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# Effect of Xanthobacter, isolated and characterized from rice roots, on growth of wetland rice

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**Abstract**

With an autotrophic, N-free medium, Xanthobacter populations were isolated from the roots of wetland rice grown under field conditions. Xanthobacter populations ranged from $3.2 \times 10^4$ to $5.1 \times 10^5$ colony-forming units (cfu) $g^{-1}$ of root and averaged 47-fold higher on the root or rhizoplane than in the neighbouring nonrhizosphere. Characterization studies indicated dissimilarities in carbon utilization and motility among the isolated Xanthobacter strains and other recognized Xanthobacter species. Under gnotobiotic conditions, the population of one isolate, *Xanthobacter* sp. JW-KR1, increased from $10^5$ to $10^7$ cfu $plant^{-1}$ 1 d after inoculation when a rice plant was present, but declined to numbers below the limit of detection ($<10^4$ cfu $assembly^{-1}$) after 3 d in the absence of a plant. Scanning electron microscopy revealed Xanthobacter as pleomorphic forms on the rhizoplane. To assess the effect of Xanthobacter on plant growth, rice plants were grown under greenhouse conditions in plant assemblies containing sand and half-strength Hoagland's nutrient solution with and without nitrogen. Plants were either inoculated with $10^5$ cfu Xanthobacter $g^{-1}$ of sand or left uninoculated. After 40 d, plants without nitrogen showed no significant differences in top or root dry weight, plant height, root length, or number of tillers or leaves, whether the plants were inoculated or uninoculated. However, when nitrogen was added, inoculated plants had a significantly larger top dry weight (15%) and number of leaves (19%) than uninoculated plants. Under conditions of added and no added nitrogen, acetylene reduction assays showed *Xanthobacter* sp. JW-KR1 produced <0.1 (below detection limit) and 7 nmol $C_2H_4$ $plant^{-1}$ $h^{-1}$, respectively. Under the conditions studied, the results suggest that both Xanthobacter and wetland rice derive some benefits from their association.

## Introduction

Diazotrophs isolated frequently from the rhizosphere of cereal crops include *Azospirillum* sp. (Bally et al., 1983; Shawky, 1989; Thomas-Bauzon et al., 1982), *Bacillus polymyxa* and *Enterobacter agglomerans* (Lindberg and Granhall, 1984), *Enterobacter cloacae* (Pederson et al., 1978, Thomas-Bauzon et al., 1982), *Erwinia herbicola* and *Klebsiella pneumoniae* (Pederson et al., 1978), *Klebsiella oxytoca* (Thomas-Bauzon et al., 1982), and *Pseudomonas* sp. (Barraquio et al., 1983; Thomas-Bauzon et al., 1982, Watanabe et al., 1982). Recently, Oyaizu-Masuchi and Komagata (1988) isolated *Xanthobacter* sp. from the rhizosphere of rice (*Oryza sativa* L.) in Japan.

The genus Xanthobacter comprises gram-type negative (Wiegel and Mayer, 1978), pleomorphic bacteria capable of fixing $N_2$ while growing

chemolithotrophically (Gogotov and Schlegel, 1974). Xanthobacter species have been isolated from marine (Lidstrom-O'Connor et al., 1983) and freshwater (Aragno et al., 1977) sediments, sewage samples (Jenni and Aragno, 1987; White et al., 1987), street ditches and soils of meadows (De Bont and Leijten, 1976; Wiegel and Schlegel, 1976, 1984).

Little is known about the effects of Xanthobacter on rice growth. The necessity of a selective medium and selective growth conditions probably account for the appearance of only a single report for the isolation of Xanthobacter species from plant roots. Nandi and Sen (1981) reported that rice yield was doubled when *Mycobacterium flavum* (now *Xanthobacter flavus*) was sprayed onto rice leaves. In an $^{15}N$ study of soil diazotrophs, rice plants, grown in sterile soil amended with lactose, received 18% of the $N_2$ fixed by *Xanthobacter flavus* (Kalininskaya et al., 1989).

Here we report the isolation, enumeration, and characterization of Xanthobacter from the rhizosphere of wetland rice. In addition, we test the ability of one of these Xanthobacter isolates to enhance the growth of rice with and without added nitrogen.

## Materials and methods

### *Sampling site*

In 1988, prior to planting, a 0.5–1.0-m portion of top soil was removed to make the land level. Soil and root samples of *Oryza sativa* var. Lemont were taken in September 1988 (rice at heading stage), April 1989 (before second planting), and August 1989 (rice at heading stage) from a rice paddy in southeastern Arkansas, USA. The soil was a Rilla silt loam (pH 5.5, 0.3% organic matter, 72.8% silt, 7.4% clay), a Typic Hapludalf of a fine-silty, mixed, thermic family. The soil was flooded with approximately 5 cm of water.

### *Sampling of roots and soil*

Roots were removed gently from the soil and were washed in the standing water to remove all of the visible soil. Nonrhizosphere soil samples were taken from areas between widely spaced plants. The roots and soil from five different sites in the same field were pooled and placed in polyethylene bags. The bags were sealed and kept on ice for 2 d during transport before the samples were assayed.

### *Isolation of Xanthobacter*

A 100-mL solution of Medium A (Wiegel and Schlegel, 1976) was inoculated with either 2–3 g of roots or soil and incubated autotrophically under $N_2$-fixing conditions at 30°C in a 2-L serum bottle with a headspace of 10% $H_2$, 10% $CO_2$, 15% air, and 65% $N_2$. Inocula were transferred sequentially three times into fresh medium with 7 d of incubation between each transfer. To isolate axenic cultures, samples were adjusted to pH 11.0 with 3 *M* NaOH and stirred for 5 min to dissolve the copious bacterial slime. This permits the bacteria to be separated (Wiegel and Schlegel, 1984). The cultures were streaked onto sterile nutrient agar (Difco Laboratories, Detroit, MI) or Medium A amended with agar (15 g $L^{-1}$). The nutrient agar plates were incubated aerobically; the Medium A plates were incubated in desiccator jars (without a desiccant) under the gas atmosphere as previously described. All samples were incubated at 30°C for at least 7 d.

### *Characterization of Xanthobacter isolates*

Xanthobacter strains were identified by their ability (a) to fix $N_2$ during autotrophic growth, (b) to form characteristic yellow colonies due to the presence of zeaxanthin dirhamnoside, (c) to grow on $C_1$–$C_4$ primary alcohols, and (d) to display pleomorphic cellular morphology when grown on nutrient agar amended with 0.35% (w/v) succinate (Wiegel and Schlegel, 1984). *Xanthobacter autotrophicus* 7C (ATCC 35674) and *Xanthobacter flavus* 301 (ATCC 35876) from the American Type Culture Collection (Rockville, MD) were used as standards.

### *Enumeration of Xanthobacter*

A 2–3 g sample of root was washed successively

in 20 mL of 20 m*M* $KH_2PO_4$ (pH 6.8) using a Stomacher blender (Seward Medical, London, England) for 1, 5, and 10 min (for a total of 16 min) before being ground. The diluent from each sample was serially diluted in phosphate buffer, plated onto Medium A, and incubated as described previously. To quantify populations of heterotrophic bacteria, the diluent was also plated onto tryptic soy agar (Difco) amended with 0.5% yeast extract (TSAYE) and incubated aerobically at 30°C. Bacterial colonies were counted after 7 d.

*Growth of Xanthobacter in the rice rhizosphere*

Rice seeds were manually hulled and surface-sterilized with 1.58% NaOCl for 30 min, rinsed with distilled water, and germinated for 2 d in the dark at 30°C. One seedling was transplanted into each gnotobiotic assembly. Control assemblies received no plants. Briefly, each assembly consisted of a rice plant grown between 0.2 $\mu$m pore-size membranes (Versapor, Gelman Sciences, Inc., Ann Arbor, MI) housed in a pyrex tube (200 × 52 mm) containing sand and half-strength Hoagland's solution (Wetter and Constabel, 1982) with and without 0.05% (w/v) $KNO_3$. With this assembly, the Xanthobacter free of the root was contained within the membrane packet and free of the surrounding sand.

To assay for growth in the rhizosphere, one of the Xanthobacter strains isolated in this study, JW-KR1, was grown for 5 d in Medium A. The late log-phase cells were starved for 2 d by removing the $H_2$ and $CO_2$ to minimize bacterial utilization of intracellular storage compounds, such as poly-$\beta$-hydroxybutyrate, after transfer. A 0.1-mL portion of a suspension of the starved cells was added to the membrane packet of each assembly to give a final density of approximately $10^5$ cfu assembly$^{-1}$. Three assemblies were assayed for each treatment at 0, 1, 2, 3, and 7 d. For assemblies with plants, the plant tops were cut off and the roots were washed in 20 m*M* $K_2HPO_4$(pH 6.8) for 1 min in a Stomacher blender. Bacteria were serially diluted onto both TSAYE and Medium A agar with a Spiral plater (Spiral System Instruments, Bethesda, MD). Plates were incubated and bacteria were counted as previously described.

*Scanning electron microscopy (SEM) of roots colonized by Xanthobacter*

Plants were inoculated with *Xanthobacter* sp. JW-KR1 in the gnotobiotic assemblies as previously described. After 7 d, the plants were removed from the gnotobiotic assemblies. The roots were excised and fixed for 2 min in Parducz's fixative (4 parts 2% $OsO_4$ and 1 part saturated $HgCl_2$) (Parducz, 1967). The roots were dehydrated in a graded ethanol series and dried at the critical point. Root sections were coated with 40 nm gold-palladium and examined using a Philips 505 scanning electron microscope at 20 KeV. To view nondehydrated samples, fixed roots were plunge-frozen in liquid $N_2$ slush, coated with 40 nm gold-palladium, and observed at −70°C using a Polaron cryostage (Polaron Instruments Inc., Hatfield, PA). Populations on root samples were streaked onto TSAYE to assay for contamination before samples were prepared for viewing by SEM. Phase-constrast light photomicrographs of *Xanthobacter* sp. JW-KR1 from the plant growth medium in the gnotobiotic assembly were taken using a Olympus Vanox light microscope (Olympus Corp., New Hyde Park, NY).

*Effect of Xanthobacter sp. JW-KR1 inoculum on rice grown with or without nitrogen*

*Xanthobacter* sp. JW-KR1 was grown to early stationary phase (48 h) in Medium A containing 0.2% (w/v) sodium gluconate and 0.1% (w/v) $NH_4Cl$. The culture was centrifuged at 7,000 × *g* at 4°C for 30 min and the supernatant discarded. The pellet was resuspended in sterile 2 m*M* $K_2HPO_4$ (pH 6.8) and the centrifugation was repeated twice. In this manner, all traces of nitrogen in the culture medium were removed. Bacteria were brought to a density of $10^8$ cfu mL$^{-1}$ for plant inoculation.

Rice seeds were sterilized and germinated as previously described. Three seedlings were transplanted after 3 d into 1-L Mason jars containing 1 kg of autoclaved sand and 370 mL of half-strength Hoagland's solution with or without 2 m*M* $KNO_3$. The amount of nutrient solution was sufficient to cover the sand to a depth of 2 cm. Rice plants were either inoculated immedi-

ately with 2 mL of bacterial suspension to yield $1 \times 10^5$ cfu $g^{-1}$ of sand or left uninoculated. In this manner, it was possible to assess if Xanthobacter affected rice growth by $N_2$ fixation or some other mechanism. The tops of all jars were covered with sterile polyethylene bags and the jars were placed in a light room at 30°C. Light room conditions were as described by Yeung et al. (1989). After 7 d, each jar was thinned to one plant, and the polyethylene bag was replaced with a Mason-jar lid modified with a hole for the plant, and a hole for air exchange and nitrogen or water additions. A sterile rubber septum with a hole in the middle was placed in each hole, and the septum was packed with sterile cotton. Plants were transferred to a greenhouse and arranged in a randomized block design. Plants were given sterile water as needed. At 21 d after planting, plants receiving N were given additional N to increase the N content by 1.25 m*M*.

At 40 d after planting, the plants were removed from the assemblies. Plant tops were cut off and measured for height and number of tillers and leaves. Roots were measured for length and Xanthobacter counts were determined as previously described. Five replicates of roots from each treatment were assayed for acetylene reduction (Bouton et al., 1981) except the roots were incubated under microaerophilic conditions (87% $N_2$, 10% acetylene, and 3% $O_2$). Both plant tops and roots were dried at 60°C for 7 d before weighing. The experiment was repeated twice. Data were analysed by ANOVA and means were separated by the Duncan's Multiple Range Test.

## Results

### *Isolation and enumeration of Xanthobacter*

Repeated plating of either soil or rice root samples, which were introduced into Medium A and incubated under autotrophic $N_2$-fixing conditions, resulted in axenic colonies of Xanthobacter in 1988 and 1989. Numbers of Xanthobacter obtained after washing in a Stomacher blender for 1, 5, and 10 min (for a total of 16 min) were $5.1 \times 10^5$, $1.1 \times 10^5$, and $2.2 \times 10^3$ cfu $g^{-1}$ dry weight of root, respectively. Numbers of Xanthobacter obtained after 16 min of washing and then grinding with a mortar and pestle were $1.0 \times 10^3$ cfu $g^{-1}$ dry weight of root.

The numbers of Xanthobacter and heterotrophic bacteria on the root and in nonrhizosphere soil samples increased over time for the three sampling periods (Table 1). Compared to the neighboring nonrhizosphere over the 2-year period, Xanthobacter and heterotrophic bacterial populations in the rhizosphere of rice average 47- and 48-fold higher numbers, respectively.

*Table 1.* Numbers of Xanthobacter and heterotrophic bacteria on the roots of rice and in nonrhizosphere soil (cfu $g^{-1}$ dry root or soil[a])

| Sample | Sampling times | | |
|---|---|---|---|
| | First growing season (1988) | Between growing seasons (1989) | Second growing season (1989) |
| **Rhizosphere** | | | |
| Xanthobacter | $3.2 \times 10^4$ | NA[b] | $5.1 \times 10^5$ |
| Heterotrophic bacteria | $6.1 \times 10^7$ | NA | $1.5 \times 10^8$ |
| **Nonrhizosphere** | | | |
| Xanthobacter | $5.5 \times 10^2$ | $1.4 \times 10^3$ | $1.4 \times 10^4$ |
| Heterotrophic bacteria | $8.4 \times 10^5$ | $4.4 \times 10^6$ | $6.2 \times 10^6$ |

[a] Populations in samples were enumerated using a 1 min root wash. All values represent the mean of at least two replicates.
[b] NA, not applicable (no plants present between growing seasons).

*Colonization of the root surface and growth of* Xanthobacter *sp. JW-KR1 cultured with rice*

In the gnotobiotic assemblies, numbers of *Xanthobacter* sp. JW-KR1 increased more than 100-fold after 1 d and populations remained at that level for the remainder of the study whether or not nitrate was present (Fig. 1). In the absence of a plant, Xanthobacter numbers in the assemblies declined to less than detectable levels ($10^4$ cfu assembly$^{-1}$) after 3 d.

Xanthobacter colonized a large area of the root, irrespective of the absence (Fig. 2A) or presence of added nitrate (data not shown). Xanthobacter appeared on the main root (Fig. 2A), lateral roots, and root hairs (Fig. 2B) with both coccal and rod-shaped cells bound on the rhizoplane and more deeply embedded into the root (Fig. 2C). In addition, the bacterial cells were interconnected by an extracellular matrix. In plant growth medium, phase contrast microscopy showed the same morphologies of Xanthobacter (Fig. 3). Samples of roots streaked on TSAYE yielded only Xanthobacter colonies.

*Characterization of Xanthobacter rice isolates*

Xanthobacter isolates from the roots of rice grew well on TCA cycle intermediates and alcohols but generally not well on sugars. The substrate utilization profiles of the rice isolates differed from the type strains of *X. autotrophicus* and *X. flavus* (Table 2). In addition, Xanthobacter isolates grown in the rhizosphere of gnotobiotic rice and in culture media containing alcohol, especially 1-propanol, yielded motile isolates.

*Effect of Xanthobacter inoculum on the growth of rice*

After 40 d, plants without nitrogen showed no significant differences in top or root dry weight, plant height, root length, number of tillers or leaves, whether the plants were inoculated or uninoculated (Table 3). However, when nitrogen was added, inoculated plants had a significantly larger top dry weight (15%) and number of leaves (19%) compared to uninoculated plants. No other significant differences were observed.

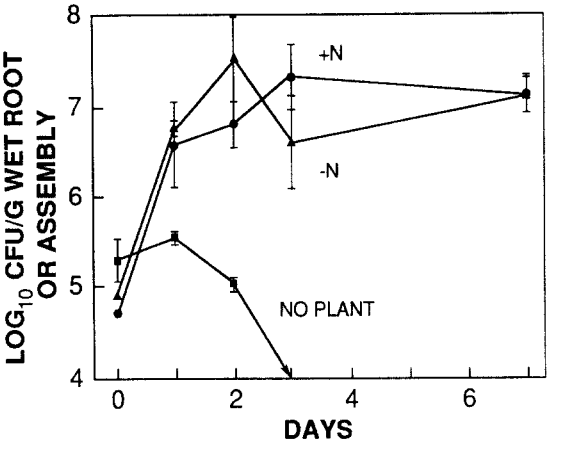


*Fig. 1.* Counts of *Xanthobacter* sp. JW-KR1 cultured with gnotobiotically grown rice roots with (●) and without (▲) added nitrate or with no plant (■). Error *bar* represents +/− 1 SE.

Similar results were observed when the experiment was repeated (data not shown).

Xanthobacter numbers from inoculated plants were $1.3 \times 10^8$ and $4.2 \times 10^7$ cfu plant$^{-1}$ in medium with and without N, respectively. Under conditions of added and no added nitrogen, acetylene reduction by *Xanthobacter* sp. JW-KR1 produced <0.1 (below detection limit) and 7 nmol $C_2H_4$ plant$^{-1}$ h$^{-1}$, respectively. Thus, Xanthobacter increased at least 100-fold in the rhizosphere of rice and produced small amounts of ethylene under $N_2$-limiting conditions.

Under the conditions tested, no phytopathogenicity was seen.

## Discussion

*Isolation and enumeration of Xanthobacter*

This is the first report of Xanthobacter isolated from wetland rice under field conditions. Incubation of the rice roots and soil under chemolithotrophic, $N_2$-fixing conditions provided an easy method to isolate pure cultures from the soil and rice rhizosphere. Xanthobacter and heterotrophic bacterial populations in the soil and rhizosphere increased from the 1988 to the 1989 growing season and this may represent establishment of these populations after the field was levelled. Although the numbers of Xanthobacter were

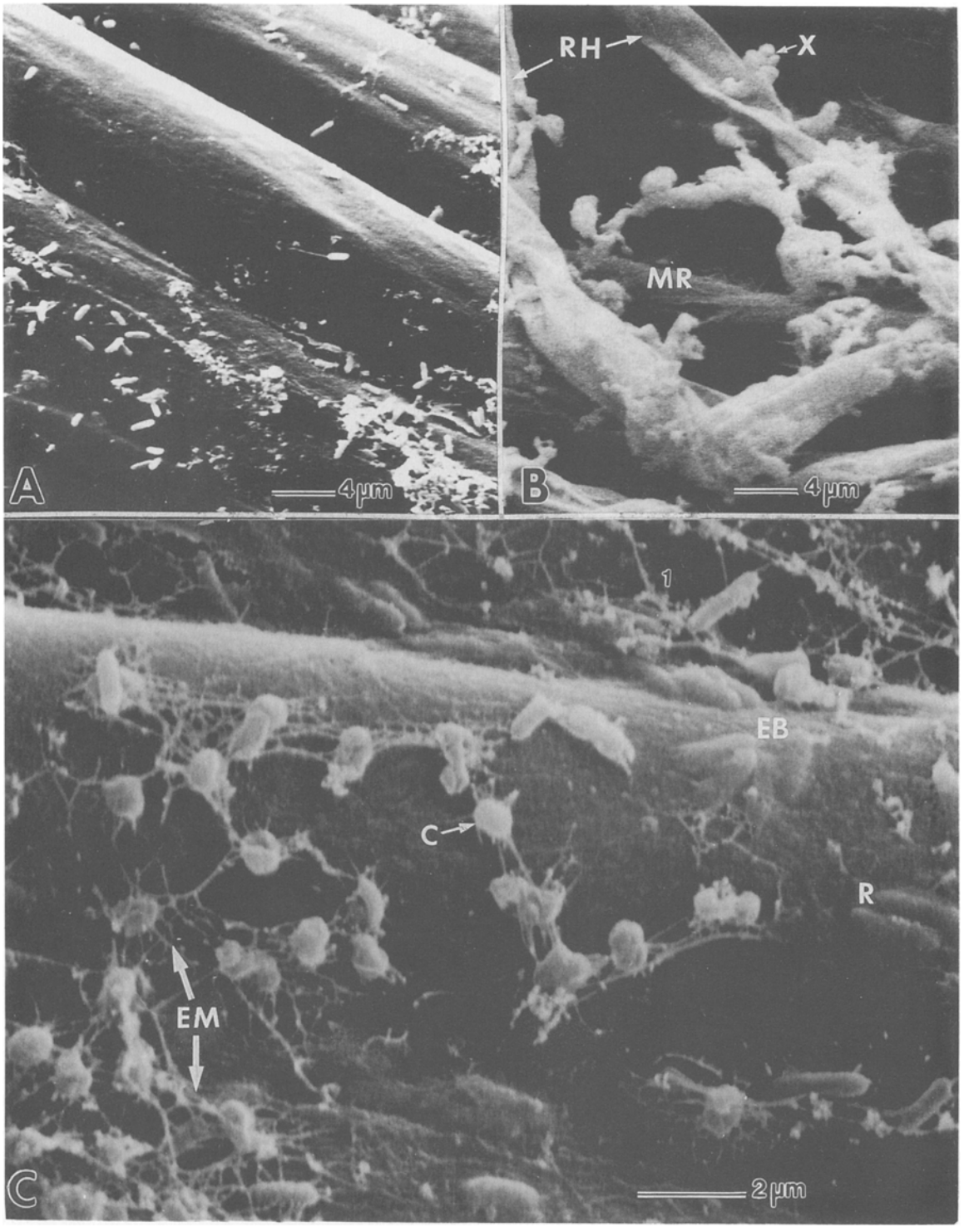


*Fig. 2.* Colonization of the main root (**A**) and root hairs (**B**) of gnotobiotically grown rice by *Xanthobacter* sp. JW-KR1 cells. Symbols: MR, main root; RH, root hair; X, Xanthobacter. (**C**) Magnified micrograph of rhizoplane colonization by pleomorphic Xanthobacter. Symbols: C, coccal Xanthobacter; EB, embedded bacteria; EM, extracellular matrix; R, rod-shaped Xanthobacter.

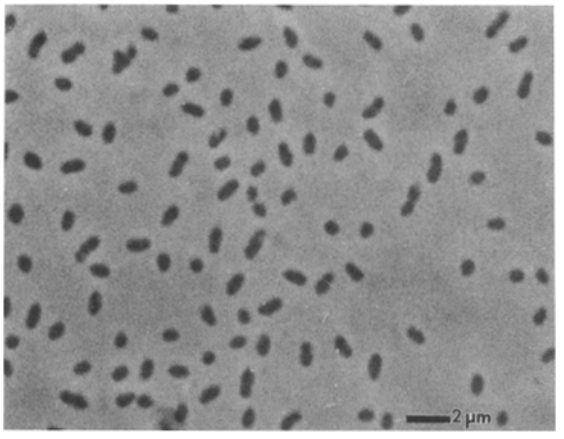


*Fig. 3.* Phase-contrast light micrograph of *Xanthobacter* sp. JW-KR1 cells from the plant growth medium in the gnotobiotic assembly containing a rice plant.

higher on the root compared to the soil, this does not represent a selective enrichment of Xanthobacter in the rhizosphere because heterotrophic bacterial numbers were also higher to a similar extent. Furthermore, the Xanthobacter rhizoplane:soil ratio approximates ratios displayed by other rhizosphere bacterial isolates (McClung et al., 1983; Watanabe et al., 1982).

### *Colonization of rice roots by Xanthobacter*

Xanthobacter cells were pleomorphic and strongly attached on the rhizoplane. While washing the roots removed most of the Xanthobacter after 6 min of washing, $1 \times 10^3$ Xanthobacter cfu $g^{-1}$ of root remained even after 16 min of washing. The ability of Xanthobacter to colonize the rhizoplane was confirmed by SEM. Xanthobacter colonized the rhizoplane in the presence and absence of nitrate. In contrast, the presence of nitrate reduced colonization of pearl millet (*Pennisetum glaucum* [L.] R. Br.) by *Azospirillum brasilense* (Umali-Garcia et al., 1980). The pleomorphic forms of Xanthobacter seen on the rhizoplane were also observed in samples taken from the plant growth medium. This diminishes

*Table 2.* Physiological properties of Xanthobacter rice isolates compared to *X. autotrophicus* and *X. flavus*[a]

| Carbon source | *X. autotrophicus* 7C | *X. flavus* 301 | Xanthobacter rice isolates | | | | |
|---|---|---|---|---|---|---|---|
| | | | JW-KR1 | JW-KR2 | JW-KR3 | JW-KR4 | JW-KR5 |
| Alanine | − | + | + | + | + | + | + |
| Aspartate | + | − | + | + | + | + | − |
| Butyrate | − | + | + | + | + | + | + |
| Formate | + | + | + | + | + | − | − |
| Fructose | + | − | − | − | − | − | − |
| Fumarate | + | + | + | + | + | − | + |
| Galactose | + | − | − | − | − | − | − |
| Glutamine | + | + | + | + | + | + | − |
| Glycerol | + | − | + | − | − | − | − |
| Heptanol | + | + | − | − | − | − | − |
| Isobutanol | + | + | − | − | − | − | + |
| Isopropanol | + | + | − | − | − | − | − |
| M-tartrate | + | − | − | − | + | − | + |
| Malate | + | + | − | + | + | − | + |
| Malonate | + | + | + | − | + | − | − |
| Mannose | − | + | + | − | − | − | − |
| Proline | − | − | + | + | + | + | − |
| Biotin[b] | − | + | + | − | + | − | + |
| Motility[c] | − | − | + | + | + | + | + |

[a] Symbols: +, growth; −, no growth. All strains grew on acetate, butanol, citrate, ethanol, gluconate, glutamate, $H_2/CO_2/O_2/N_2$, methanol, 2-oxoglutarate, 1-propanol and succinate. Colonies of all these isolates were yellow, and all strains became pleomorphic when grown on nutrient agar with 0.35% succinate. None of these strains grew on D-arabinose, arginine, asparagine, galactose, glycine, glucose, histidine, lactose, lysine, mannitol, methionine, raffinose, D-ribose, threonine, serine, sorbose, rhamnose, sorbitol, sucrose, valine, or xylose.

[b] Requirement for biotin (10 $\mu g\ L^{-1}$) was tested using succinate as the carbon source.

[c] Cells were motile when grown on all alcohols tested but were otherwise nonmotile.

*Table 3.* Effect of *Xanthobacter* sp. JW-KR1 inoculation on rice plants grown in sand after 40 d with and without nitrogen. Values are means of at least 25 plants

| Treatment | Inoculated | Top dry weight (mg) | Plant height (cm) | No. of Tillers | Leaves plant$^{-1}$ | Root Length (cm) | Root Dry Weight (mg) |
|---|---|---|---|---|---|---|---|
| −N | − | 21 a[a] | 20.3 a | 1.0 a | 3.0 a | 15.5 a | 27 a |
| | + | 22 a | 20.8 a | 1.0 a | 3.0 a | 15.5 a | 25 a |
| +N | − | 34 b | 36.4 b | 3.8 b | 8.7 b | 15.5 a | 230 b |
| | + | 41 c | 37.4 b | 3.9 b | 10.3 c | 15.4 a | 250 b |

[a] Means followed by the same letter within a column are not significantly different based on a Duncan's Multiple Range Test ($p = 0.05$).

the likelihood that the observed pleomorphism is an artifact of the SEM preparation. In culture medium, Xanthobacter cellular morphology depends on the carbon substrate used (Wiegel and Schlegel, 1984), so the various shapes of Xanthobacter on the root surface may reflect enrichment of specific carbon exudates from different root regions.

*Characterization of Xanthobacter rice isolates*

The Xanthobacter rice isolates varied from the type strains of *X. autotrophicus* and *X. flavus* with respect to carbon utilization profile and motility (Wiegel and Schlegel, 1984). Most or all of the Xanthobacter isolates from rice roots differed from *X. autotrophicus* and *X. flavus* in heptanol, isobutanol, isopropanol, and proline utilization. All Xanthobacter isolates from rice roots were motile, whereas *X. autotrophicus* and *X. flavus* are nonmotile. The only known motile species of Xanthobacter is *Xanthobacter agilis* (Jenni and Aragno, 1987). In contrast to the rice isolates, strains of *X. agilis* show weak or no pleomorphism when cultured on media containing succinate (Jenni and Aragno, 1987). This suggests that the isolates from rice are different from strains of the other Xanthobacter species. Further research is necessary to determine the taxonomic position of the new isolates.

*Xanthobacter* sp. JW-KR1 had a small, but significant effect on rice growth by increasing top dry weight and leaves plant$^{-1}$. Because this Xanthobacter strain reduces a small amount of acetylene only under nitrogen-limiting conditions and differences in plant weights were observed only under nitrogen addition, the results suggest that the effect of Xanthobacter inoculation is not $N_2$ fixation but some other mechanism. In $^{15}N$ studies, Kalininskaya et al. (1989) observed that rice plants assimilated only 18% of the nitrogen fixed by *Xanthobacter flavus*; any effect on plant growth was not reported.

Xanthobacter may produce plant growth regulators. In preliminary experiments, we have detected indoleacetic acid in Xanthobacter cultures grown in medium containing tryptophan. However our results of the effect of Xanthobacter on plant growth are atypical in that indoleacetic acid normally alters root morphology in plants (Martin et al., 1989). This effect was not observed in our study. Further investigation of the production of possible plant growth regulators by Xanthobacter is in progress.

In conclusion, Xanthobacter isolated from the roots of rice multiplied in the rhizosphere of rice and colonized the rhizoplane. Plants inoculated with Xanthobacter in the presence of nitrogen had a small but significant increase in top dry weight and leaves plant$^{-1}$ after 40 d. Therefore, under the conditions tested, the results suggest that both the bacterium and the plant derive some benefits from their association.

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