APPLIED AND ENVIRONMENTAL MICROBIOLOGY, June 1988, p. 1536–1540
0099-2240/88/061536-05$02.00/0

Vol. 54, No. 6

An Acidophilic and a Neutrophilic *Nitrobacter* Strain Isolated from the Numerically Predominant Nitrite-Oxidizing Population of an Acid Forest Soil†

T. R. HANKINSON AND E. L. SCHMIDT*

Department of Microbiology and Department of Soil Science, University of Minnesota, St. Paul, Minnesota 55108

Received 14 December 1987/Accepted 18 March 1988

Two physiologically and serologically distinct strains of chemoautotrophic nitrite-oxidizing bacteria were isolated as numerically predominant members of the nitrite-oxidizer population of an undisturbed forest soil with a pH range of 4.3 to 5.2. One isolate responded as a neutrophile, characteristic of the family *Nitrobacteraceae*, and cross-reacted strongly with fluorescent antibody to *Nitrobacter* strain Engel. The second isolate responded as an acidophile in pure culture, demonstrated maximal nitrite oxidation activity at pH 5.5, and had a pH tolerance range of pH 4.1 to 7.2. Nitrite oxidase in whole cells of the acidophile sustained activity to at least pH 3.5. Cell morphology of both strains typified the genus *Nitrobacter* in all respects when cultured at pH 7. However, under more acidic conditions the acidophile tended to elongate and at times appeared to branch. These data provide the first evidence for the existence of an acidophilic chemoautotrophic nitrifying bacterium. Isolation of the neutrophilic *Nitrobacter* strain reported here complements the earlier isolation of a neutrophilic *Nitrosospira* strain to provide further evidence of a prominent acid-intolerant population of chemoautotrophic nitrifiers in this acid forest soil.

Chemoautotrophic nitrifying bacteria have long been characterized as acid sensitive. Their acid intolerance is well documented and is considered to be a general characteristic of the family *Nitrobacteraceae*, with growth in pure culture generally limited to pH 6.5 or higher (21). Nevertheless, nitrification occurs in some acid soils at pH levels far below the reported acid tolerance of the chemoautotrophic nitrifiers (5, 19, 22).

The microbiological basis for nitrification in highly acidic soils has yet to be resolved. One possibility is that heterotrophic microorganisms may be contributing or even exclusive agents of nitrification in some environments (13). Several studies have provided strong indirect evidence of heterotrophic nitrification in acid forest soils (1, 12, 17), but no microbiological data were reported. However, recent isolation (18) from an actively nitrifying acid forest soil of a fungus that formed nitrite in pure culture is of particular interest since this is the first fungal isolate shown to nitrify in acid media.

Evidence for participation of the chemoautotrophic nitrifiers in acidic nitrification also has remained inconclusive. Very few autotrophic nitrifiers have been isolated from acid soils, but they nevertheless are representative of most of the generic diversity known among the *Nitrobacteraceae*. The genus *Nitrosospira* has been isolated most frequently (3, 9, 20), followed by *Nitrosolobus* and *Nitrosomonas* (4, 20), *Nitrosovibrio* (19), and *Nitrobacter* (7, 8). All of these isolations were obtained by enrichment culture so that the relative abundance of the nitrifier in the acid soil prior to enrichment could not be estimated. Moreover, none of the isolates was characterized with respect to acid tolerance in pure culture.

We investigated an acid oak forest soil in Indiana and reported (6) the isolation of a *Nitrosospira* strain from the most abundant ammonia-oxidizing population. Despite the predominance of this isolate in most-probable-number (MPN) soil dilution tubes, its growth in pure culture was limited to pH 6.2 and higher. Thus, no acidophilic or even acid-tolerant chemoautotrophic nitrifier has been identified among isolates from nitrifying acid environments. Based on further studies of the most abundant nitrifiers of the same Indiana oak site, we now report the first isolation of an acidophilic autotrophic nitrifier, a nitrite oxidizer of the genus *Nitrobacter*. In addition, we report the isolation of an acid-intolerant strain of the same genus coexisting in this acid soil.

MATERIALS AND METHODS

The soil examined was from an undisturbed 50-year-old mixed oak stand in the Hoosier National Forest in southern Indiana. This site was included in a study of nitrogen cycling and the nitrification process in disturbed and undisturbed forest ecosystems (19). Samples were collected either as intact columns of soil in coffee cans (6) or separated into O and A1 horizons and stored in Whirlpak bags. Samples were transported at ambient temperature to the laboratory for pH measurements and inoculation into MPN dilution tubes within 48 h after collection. Soil pH was determined after suspension and equilibration of 1 volume of soil with 2 volumes of 0.01 M $CaCl_2$. Individual samples were within the pH range of 4.3 to 5.2, somewhat less acid than the samples collected from the same site 2 years earlier for previous study (6).

The MPN method for chemoautrophic nitrifier enumeration was performed as described by Schmidt and Belser (14) and modified for enumeration of acid-tolerant nitrifiers (6). Parallel MPN estimates were performed with pH 7 medium or medium adjusted to pH 5 by adding 1.8 ml of 0.5 N HCl per liter of medium. Autoclaving tended to increase pH of the acid medium by 0.2 to 0.4 units, so the final pH was often achieved by amending autoclaved medium with sterile 0.05 N HCl.

* Corresponding author.

† Paper no. 15740 in the Scientific Journal Series of the Minnesota Agricultural Experiment Station, St. Paul.

Nitrifying activity in the MPN dilution tubes was determined after 6, 8, and 12 weeks of incubation. In some cases, activity in the highest-dilution MPN tubes was not detected until week 8 or 12. Activity was measured qualitatively using the Griess-Ilosvay colorimetric spot test to observe the disappearance of nitrite (14). Nitrite oxidation was confirmed with the diphenylamine spot test for nitrate (14). The confirming test for nitrate was particularly useful in the most acidic pH medium to distinguish between biological oxidation and the slow but detectable acid- induced chemical destruction of nitrite during this extended period of incubation (6). It was also useful to insure that loss of nitrite from the medium was the result of nitrification rather than nitrite assimilation by soil heterotrophs associated with soluble soil organic material in the less-dilute MPN tubes. The latter was not observed in this study.

Pure culture isolations were performed by the nonenrichment approach of Belser and Schmidt (2). Isolations were initiated from the highest-dilution MPN enumeration tubes that were positive for nitrite oxidation in both pH 4.0 and 7.0 media (6). One such tube from the pH 4.0 MPN series was transferred into pH 5.0 medium to encourage more rapid growth, and a counterpart tube from the pH 7.0 series was transferred into pH 7.0 autotrophic nitrite oxidizer enrichment media. Both were amended periodically with 100 μM KNO_2 to increase populations before pure culture isolation. Bacteria were concentrated on polycarbonate filters (0.2-μm pore size, 25-mm diameter; Nuclepore Corp., Pleasanton, Calif.) pretreated with irgalan black (11) and enumerated by fluorescence microscopy after staining with acridine orange (15). Samples from enriched cultures were diluted to 0.5 cells per ml in sterile saline and inoculated into 25 tubes containing 4 ml of autotrophic media of appropriate pH at a rate of 1 ml per tube. These isolation tubes were incubated for 4 to 6 weeks before screening for NO_2^- oxidizing activity.

All active cultures were examined for contamination by heterotrophs. Trypticase soy agar (BBL Microbiology Systems, Cockeysville, Md.) plates were spread with 0.1 ml of culture, and 9 ml of sterility no. 4 medium (14) was inoculated with 1.0 ml of culture. Absence of colonies on Trypticase soy agar plates and absence of turbidity in sterility broth after 7 days of incubation at 23°C was considered preliminary evidence of a pure culture. Each culture was examined further for purity by phase-contrast and fluorescence microscopy.

Characterization of isolates. Inoculum cultures were grown in 250-ml flasks containing 100 ml of autotrophic nitrite-oxidizer medium at the appropriate pH. Cultures were incubated at 23°C on an orbital shaker until nitrite was completely consumed. Except where noted, the substrate concentration for all inoculum cultures was 100 μM nitrite. Experiments were initiated within 24 h after complete oxidation of nitrite by the inoculum culture (10 μmol of NO_2^- per 100 ml of culture).

The effect of acidity on nitrite oxidation by the two isolates was determined by using 10-ml samples of inoculum transferred to 250-ml flasks containing 100 ml of nitrite-oxidizer medium at various pHs in the range of 4.6 to 8.0. Consumption of nitrite was quantified by the modified Griess-Ilosvay technique (4). The pH was monitored during all pure culture experiments and remained essentially unchanged.

For the nitrite oxidase pH tolerance assay, nitrite oxidation was measured in washed whole cells over 6 h in 100 ml of nitrite-oxidizer medium adjusted to pH 3.5, 4.1, 4.5, or 5.5 with sterile 0.05 N HCl. To achieve a reasonably heavy cell suspension, a 100-ml culture in pH 5.5 medium was routinely amended with a sterile solution of KNO_2 (10 μmol of NO_2^- per ml) over 22 days until a total of 42 μmol of NO_2^- had been oxidized by the culture. Amendments were increased progressively from 1 to 5 ml per culture over 22 days of incubation. Samples (10 ml) of the culture were then filtered through each of 4 sterile, 0.2-μm-pore-size polycarbonate filters (Nuclepore) and washed with 10 ml sterile saline, and the total filter residue was used to inoculate each of the four flasks of nitrite-oxidizer medium. Enzyme activity was recorded as the cumulative change in nitrite concentration in the standard nitrite medium every 2 h for an 6-h time period. Duplicate flasks containing sterile medium at each pH value were included to account for any pH-dependent chemical destruction of nitrite.

Both isolates were serotyped by immunofluorescence with fluorescent antibodies (FAs) and procedures as reported previously (6).

FA preparation. It was necessary only to prepare FA against the acidic MPN-derived isolate. That isolate was cultured in 1,200 ml of nitrite-oxidizer medium at pH 5.8 in a Fernbach flask for 58 days. The culture was amended periodically with a sterile solution of nitrite to compensate for nitrite oxidized beginning with amendments of 85 mg of NO_2^- per 1,200 ml of culture and gradually increasing to 425 mg of NO_2^- per 1,200 ml of culture. The culture received a total of 2.28 g of NO_2^- before cell harvest and antigen preparation. Production of FAs was performed by the methods of Belser and Schmidt (2).

Screening of MPN dilution tubes with FAs. Confirmation of the presence of the isolate strains as predominant nitrite oxidizers in the Indiana oak soil was sought by FA screening of MPN dilution cultures. MPN dilution series at pH 7.0 and 5.0 were inoculated with soil obtained from the same undisturbed forest site from which isolate strains had originated. After incubation, samples of 0.5 ml from highest-dilution NO_2^- oxidation-positive tubes were placed on irgalan black-treated Nuclepore filters, stained with FAs, washed, and examined by immunofluorescence (6).

RESULTS

The dilution isolation technique was applied successfully to isolate pure cultures of nitrite oxidizers from the highest positive dilutions of soil-inoculated MPN determinations in both acid and neutral media.

The pH 7 isolate showed typical *Nitrobacter* morphology when observed by phase and fluorescence microscopy. Cells were small, approximately 0.8 μm in length, with one end slightly enlarged and roughly 0.6 μm in diameter. Based on cell morphology and chemoautotrophic metabolism, the organism appeared to be a member of the genus *Nitrobacter* and was designated *Nitrobacter* strain IOneut.

The isolate obtained from acidic MPN and isolation media also resembled *Nitrobacter* species, but most cells appeared elongated, up to 2 times the normal average cell length of the other isolate. Pending further study, the acid isolate was tentatively labeled *Nitrobacter* strain IOacid.

Serological examination of strains IOneut and IOacid. Both nitrite-oxidizer strains were checked for cross-reactivity against FAs raised against 12 *Nitrobacter* strains (Table 1). *Nitrobacter* strain IOneut gave a strong 4+ reaction when stained with *Nitrobacter* strain Engle FA, placing it in the Engel serogroup described by Stanley and Schmidt (16). Strain IOneut also gave a barely detectable reaction when stained with *Nitrobacter* strain 7R FA and did not react with the remaining nine FAs.

TABLE 1. Serological cross-reactivity of acid forest soil isolates *Nitrobacter* strains IOneut and IOacid against stock culture autotrophic nitrite-oxidizer FAs

Nitrite-oxidizer FA	Titer[a]	Homologous reaction[b]	FA cross-reaction against strain:	
			IOneut	IOacid
6R	1:8	4+	0	0
7R	1:8	4+	±	0
Agilis	1:8	4+	0	0
844	1:16	4+	0	±
622	1:16	4+	0	0
Winogradsky	1:16	4+	0	0
W63	1:32	4+	0	0
Cl-19	1:16	4+	0	0
Engel	1:32	4+	4+	±
Bock	1:16	4+	0	1+
CalA	1:32	4+	0	0
IOacid[c]	1:8	3+	0	3+

[a] FAs were diluted by twofold increments with filtered saline to the highest dilution which still provided a 4+ (or best possible) reaction with the strain against which the FA was raised.
[b] 0, No fluorescence; ± to 4+, trace to bright fluorescence.
[c] Prepared during this study.

Nitrobacter strain IOacid gave a 1+ reaction when stained with *Nitrobacter* strain Bock FA and reacted <1+ against FAs CH844 and Engel. Strain IOacid was completely unreactive against the nine remaining FAs. FA raised against strain IOacid provided a 3+ homologous reaction and was unreactive against strain IOneut. *Nitrobacter* strains IOneut and IOacid thus appear to belong to separate serogroups which do not share common antigens.

Acid tolerance of strains IOneut and IOacid. Under pure culture conditions the pH optimum for nitrite oxidation by IOneut was at least 7.2 (Fig. 1). Nitrite oxidation decreased progressively with decreasing pH. The limit of acid tolerance for IOneut was near pH 5.5 or slightly below. In contrast, IOacid was most active at a pH of about 5.5 and was decreasingly active above and below that pH (Fig. 2). No activity was observed at pH 8. Strain IOneut responded as an obligate neutrophile under moderately acid conditions, whereas strain IOacid can be characterized as a moderate acidophile.

Acid tolerance of the nitrite oxidase function in *Nitrobacter* strain IOacid is shown in Fig. 3 as the rates of nitrite oxidation by washed whole cells suspended in nitrite-oxidizer medium containing 100 μmol of NO_2^- per liter. Rates

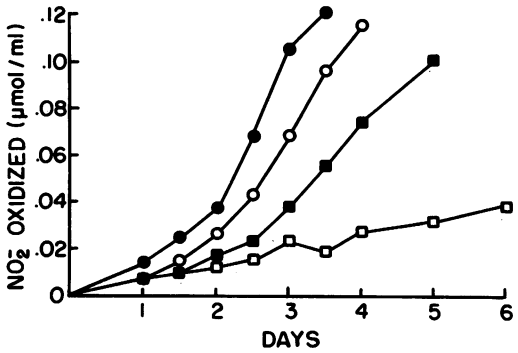

FIG. 1. Effect of pH on oxidation of nitrite by *Nitrobacter* strain IOneut. Symbols: ●, pH 7.2; ○, pH 6.4; ■, pH 6.0; □, pH 5.5.

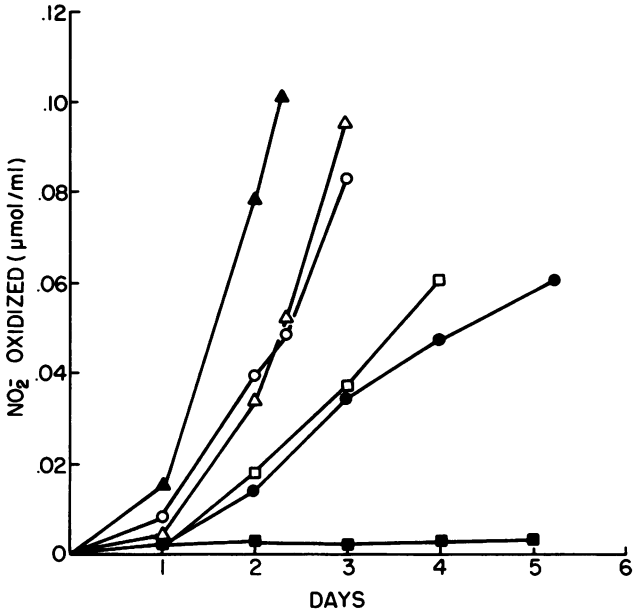

FIG. 2. Effect of pH on oxidation of nitrite by *Nitrobacter* strain IOacid. Symbols: ▲, pH 5.5; △, pH 5.2; ○, pH 6.5; □, pH 7.0; ●, pH 4.6; ■, pH 8.0.

at pH 5.5, 4.5, 4.1, and 3.5 were 11.98, 8.71, 4.86, and 1.44 mol of NO_2^- oxidized per h, respectively (the rate for pH 3.5 was corrected for chemical destruction of NO_2^-). The curve representing nitrite oxidation at pH 3.5 was adjusted to account for chemical destruction of nitrite under very acid conditions. Chemical destruction at pH values higher than 3.5 was insignificant over the 6-h assay period. Nitrite oxidase activity in strain IOacid whole cells decreased with increasing acidity, but activity persisted at pH 3.5 and possibly below, almost 1 full pH unit below the limit observed in growing cells.

Cell morphology and pH. *Nitrobacter* strain IOacid manifested interesting morphological changes when grown under acid conditions. When cultured at pH 7 and stained with homologous FA, IOacid possessed a typical pear shape and was morphologically indistinguishable from strain IOneut (Fig. 4a). In pH 6 medium (Fig. 4b) some cells appeared longer but often retained the enlarged end characteristic of *Nitrobacter*. In pH 5.5 medium (Fig. 4c) a majority of the cells appeared 3 to 5 times the original cell length or longer and in some cases appeared to branch. Cell width also appeared to increase slightly.

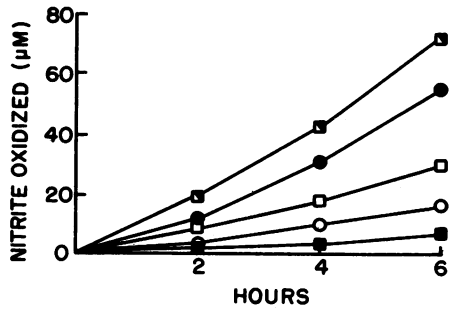

FIG. 3. Acid tolerance of nitrite oxidase in whole cells of *Nitrobacter* strain IOacid. Symbols: ◥, pH 5.5; ●, pH 4.5; □, pH 4.1; ○, pH 3.5 uncorrected; ■, pH 3.5 corrected.

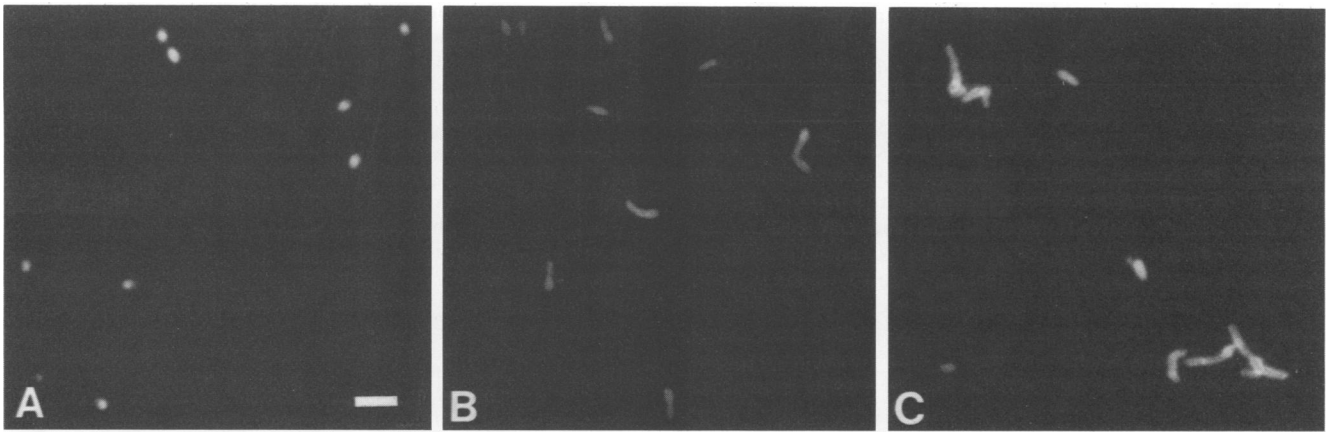

FIG. 4. Morphological response of *Nitrobacter* strain IOacid to the acidity of the growth medium. Photomicrographs of cells stained by homologous fluorescent antibodies. A, pH 7.0; B, pH 6.0; C, pH 5.5. Bar, 2 μm for all micrographs.

Relative predominance of the isolates in the acid soil. The occurrence of both *Nitrobacter* strains IOacid and IOneut as predominant nitrifiers in the Indiana oak soil was verified by immunofluorescence (Table 2). Soil samples freshly collected in 1984 from the same site that yielded the isolates in a 1982 collection contained nitrifier populations cross-reactive to FAs for both IOacid and IOneut. All 16 of the highest-dilution pH 5.0 MPN tubes that showed nitrite oxidation contained cells reactive to IOacid FA and were presumed positive for the presence of strain IOacid. Similarly, 11 of the 16 positive dilution tubes from the pH 7.0 series were also presumed positive for the occurrence of strain IOneut.

TABLE 2. Confirmation of the occurrence and numerical predominance of isolates IOacid and IOneut by FA examination of the highest dilution, nitrite oxidation-positive tubes from MPN analyses of Indiana oak acid forest soil

Soil sample[a]	Soil pH	MPN pH	MPN dilution[b]	FA[c] of strain:	
				IOacid	IOneut
IO 4a O	4.6	5	10^{-4}	2/2	0/2
		7	10^{-4}	0/2	0/2
IO 4a A1	4.3	5	10^{-3}	2/2	0/2
		7	10^{-3}	0/2	1/1
		7	10^{-4}	0/2	1/1
IO 4b O	5.1	5	10^{-3}	1/1	0/2
		5	10^{-4}	1/1	0/2
		7	10^{-5}	0/2	0/2
IO 4b A1	4.8	5	10^{-3}	1/1	0/2
		5	10^{-5}	1/1	0/2
		7	10^{-5}	0/2	1/2
IO 4c O	4.8	5	10^{-4}	2/2	0/2
		7	10^{-5}	0/2	2/2
IO 4c A1	4.7	5	10^{-3}	2/2	0/2
		7	10^{-3}	0/2	1/1
		7	10^{-4}	0/2	1/1
IO 4d O	5.2	5	10^{-3}	1/1	0/2
		5	10^{-4}	1/1	0/2
		7	10^{-4}	0/2	2/2
IO 4d A1	4.9	5	10^{-3}	2/2	0/2
		7	10^{-3}	0/2	1/1
		7	10^{-5}	0/2	1/1

[a] Indiana oak (IO) sample series 4; O is organic layer, A1 is A_1 horizon.
[b] Nitrite negative by the Griess-Ilosvay test.
[c] IOacid FA was diluted 1:8; Engel FA (cross-reacts with IOneut) was diluted 1:32. Results are expressed as the number of FA positive tubes/total number of tubes examined.

DISCUSSION

One difficulty in relating acidic nitrification to the activities of classical autotrophic nitrifiers has been the absence of any acid-tolerant isolate. The importance of this, however, must not be exaggerated since the nitrifiers remain very difficult to isolate even from circumneutral environments. In any case, we now report the first isolation of an acidophilic chemoautotrophic nitrifier, *Nitrobacter* strain IOacid, and suggest the likelihood of the occurrence of other such nitrifiers in acid habitats. Rather than a transient resident selected by enrichment, the isolate was a predominant nitrifier in the soil when collected and equally predominant in the same soil 2 years later (Table 2).

Isolation of the acidophile, strain IOacid, was attained by the use throughout of MPN and isolation media at or near the ambient pH of the soil. Care was also taken to insure that the concentration of nitrite never exceeded 0.1 μmol of NO_2^- per ml during the initial stages of isolation. Attempts to transfer several acidoduric nitrite oxidizers from our stock collection to moderately acid media were unsuccessful at nitrite concentrations higher than 0.1 μmol/ml (unpublished data). Substrate nitrite is likely to remain at very low concentrations in the acid forest soil studied in view of its relatively high nitrite-oxidizing population and its apparently low ammonia-oxidizing activity (6).

In terms of nitrite oxidation by growing cultures relative to the pH of its natural habitat, the *Nitrobacter* strain IOacid must be considered as moderately acidophilic. However, with optimal activity at pH 5.5 in culture it is about 100-fold more acidophilic than any previously isolated *Nitrobacter* strain. The acid tolerance of this isolate is noteworthy in that growth in pure culture was sustained in the range of pH 4.6 to 7.0 despite evidence of stress near its nitrite oxidation optimum (Fig. 4). These features, combined with the indications of nitrite oxidase activity extending at least to pH 3.5, strongly suggest the likelihood that oxidation of nitrite in some extremely acidic terrestrial environments can be attributed to acid-adapted *Nitrobacter* strains. Further reinforcement of this likelihood is found in the MPN enumeration of nitrite oxidizers in the Indiana oak soil. The same acid MPN medium that led to the isolation of *Nitrobacter* strain IOacid developed estimated populations of a few thousand to several hundred thousand nitrite oxidizers per gram of the acid soil (6).

Isolation of *Nitrobacter* strain IOneut as a predominant nitrite oxidizer from pH 7 MPN medium attests to further diversity of the autotrophic nitrite-oxidizing population of this soil. Strain IOneut was relatively intolerant of acid conditions and required a pH of about 7.2 to attain maximum nitrite oxidation (Fig. 1). Its pure culture response to acidity is reminiscent of that of the ammonia-oxidizing isolate *Nitrosospira* strain IO1a, also a predominant acid-sensitive nitrifier in the same soil (6). The occurrence of neutrophilic populations of both ammonia and nitrite oxidizers reinforces the possibility that some nitrification in very acid soils may emanate from microsites less acid than the soil homogenate prepared for pH measurement.

ACKNOWLEDGMENTS

This research was supported by grant DEB8121642 from the National Science Foundation and project 25-072 of the Minnesota Agricultural Experiment Station.

LITERATURE CITED

1. **Adams, J. A.** 1986. Identification of heterotrophic nitrification in strongly acid larch humus. Soil Biol. Biochem. **18**:339–341.
2. **Belser, L. W., and E. L. Schmidt.** 1978. Diversity of the ammonia-oxidizing nitrifier population of a soil. Appl. Environ. Microbiol. **36**:584–588.
3. **Bhuiya, Z. H., and N. Walker.** 1977. Autotrophic nitrifying bacteria in acid tea soils from Bangladesh and Sri Lanka. J. Appl. Bacteriol. **42**:253–257.
4. **Bremner, J. M.** 1965. Inorganic forms of nitrogen, p. 1179–1237. *In* C. A. Black, D. D. Evans, J. L. White, L. E. Ensminger, and F. E. Clark (ed.), Methods of soil analysis, part 2. American Society of Agronomy, Madison, Wis.
5. **Federer, C. A.** 1983. Nitrogen mineralization and nitrification: depth variation in four New England forest soils. Soil Sci. Soc. Am. J. **47**:1008–1014.
6. **Hankinson, T. R., and E. L. Schmidt.** 1984. Examination of an acid forest soil for ammonia- and nitrite-oxidizing autotrophic bacteria. Can. J. Microbiol. **30**:1125–1132.
7. **Josserand, A., and R. Bardin.** 1981. Nitrification in acid soil. I. Evidence for autotrophic nitrifying bacteria genus *Nitrobacter* in a coniferous forest soil. Rev. Ecol. Biol. Sol. **18**:435–446.
8. **Josserand, A., and J. C. Cleyet-Marel.** 1979. Isolation from soils of *Nitrobacter* and evidence for novel serotypes using immunofluorescence. Microb. Ecol. **5**:197–205.
9. **Martikainen, P. J., and E.-L. Nurmiaho-Lassila.** 1985. *Nitrosospira*—an important ammonium-oxidizing bacterium in fertilized coniferous forest soil. Can. J. Microbiol. **31**:190–197.
10. **Matson, P. A., and P. M. Vitousek.** 1981. Nitrogen mineralization and nitrification potentials following clearcutting in the Hoosier National Forest, Indiana. Forest Sci. **27**:781–791.
11. **Moawad, H. A., W. R. Ellis, and E. L. Schmidt.** 1984. Rhizosphere response as a factor in competition among three serogroups of indigenous *Rhizobium japonicum* for nodulation of field-grown soybeans. Appl. Environ. Microbiol. **47**:607–612.
12. **Schimel, J. P., M. K. Firestone, and K. S. Killham.** 1984. Identification of heterotrophic nitrification in a Sierran forest soil. Appl. Environ. Microbiol. **48**:802–806.
13. **Schmidt, E. L.** 1982. Nitrification in soil, p. 253–288. *In* F. J. Stevenson (ed.), Nitrogen in agricultural soils. Agronomy monograph no. 22. American Society of Agronomy, Madison, Wis.
14. **Schmidt, E. L., and L. W. Belser.** 1982. Nitrifying bacteria, p. 1027–1042. *In* A. L. Page, R. H. Miller, and D. R. Keeney (ed.), Methods of soil analysis, part 2. Chemical and microbiological properties. Agronomy monograph no. 9, 2nd ed. American Society of Agronomy, Madison, Wis.
15. **Schmidt, E. L., and E. A. Paul.** 1982. Microscopic methods for soil microorganisms, p. 803–814. *In* A. L. Page, R. H. Miller, and D. R. Keeney (ed.), Methods of soil analysis, part 2. Chemical and microbiological properties. American Society of Agronomy, Madison, Wis.
16. **Stanley, P. M., and E. L. Schmidt.** 1981. Serological diversity of *Nitrobacter* spp. from soil and aquatic habitats. Appl. Environ. Microbiol. **41**:1069–1071.
17. **Strayer, R. F., C. J. Lin, and M. Alexander.** 1981. Effect of simulated acid rain on nitrification and nitrogen mineralization in forest soils. J. Environ. Qual. **10**:547–551.
18. **Stroo, H. F., T. M. Klein, and M. Alexander.** 1986. Heterotrophic nitrification in an acid forest soil and by an acid-tolerant fungus. Appl. Environ. Microbiol. **52**:1107–1111.
19. **Vitousek, P. M., J. R. Gosz, C. C. Grier, J. M. Melillo, and W. A. Reiners.** 1982. A comparative analysis of potential nitrification and nitrate mobility in forest ecosystems. Ecol. Monogr. **52**:155–177.
20. **Walker, N., and K. N. Wickramasinghe.** 1979. Nitrification and autotrophic nitrifying bacteria in acid tea soils. Soil Biol. Biochem. **11**:231–236.
21. **Watson, S. W.** 1974. Gram-negative chemolithotrophic bacteria. Family 1, p. 450–456. *In* R. E. Buchanan and N. E. Gibbons (ed.), Bergey's manual of determinative bacteriology, 8th ed. The Williams and Wilkins Co., Baltimore.
22. **Weber, D. F., and P. L. Gainey.** 1962. Relative sensitivity of nitrifying organisms to hydrogen ions in soils and in solutions. Soil Sci. **94**:138–145.