin-Elmer, Foster City, Calif.). Sequencing products were analyzed on an Applied Biosystems model 310 DNA sequencer following the manufacturer’s recommendations. Phylogenetic analyses based on either 450-nucleotide (excluding primers) or deduced 150-amino-acid sequences were performed using algorithms in PHYLIP version 3.57c (J. Felsenstein, University of Washington, Seattle). Evolutionary distances between amino acid sequences were generated using the point-accepted mutation–Dayhoff substitution model implemented in PROTDIST, while distances between nucleotide sequences were generated by a maximum-likelihood method (Quick-CLUSTAL). Distance dendrograms based on the Fitch-Margoliash method were generated using the FITCH algorithm. The topologies of the resulting trees were consistent with those presented below. Robustness of derived groupings was tested by bootstrap using 100 replications.


**RESULTS AND DISCUSSION**

The PCR-DGGE-cloning approach. The combination of PCR and DGGE targeting the 16S rRNA gene has been widely used to detect and characterize AOB populations from various environments (16, 28–30, 51). More recently, the approach has also been demonstrated to be feasible when targeting the amoA gene (36). In this study, DGGE was used to screen libraries of amoA clones and to profile the AOB populations based on amoA retrieved from the root and soil environments.

Bands corresponding to *Nitrosomonas*-like and *Nitrospira*-like sequences migrated to the top and bottom halves, respectively, of DGGE gels (Fig. 1). This agrees with results of Oved et al. (36), although our environmental profiles were generally more complex. This might be explained by the two-step amplification procedure that was performed to link *amoA* with the GC clamp as described above. During preliminary experiments, we found that amplification of environmental samples using the primer pair with the GC clamp was sometimes less efficient compared to that with the original primers. Furthermore, the introduction of a second PCR step allowed us to load more-uniform amounts of DNA in the DGGE gels, since we could use a measured amount of template (1.5 to 2 ng) for the second PCR and limit the additional cycles in order to minimize errors in amplification. This strategy probably increased the yield of nonspecific amplification products, although the detection of less-predominant authentic sequences was also probably increased. Since *amoA* library clones could be used as reference bands, we confined our analysis to bands with corresponding clones. Based on comparisons between individual *amoA* clones and the environmental profiles, we were able to determine that the two most prominent bands in

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**Oxygen measurements from roots of Mahsuri and IR63087-1-17.** Plants were grown for 3 weeks in liquid culture solution having the following composition: (NH₄)₂SO₄ 50 μM; K₂SO₄ 1 mM; MgSO₄ 2 mM; CaCl₂ 1 mM; NaH₂PO₄ 300 μM; Fe-EDTA 100 μM; MnCl₂ 9 μM; (Na)₂MoO₄·2 25 μM; H₂BO₃ 20 μM; ZnSO₄ 1.5 μM; CuSO₄ 1.5 μM. The pH of the medium was adjusted to 6.5 with NaOH and replenished every 2 days. In order to immobilize the roots during microelectrode measurements, the plants were transplanted to 1% agar blocks containing the same components as the growth medium prior to microelectrode measurements. Microtype oxygen microelectrodes with tip diameters of about 10 μm were prepared and calibrated as described by Retsbech and Jorgensen (41). Oxygen profiles were obtained using methods described by de Beer and van den Heuvel (20). Concentration profiles were obtained by using a motor-driven micromanipulator (model ACV-104-HP, Chuo Precision Industrial, Tokyo, Japan) at intervals of 100 to 200 μm from the surface of selected plant roots. Microprofiles were determined three times on different roots at points 2 to 3 cm from the base of three plants.

**Data analysis.** Data for 15N analysis (N pools, 15N excess abundance; n = 3) for each treatment within each N source were compared by analysis of variance and least significant difference comparison of means using Statistica for Windows (version 2.0; Analytical Software, Tallahassee, Fla.). The cell densities from FISH were tested for evenness of distribution by applying Kruskal-Wallis tests and constructing histograms. Both tests showed nonnormal distribution in all cases. Therefore, differences between means were verified by Kruskal-Wallis analysis of variance using Statistica for Windows.

**Nucleotide sequence accession numbers.** The environmental *amoA* sequences generated in this study have been deposited in the GenBank nucleotide sequence database under accession numbers AY050674 through AY050688.
Fig. 1 may actually correspond to up to two and five different amoA clones, respectively.

The environmental profiles show that certain AOB strains are more prominent in the presence of rice roots. In particular, bands represented by Nitrosospira clones I1, K4, and J4 appear prominently only in the planted treatments (Fig. 1A). The levels of detection of amoA by PCR and Nitrosomonas by DGGE are compared to their respective detection levels by using FISH in Table 1. In general, amoA was detected in all (roots, rhizosphere, and unplanted soil) samples. DGGE bands corresponding to Nitrosomonas, however, were consistently detected only in the root environment of IR63087-1-17, particularly in the root samples. This was supported by FISH estimates, which detected around three times as many Nitrospira cells on the roots of IR63087-1-17 as on the roots of the traditional varieties.

Phylogenetic analysis of all the sequenced amoA clones related one major cluster (cluster A) of sequences to Nitrosomonas europaea and two clusters (clusters B and C) with two different strains of Nitrosospira multiformis (Fig. 2). There was rough agreement between clusters generated by phylogenetic analysis and clone mobility in DGGE gels, indicating that a limited amount of phylogenetic information could be derived from DGGE analysis. The similarities of the retrieved sequences to the DNA and amino acid sequences of the cultured sequences were greater than 80 and 85%, respectively. Based on this criterion (39), all the sequences described in this study are likely to be different strains of either N. europaea or N. multiformis. Based on clone frequency, Nitrosomonas sequences were retrieved at a higher frequency from IR63087-1-17 roots, while Nitrosospira-like amoA sequences were predominant in all other cases (Fig. 3). While these frequencies are based only on single libraries consisting of a small number of clones (20 to 38) from each DNA source, the contrast in AOB population structure associated with roots is partially supported by FISH data (below).

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roots themselves were strongly autofluorescent, and using epifluorescence microscopy, it was possible to see the biofilm as a dark coating on the root surface (Fig. 4A). On whole root sections, it was possible to detect individual cells and cell clusters stained by Nso190 (Fig. 4C and D). However, the abundance of AOB was relatively low, their distribution was uneven, and root autofluorescence was strong, making quantification impossible on whole root sections. These problems were solved by applying moderate sonication to root samples and probing only the detached biofilm, which could be concentrated on a small surface area (diameter, 8 mm) on glass slides. After sonication, fine root hairs that are typically embedded within the biofilm were revealed (Fig. 4B). This suggests that the increase in root surface area attributed to root hairs may be highly relevant in relation to processes occurring within the biofilm.

Differences were detected in the general bacterial constituents of the root biofilms of different cultivars. The cells on Mahsuri biofilms were generally larger, with clearer outlines and brighter fluorescence compared to indigenous populations on the other two varieties (Fig. 4E and F). No significant differences were detected in the total abundance of cells on the root biofilms of the three varieties. However, because cells on Mahsuri biofilms were generally larger, the total bacterial biovolume was nearly twice that present in the other varieties (Fig. 4I). These observations suggest that root exudates from Mahsuri favor the development of more heterotrophic copiotrophs.

Detection and quantification of AOB and *Nitrosomonas* spp.

Under conditions favorable to nitrification, AOB generally occur as large aggregates within thick (>100-μm-thick) biofilms and are frequently associated with nitrite-oxidizing bacteria (22, 35, 46). In whole root sections, we were able to detect clusters of AOB (Fig. 4C and D), although individual cells dispersed unevenly within the biofilm were also frequently observed. It was difficult to make a more detailed analysis of their distribution in situ because of root autofluorescence and
their scant and uneven distribution relative to the total bacterial community. While our in situ observations were limited, they do suggest that a mixed community of AOB is present on rice roots, based on cell aggregation characteristics. After sonication, the AOB were mostly dispersed; hence, we should assume that dispersal of aggregates and some cell loss could have occurred during sonication. This may have led to a certain degree of underestimation in our counts. The AOB cells had diameters between 0.5 and 1.0 μm and were usually short or curved rods and spheres (Fig. 4D and G).

We detected no significant differences in the abundances of AOB among the three rice cultivars (Table 1). However, significantly higher ($P < 0.05$) levels of Nitrosomonas cells were detected in the root biofilms of IR63087-1-17 (Table 1). In this study, we considered the Nitrosomonas density subtracted from the total AOB cell density to reflect the abundance of Nitrospira spp., since all terrestrial AOB are comprised of either Nitrosomonas or Nitrospira spp. based on 16S rRNA sequences (24, 52). The percentage of Nitrosomonas associated with IR63087-1-17 ranged from 40 to 69% of the total AOB (based on the ratio of ranges between 95% confidence intervals). In the case of KDML 105 and Mahsuri, the Nitrosomonas cells ranged from 7 to 23% of the total AOB, and therefore, the predominant AOB present on roots of these varieties are Nitrospira spp. The presence of a higher proportion of Nitrosomonas cells on roots of IR63087-1-17 corresponded with the highest gross nitrification rates measured among the three varieties and unplanted soil (Table 2). Population estimates of...
Nitrosomonas on root biofilms were positively correlated ($R^2 = 0.93$) with gross nitrification rate estimates. The latter lacked enough sensitivity to provide reliable estimates for Mahsuri and unplanted soil. This may be attributed to low soil ammonium N pools (5 to 8 μg of NH$_4^+$-N [cm$^3$ of soil]$^{-1}$) relative to the added $^{15}$NO$_3^-$ (30 μg NO$_3^-$-N [cm$^3$ of soil]$^{-1}$). The relative differences between estimates, however, should be valid and indicate that significantly lower nitrification rates are associated with Mahsuri and unplanted soil than with the other treatments.

We did not attempt to estimate the abundance of AOB in the rhizosphere soil. PCR for $amoA$, however, did not yield significantly more product from the rhizosphere soil of IR63087-1-17 (data not shown). Since the PCR conducted in this study was not meant to be quantitative, we cannot make meaningful comparisons based on these results. We also failed to detect significant differences in nitrification rates obtained by the different treatments (Table 2). Collectively, therefore, our data indicate that root surface populations of AOB play a major role in determining the nitrification rates in the root environment.

It is recognized that AOB tend to be surface attached rather than free living (1, 3), and the degree of surface attachment is stronger than that of heterotrophic bacteria (1). In general, bacteria attached within biofilms are more resistant to a variety of environmental stresses (19). Such an advantage would be necessary for slow-growing bacteria such as AOB. Notably, it has been shown that biofilm populations of *N. europaea* recover faster from starvation than do their free-living counterparts (12). Another response to starvation is the production of exopolysaccharides (48), which would promote stronger surface attachment. This characteristic would be relevant for AOB attached to rice roots, since we would expect the bacteria to be in a starved state between periods of fertilization. Between the two genera of AOB, enhanced aggregate formation has been observed for *Nitrosomonas* in the marine environment (37) and on membrane filters (26). Since cell aggregates within biofilms may be more resistant to extraction, the failure of DGGE to accurately reflect the levels of *Nitrosomonas* associated with the rice roots may have arisen from difficulties in extracting the *Nitrosomonas* DNA from the root samples.

**Oxygen excretion from roots.** The existence of intervarietal differences in the flux of oxygen from rice roots has been recognized since 1969 (8), although such differences have never been convincingly linked to the ecology of any group of soil aerobes. *Nitrosomonas* strains have been described as $r$ strategist, with low substrate affinities and high maximum activity compared to the $K$ strategist *Nitrosospira* (6, 45, 46). In pure culture, *N. europaea* exhibits $K_{\text{m}}$ (O$_2$) values between 6.9 and 17.4 μM. Oxygen concentration measurements on the rice root surface were generally higher than these values (Fig. 5), but in the case of Mahsuri, oxygen could become more limiting in the presence of heterotrophic bacteria, which are generally superior competitors for oxygen compared to AOB (38, 54). We have observed large, active, and presumably heterotrophic bacteria on root biofilms derived from Mahsuri (Fig. 4E). At present, no kinetic data with respect to oxygen are available for *Nitrospira* spp. However, *Nitrospira* spp., occurring in a nitrifying fluidized bed reactor exhibited $K_{\text{m}}$ (NH$_4^+$) values that were 1 to 2 orders of magnitude lower than values for *N. europaea* (45). This might also imply that *Nitrospira* spp. have a higher affinity for oxygen, which would explain their predominance in Mahsuri. On the other hand, aside from the kinetic data cited for *N. europaea*, experiments conducted on a membrane-bound biofilm system also demonstrated the ability of *N. europaea* to outcompete *Nitrospira* spp. at high substrate and O$_2$ concentrations (44). Therefore, the greater abundance of *Nitrosomonas* spp. on the roots of IR63087-1-17 can be partly explained by the higher O$_2$ concentration on the root surface. However, since *Nitrosomonas* spp. also require relatively high NH$_4^+$ concentrations, we also expect the turnover of NH$_4^+$ in the root environment of IR63087-1-17 to be different from those of other rice varieties.

In conclusion, we have demonstrated, using molecular methods, an enrichment of AOB on rice root surfaces. The levels of AOB root abundance detected in this study are 2 to 3 orders of magnitude higher on a volume basis than densities usually encountered in soils, including estimates in an aerobic soil obtained by a FISH technique (2, 25, 40). In soils, AOB typi-

### TABLE 2. Gross nitrification and mineralization rates

<table>
<thead>
<tr>
<th>Sample</th>
<th>Gross nitrification rate (μg N·g of soil$^{-1}$·day$^{-1}$)</th>
<th>Gross mineralization rate (μg N·g of soil$^{-1}$·day$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soil from rice cultivar</td>
<td></td>
<td></td>
</tr>
<tr>
<td>KDML 105</td>
<td>0.25 A</td>
<td>1.24 A</td>
</tr>
<tr>
<td>Mahsuri</td>
<td>NSD$^{a}$</td>
<td>1.28 A</td>
</tr>
<tr>
<td>IR63087-1-17</td>
<td>1.22 B</td>
<td>1.35 A</td>
</tr>
<tr>
<td>Unplanted soil</td>
<td>NSD$^{a}$</td>
<td>0.93</td>
</tr>
</tbody>
</table>

$^{a}$ Means ($n = 3$) followed by the same letter are not significantly different based on least significant difference comparison of means ($p = 0.05$).

$^{a}$ NSD, no significant dilution of the label was detected.

![FIG. 5. Oxygen concentration profiles from the surface of roots of 3-week-old rice plants grown in culture solution.](http://aem.asm.org/)

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cally comprise <1% of the total bacterial community, while on rice roots, they constituted >10%. Based on the known physiological properties of *Nitrosomonas* and *Nitrosospira*, their respective abundances on the root surfaces of the different rice varieties could be partially explained by the differences in oxygen release from rice roots, which ultimately resulted in different rates of gross nitrification associated with each variety. We have not yet determined the temporal stability of the associations described. However, since biofilm populations are generally resistant to environmental stresses (19), these are likely to be stable through time. Studies to determine whether the nature of N uptake by the plant can affect the nature and activity of AOB on the root surface will be presented in a separate report. In an intensively cultivated crop such as rice, the microbial activities associated with roots are not likely to be trivial. Further characterization of this component of the rhizosphere communities should provide valuable insight into the nature of N cycling in the rice root environment.

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