The desert epiphyte *Tillandsia recurvata* harbours the nitrogen-fixing bacterium *Pseudomonas stutzeri*

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Pseudomonas stutzeri, a nitrogen-fixing bacterium, was isolated from the interior of the desert epiphyte *Tillandsia recurvata*, which grows on electrical cables and giant columnar cacti in the semiarid zone of Baja California, Mexico. This study is the first to indicate the possible close association between bromeliad plants and nitrogen-fixing bacteria.

Key words: beneficial bacteria, bromeliads, Bromeliaceae, nitrogen fixation, *Pseudomonas stutzeri, Tillandsia recurvata*.

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Les auteurs ont isolé le *Pseudomonas stutzeri*, une bactérie fixatrice d'azote, a partir de 1'intérieur de l'épiphyte désertique *Tillandsia recurvata* qui pousse sur les fils électriques et sur les cactus columnaires géants dans la région semi-aride de Baja California, au Mexique. Cette étude est la première à suggérer l'existence d'une étroite association entre les bromélias et des bactéries fixatrices d'azote.

Mots clés : bactéries bénéfiques, bromélias, Broméliacées, fixation de l'azote, *Pseudomonas stutzeri, Tillandsia recurvata*.

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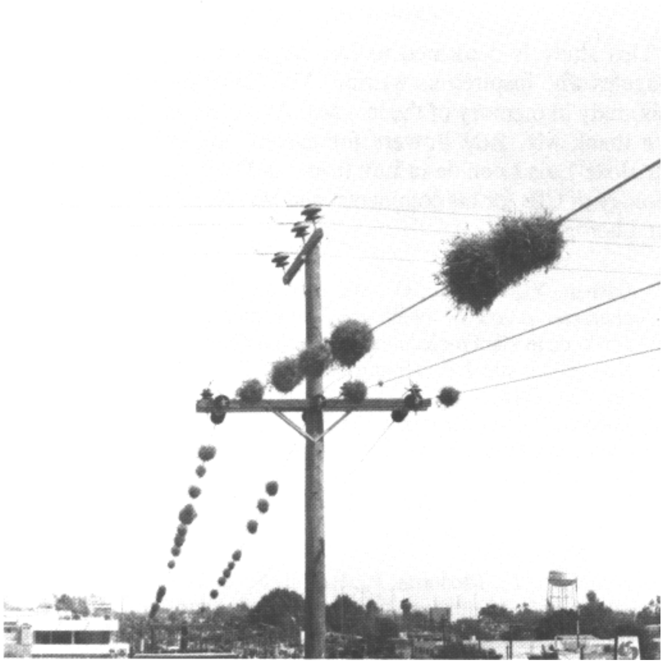

FIG. 1. Plants of *Tillandsia recurvata* attached to electrical cables in Cuidad Constitucion, Baja California Sur, Mexico.

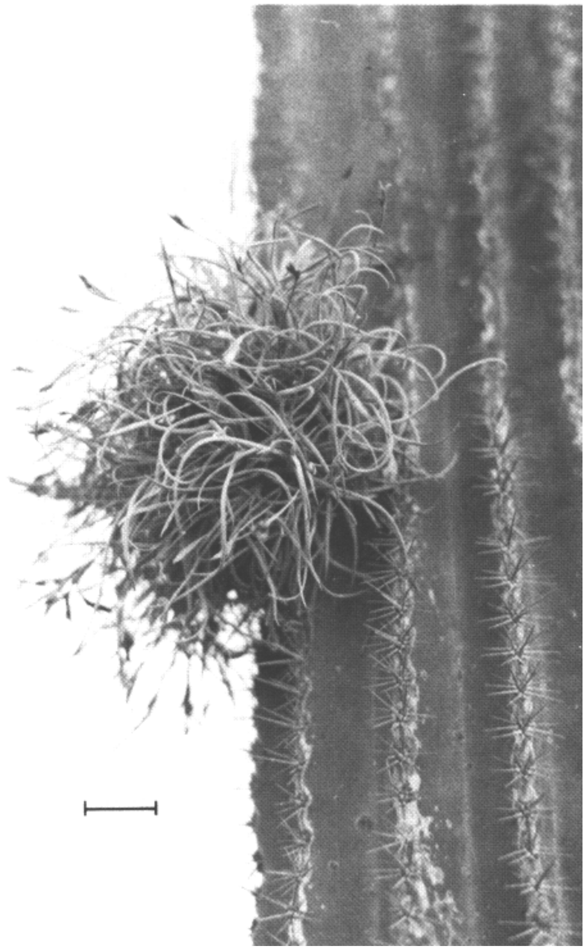

FIG. 2. Plants of *Tillandsia recurvata* attached to the giant Cardon cactus in Santo Domingo Valley, Baja California Sur, Mexico. Scale bar = 5 cm.

The epiphyte *Tillandsia recurvata* L. (Bromeliaceae) is a common plant of Mexico in the rainy zone bordering the Gulf of Mexico and the semiarid zones of southern Baja California. Relatively little has been published in scientific literature on the *Tillandsia* genus.

The nutritional metabolism of this genus and its possible association with any microorganisms are unknown. It has been assumed that in arid zones, the plants obtain water from condensation on their extensive foliar trichomes (characteristic of the Bromeliaceae) (3,5) and trace minerals from dust (14). It was further speculated that bromeliad plants derive part of their nitrogen from the decomposition of insects that take refuge inside their dense foliage (16). However, it remains unclear how these plants obtain essential elements, such as nitrogen, phosphorous, and potassium, which are required in large quantities for such profuse growth. Previous theories do not adequately explain the phenomenon in the deserts of Baja California, since (*i*) the local dust contains very little of these elements (Y. Bashan, unpublished), (*ii*) there is no evidence that *T. recurvata* plants are a preferred site for any particular insect, (*iii*) the plant foliage is dry almost all year long, preventing the rapid decomposition of any dead insects, and (*iv*) conditions favorable for phylosphere-surface nitrogen fixation such as a high carbon to nitrogen ratio, availability of an energy source, optimal temperature, and high humidity, which may be provided by tropical *Tillandsia* species, hardly exist under the harsh desert conditions of Baja California.

The aim of our study was to isolate and identify an associated, efficient nitrogen-fixing bacterium from the plant interior that may provide at least part of the nitrogen required by this prolific plant.

In June and October 1991, several plants of *T. recurvata* were removed from the giant columnar Cardon cactus *Pachycereus pringlei* at several sites in the Santo Domingo valley and from electrical cables in the town of Cuidad Constitucion, both in the state of Baja California Sur, Mexico (Figs. 1 and 2). In the laboratory, the plants were thoroughly washed with tap water and detergents to remove any dust. They were then surface disinfected with 2% NaOCl for 10 min at ambient temperature and then with 10 % calcium hypochlorite for 10 min. The disinfectants were removed by washing continuously for 10 min with sterile distilled water in a laminar flow hood. The leaves were cut with flame-sterilized tweezers and the remaining "stem" was subjected to a secondary surface-sterilization procedure (as above) followed by a flame sterilization. The stem was then cut longitudinally with a sterile razor, small pieces of plant tissue (<0.5 g) were removed from the vascular area of the plants, and transferred into flasks containing 60 mL OAB semisolid (0.5 g/L agar) nitrogen-free medium (1) and incubated for 6 days at 37°C without movement. OAB medium contains the following. Solution A: 5 g/L DL-malic acid, 3 g/L NaOH, 02 g/L $MgSO_4 \cdot 7H_2O$, 0.02 g/L $CaCl_2$, 0.1 g/L NaCl, 1 g/L NH_4Cl, 0.1 g/L yeast extract, 0.01 g/L $FeCl_3$, 2 mg/L $NaMoO_4 \cdot 2H_2O$, 2.1 mg/L $MnSO_4$, 2.8 mg/L H_3BO_3, 0.04 mg/L $Cu(NO_3)_2 \cdot 3H_2O$, 0.24 mg/L $ZnSO_4 \cdot 7H_2O$, and 900 mL distilled water. Solution B: 6 g/L K_2HPO_4, 4 g/L KH_2PO_4, and 100 mL distilled water. After autoclaving and cooling, the two solutions were mixed. The medium pH was 6.8. The bacterial pellicle that formed within the growth medium was extracted, serially diluted in 0.06 M potassium phosphate buffer supplemented with 0.15 M NaCl, and plated on the same medium, solidified with 1.5 % agar, and incubated for 48 h at 37°C. Ten different bacterial colony morphotypes were developed and purified in a conventional manner on the same solid medium lacking NH_4Cl. Since the medium lacked a nitrogen source, all these bacteria were suspected to be nitrogen-fixing bacteria. The ability of all pure morphotypes to fix nitrogen was evaluated by the acetylene reduction (ARA) technique (6). ARA-positive bacteria were further identified by gas chromatographic analysis of the cellular fatty acids (GC-FAME) (13) and further confirmed by biochemical tests listed for *Pseudomonas stutzeri* (8).

Of the 10 bacterial morphotypes that developed after several transfers on nitrogen-free medium, only 1 was ARA positive. Isolates of this morphotype reduced acetylene to ethylene at a rate of 3860 nmol $\cdot$ 50 mL^{-1} $\cdot$ 24 h^{-1}, which is similar to other associative nitrogen-fixing bacteria such as *Azospirillum*, *Pseudomonas*, *Listonella anguillarum*, or *Vibrio cambellii* (2,7,9). Fatty acid analyses and complementary biochemical tests identified this morphotype as *Pseudomonas stutzeri* (Lehmann and Neumann 1896) Sijderius 1946. The match values in the GC-FAME analysis of the two bacterial libraries were 0.769 (in the main aerobic bacteria library) and 0.606 (in the clinical bacteria library) without an alternative match. In general, a match of 0.5 or higher with no second choice is correct to the subspecies level, a match of 0.2 or higher with no second choice is correct to the species level, and a match of less than 0.2 with no second choice is correct only to the genus level (13).

This bacterial species is known as a nitrogen-fixing bacterium of "lesser importance," which is also capable of transforming nitrogen in nature (8). Its usual habitat is water and soil (11), but not the phyllosphere. The amount of nitrogen fixed by the bacteria within the plant was not evaluated in this study, nor was the size of its native population. However, from our observations of the interior site from which the bacteria were isolated, we propose that (*i*) the bacteria may form an as yet undefined positive association with the plant and (*ii*) if the bacterium fixes nitrogen at this site, it may supply part of it to the plant.

Phyllosphere diazotrophs in other plant species are known (10,12,15). Moreover, recent analysis (4) of the phyllosphere bacterial populations of *Tillandsia* species in mainland Mexico detected, but only after a liquid enrichment of the entire leaf, a single diazotrophic species, *Bacillus megatherium*. It is noteworthy that both diazotrophs known to be associated with bromeliads are considered of minor importance as diazotrophic species, both in the soil and in the rhizosphere. All the more common diazotrophs one would expect to find, such as *Azotobacter*, *Azospirillum*, *Arthrobacter*, and *Beijerinckia*, were not detected in either study.

In conclusion, *Pseudomonas stutzeri*, a nitrogen-fixing bacterium, was isolated from the interior of the epiphyte *Tillandsia recurvata*, which grew attached to electrical cables and giant columnar cacti in the semiarid zone of Baja California, Mexico. This study, together with a recent report from mainland Mexico (4), are the first reports on a possible association between bromeliad plants and nitrogen-fixing bacteria.

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