Ammonium-oxidizing nitrifying bacteria from Chilean soils.

Resumen. Se describe el aislamiento e identificación de bacterias oxidantes del amonio existentes en suelos colectados en la VIII Región, Chile. Se analizó un total de 10 muestras de suelos correspondientes a las siguientes ocho series de suelos: San Esteban, Cauquenes, San Carlos, Mirador, Collipulli, Quella, Diguillin, Santa Bárbara. El aislamiento se realizó mediante cultivos de enriquecimiento. La identificación de las bacterias se basó en las características morfológicas observadas al microscopio electrónico de transmisión. Se detectó *Nitrosomonas* solamente en una de las muestras de suelo, *Nitrosospira* en cuatro y *Nitrosolobus* en seis de ellas. No se encontró alguna relación entre los géneros aislados y las propiedades de los suelos.

Although the process of autotrophic nitrification in soils has been extensively investigated, there are relatively few reports on the ecology of nitrifying bacteria in soils in relation to the geographic distribution of the generator species.

Moreover, there is a lack of information from many areas of the world. The purpose of this study was to identify ammonium-oxidizing bacteria in Chilean soils.

Materials and methods

The soils studied (Table 1) correspond to series occurring in the provinces of Concepción and Nuble, VIII Región, Chile. They were surface sampled (0-25 cm) in field-moist condition and kept under refrigeration. Subsamples were sieved (2 mm), air-dried and analyzed. For enrichment, an amount of field-moist soil equivalent to 2 g of oven-dried material was placed in 250 ml Erlenmeyer flasks containing 50 ml of sterile ammonium sulfate growth medium (3) and incubated at 25°C, until the color of the medium changed to yellow. The pH was readjusted with sterile 5% sodium carbonate solution. When a second color change occurred, 0.5 ml of the culture was transferred to fresh medium, and a drop was spread on Bhuiya and Walker medium (3) solidified with 2% Noble (Difco) agar. This procedure was repeated three times. After the last color change, 0.5 ml of the culture was diluted tenfold up to 10^{-7} in tubes containing 4.5 ml of medium. The tubes were incubated at 25°C for three weeks and a new tenfold dilution was made from the highest dilution showing nitrite production, and a drop was spread on an agar plate. This procedure was repeated five to six times. From the agar plates showing a strong nitrite reaction to Griess-Ilosvay reagent after incubation at 25°C for three weeks, nitrifier-likely colonies were picked using microcapillary drawn Pasteur pipettes (9) and transferred to tubes of medium and incubated at 25°C.

Identification of ammonium-oxidizing bacteria at the genus level was done by transmission electron microscopy following the morphological criteria given by Buchanan and Gibbons (4).

Enrichment cultures, or cultures from successfully transferred colonies, were used to inoculate (1% inoculum) flasks containing 2 liters of medium. These cultures were incubated at 25°C and sparged with air (50 ml · L^{-1} · min^{-1}) sterilized through a 0.45 um Millipore filter. During incubation the pH was adjusted by the addition of a 5% solution of sodium carbonate, and bacterial growth was measured colorimetrically (14). Bacterial cells were processed for electron microscopy according to the method of Watson (13). After prefixation with glutaraldehyde in the growth medium, the cells were harvested by filtration through a 0.45 um membrane filter (47 mm

Table 1. Characteristics of the soils sampled.

Sample No.	Soil		Predominant Clay	pH	Organic matter %	Nitrate N ppm	P2O5 ppm	K20 meq/100 g
	Series	Subgroup						
1	San Esteban	Ultic Palexeralfs	Kaolinite	6.3	4.6	12.3	14.0	0.46
2	Cauquenes	Ultic Palexeralfs	Kaolinite	6.2	2.5	8.9	8.0	0.48
3	San Carlos	Typic Durixeralfs	Metahalloysite	5.9	3.2	15.8	14.0	0.32
4	Mirador	Ultic Palexeralfs	Metahalloyside	5.8	3.0	8.7	8.0	0.98
5	Collipulli	Ultic Palexeralfs	Metahalloysite	5.2	2.5	5.3	6.0	0.70
6	Collipulli	Ultic Palexeralfs	Metahalloysite	5.5	2.2	19.2	3.6	0.82
7	Quella	Typic Pelloxerents	Montmorillonite	5.8	3.2	5.2	20.0	0.22
8	Diguillin	Typic Dystrandepts	Allophane	6.0	10.0	12.3	16.0	0.70
9	Santa Barbara	Typic Dystrandepts	Allophane	5.9	6.9	8.8	6.0	0.30
10	Santa Barbara	Typic Dystrandepts	Allophane	6.0	9.7	35.0	7.2	0.50

in diameter) placed in a Swinnex (Millipore) holder. The steps of fixation with osmium tetroxide and staining with uranyl acetate were performed without removing the bacteria from the filter, simply by forcing the solutions through the filter using disposable syringes. The bacteria were resuspended in a few drops of buffer, enrobed in an equal volume of 3% Noble agar, dehydrated with alcohol and embedded in a mixture of Araldite-Epon 812. Sections were cut to 45 nm, placed on Formvar coated grids and stained with lead citrate. Electron micrographs were obtained from a Philips EM-200 operated at 80 kV.

Results and discussion

From each of the ten soil samples, enrichment of ammonium-oxidizing bacteria was obtained, but all attempts to obtain pure cultures through the transfer and dilution procedure, or by plating and colony picking, failed. In spite of this, identification to genera level was possible in thin sections of non-pure cultures prepared for TEM. This could be accomplished because in the enrichment cultures, the ammonium-oxidizing bacteria population far outnumbered that of heterotrophic contaminants; also their morphology is sufficiently characteristic to allow for reliable identification.

Nitrosolobus (Fig. 1a) was found to be the most common genus (Table 2), being detected in 6 of the soil samples surveyed. The genus *Nitrosospira* (Fig. 1b) was present in 4 of the samples; *Nitrosomonas* (Fig. 1c) was found in only one soil, which also contained *Nitrosolobus*. The last observation supports the findings of Belser and Schmidt (2) regarding the coexistence of multiple genera of ammonium-oxidizing bacteria in the same soil. It should be stressed that while most investigators have reported the genera *Nitrosomonas* and/or *Nitrosospira* as the most frecuently isolated (1, 2, 3, 6, 7, 8, 10, 12), in our study the most prevalent genus was *Nitrosolobus*. The predominance of the genus *Nitrosolobus* in certain soils has also been reported by other authors. Bhuiya and Walker (3) isolated *Nitrosospira* from all acid tea soils tested from Bangladesh, but in tea soils from Sri Lanka and nonacidic soils from Bangladesh they found that *Nitrosolobus* was the most common genus. Walker (11), in a search of nitrifiers from soils collected from different continents, concluded that *Nitrosolobus*, and not *Nitrosomonas*, is the dominant genus in agricultural soils. Additionally, MacDonald (5), in his study of the dyanmics of the nitrifying bacterial population in a soil from Rothamsted, isolated *Nitrosolobus* only.

In this study, we found no relationship between the occurrence of the different genera and such soil properties as pH, predominant clay mineral, and chemical composition.

We are not aware of any previous report of the identification of autotrophic ammonium-oxidizing bacteria in Chilean soils.

Table 2. Ammonium-oxidizing bacteria identified in soil samples from Chile.

Sample No.	Soils series	Genus
1	San Esteban	*Nitrosospira*
2	Cauquenes	*Nitrosolobus*
3	San Carlos	*Nitrosolobus*
4	Mirador	*Nitrosolobus*
5	Collipulli	*Nitrosolobus*
6	Collipulli	*Nitrosospira*
7	Quella	*Nitrosolobus* and *Nitrosomonas*
8	Diguillin	*Nitrosolobus*
9	Santa Bárbara	*Nitrosospira*
10	Santa Bárbara	*Nitrosospira*

Summary

The isolation and identification of ammonia-oxidizing bacteria in soils collected from the VIII Región, Chile is described.

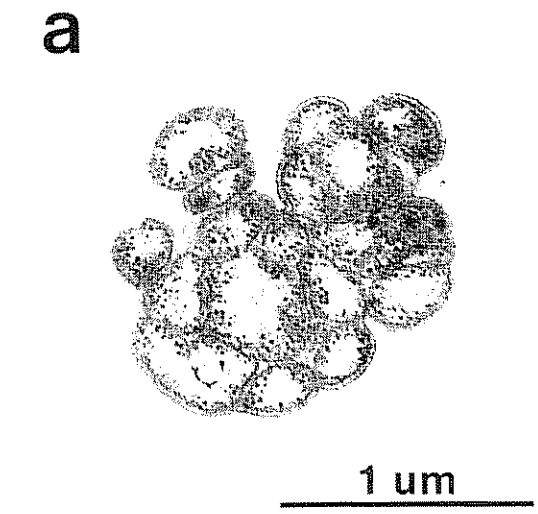

Fig. 1. Electron micrographs of lead-stained thin sections: (a) *Nitrosolobus* sp. from Mirador soil; (b) *Nitrosospira* sp. from San Estebal soil; (c) *Nitrosomonas* sp. from Quella soil.

Ten samples of soil corresponding to 8 different soil series, namely San Esteban, Cauquenes, San Carlos, Mirador, Collipulli, Quella, Diguillín and Santa Bárbara were analyzed. Isolation was done by soil enrichment cultures. Identification of bacteria was based on morphological characters as revealed by transmission electron microscopy. *Nitrosomonas* was found only in one of the soil samples, *Nitrosospira* in four, and *Nitrosolobus* in six of the soils tested. No relationship was found between the genera isolated and soil properties.

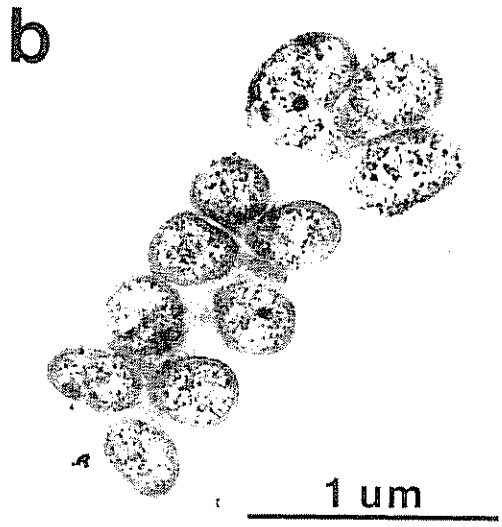

Fig. 1b

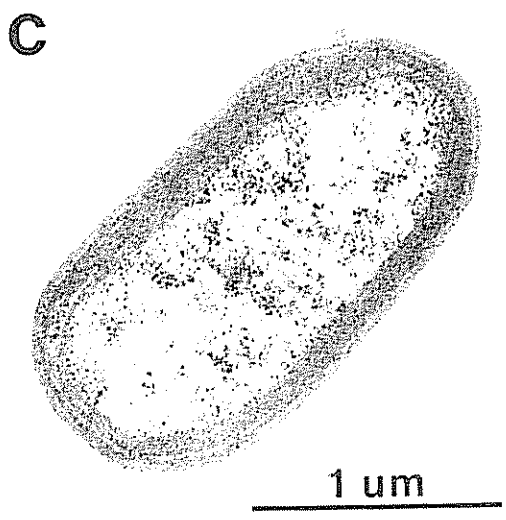

Fig. 1c

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