APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Nov. 1982, p. 1241–1243
0099-2240/82/111241-03$02.00/0

Vol. 44, No. 5

Ammonium Production by Dissimilatory Nitrate Reducers Isolated from Baltic Sea Water, as Indicated by ^{15}N Study

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Received 2 June 1982/Accepted 14 June 1982

Bacteria able to perform dissimilatory nitrate reduction to ammonium were isolated from low-oxygen masses in the Baltic Sea. In liquid media enriched with $^{15}NO_3^-$ and incubated anaerobically, the NH_4^+-producing isolates transformed 25 to 72% of the $^{15}NO_3^-$ to $^{15}NH_4^+$.

During the last few years there has been renewed interest in the dissimilatory reduction of nitrate to ammonium (15). Several papers have dealt with this process in marine (7, 9, 13) and freshwater (6) sediments and in soil (2, 4). The dissimilation of nitrate to ammonium has also been investigated in pure cultures (5, 7, 8). The present ^{15}N study demonstrates that bacteria able to perform this dissimilatory reduction are also present in the water column and discusses the probable occurrence of this process in the Baltic waters.

Water samples used to isolate the NO_3^--reducing bacteria were obtained from the Baltic Sea at a depth of 400 m (Landsort deep) and 100 m (Karlsö deep), 40 and 10 m above the sediment, respectively. The water samples were collected with a 1.7-liter Nansen sampler. Subsamples were put into air-tight bottles and kept in a refrigerator at 4°C. The chemical properties of these two waters are given in Table 1. The following methods were used for the determinations: oxygen, the Winkler method; ammonium, the indophenol blue method modified by Koroleff; and nitrate and nitrite, a method based on Strickland and Parsons (1968). The above methods are specified in the *New Baltic Manual* (3).

Subsamples of the Baltic waters enriched with glucose and nitrate to an initial concentration of 500 μmol/liter and 490 μmol/liter, respectively, showed considerable ammonium formation; a production of approximately 100 μmol of NH_4^+ per liter was measured. The bottom waters at these stations contained a low oxygen concentration and yet some NO_3^- (Table 1), both prerequisites for denitrification and dissimilatory NO_3^- reduction to NH_4^+ (14). In water where the nitrite concentration was higher than the nitrate concentration, and an even higher ammonium formation was noticed (unpublished data). This indicated the presence of organisms able to reduce nitrate to ammonium.

Isolation. Of 36 glucose-NO_3^--enriched flasks (above), 27 showed ammonium formation. Six of these flasks were used for the isolation of bacteria. One-milliliter subsamples were plated onto agar medium containing 0.5% (wt/vol) tryptic soy broth (Difco Laboratories), 3.5 mmol of KNO_3 per liter, 0.5% (wt/vol) sodium thioglycolate, and, 1.5% (wt/vol) agar (4) prepared in artificial seawater (10) supplied with an ordinary vitamin and metal solution. The plates were incubated at room temperature in anaerobic containers provided with hydrogen as the gas phase. After incubation, 30 different colonies were picked and streaked out at least twice. The isolates were inoculated into a liquid medium containing the same components as for the agar medium and hydrogen as the gas phase. The cultures were then tested for ammonium and nitrite formation with Nesslers reagent (Merck Sharp & Dohme) and Griess reaction (3), respectively. Twelve cultures were found to be ammonium and nitrite producers (herein denoted 1 to 12). They were facultative anaerobes, motile and rod shaped. No spore formers were present since the cultures did not survive a treatment of 75°C for 10 min. The ability of the isolates to form NH_4^+ from NO_3^- under anaerobic conditions was tested in the two following experiments.

Experiment with unlabeled nitrate. Samples (70 ml) of artificial seawater enriched with glucose and NO_3^- to concentrations of 7.38 mmol/liter and 130 μmol/liter, respectively, were inoculated with each of the 12 ammonium-producing cultures. The inocula contained amounts of NH_4^+ and NO_3^- up to 25.5 and 10 μmol/liter, respectively. After 3 days of incubation, NO_3^- was completely consumed in spite of no or only slight growth, and eight cultures had reached their maximum NH_4^+ concentrations (Fig. 1). It is apparent that NH_4^+ production lagged behind NO_3^- disappearance. The reason for this is

TABLE 1. Ambient concentrations of O_2, NO_3^-, NO_2^-, and NH_4^+ at Landsort deep and Karlsö deep

Site	O_2 (ml/liter)	NO_3^- (μmol/liter)	NO_2^- (μmol/liter)	NH_4^+ (μmol/liter)
Landsort deep (400 m)	0.22	3.29	0.02	0.21
Karlsö deep (100 m)	0.16	0.12	0.39	1.70

unknown, but it could be due to a delay in the excretion of the accumulated N-intermediate(s). In culture 4 (typical for the group of eight), NH_4^+ amounted to 160 μmol/liter (Fig. 1); 24 μmol/liter was supplied with the inoculum, and therefore 136 μmol/liter was likely derived from NO_3^- reduction since no organic nitrogen was supplied. This NH_4^+ concentration corresponds reasonably well with the initial NO_3^- value. The concentration of NO_2^- stabilized at a value between 0.4 and 0.8 μmol/liter during the incubation. The remaining four cultures showed a decrease in nitrate without any increase in NH_4^+ (e.g., Fig. 1, culture 2); the cells perhaps used NO_3^- to produce N_2O, N_2, or organic nitrogen.

^{15}N experiment. A ^{15}N study was undertaken to verify that the NH_4^+ was derived from NO_3^-.

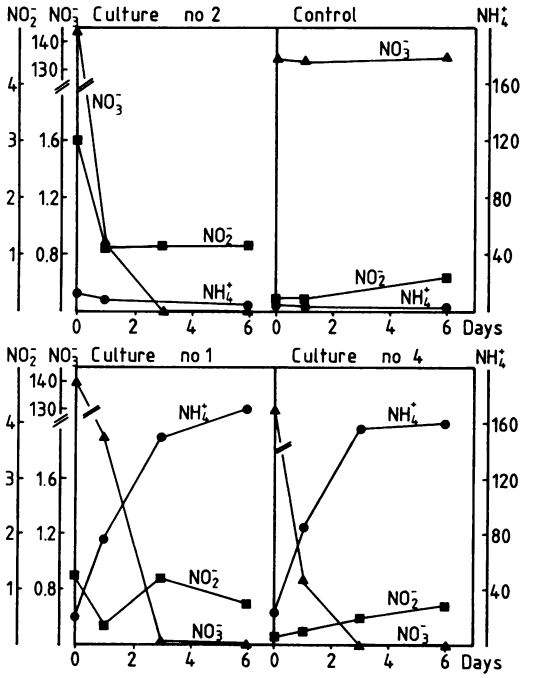

FIG. 1. Consumption of nitrate and production of nitrite and ammonia during 6 days of incubation. Symbols: ▲, NO_3^-; ■, NO_2^-; and ●, NH_4^+. Amounts are given in micromoles per liter.

TABLE 2. Concentrations of ^{15}N-labeled nitrogen compounds in the liquid media after 3 days of incubation of 12 different isolates in an artificial seawater medium initially enriched with 147 μmol of $^{15}NO_3^-$ per liter

Group no.	Isolate no.	^{15}N ions found (μmol/liter)			% $^{15}NO_3^-$ recovered as $^{15}NH_4^+$
		$^{15}NH_4^+$	$^{15}NO_3^-$	$^{15}NO_2^-$	
I	1	36.7	0.13	0.08	25.0
	3	78.5	22.0	0	53.4
	4	105.5	11.8	0	71.8
	6	85.9	0.11	0	58.4
	7	86.4	0	0	58.8
	9	76.8	0	0	52.3
	10	93.8	0.20	0	63.8
	11	68.9	0.14	0.08	46.8
	12	91.3	0	0.14	62.1
II	2	ND[a]	70		
	5	ND	100		

[a] ND, Not detected.

The conditions were similar to the above experiment. The ^{15}N analysis was performed by the method of Blackburn (1), where acid-washed glass capillaries in the experimental bottles trapped the diffusing NH_4^+ for 24 h. The capillaries were transferred to ampoules, which were heated to 550°C for 3 h to convert NH_4^+ to N_2. The $^{14}N/^{15}N$ ratios were then determined with a Statron NOI-5 emission spectrometer. The percentage of [^{15}N]nitrate transformed to [^{15}N]ammonium varied between 25 and 72% for the NH_4^+-producing isolates (group I, Table 2; the group II isolates did not produce $^{15}NH_4^+$). The unrecovered ^{15}N was probably as cell nitrogen or N_2O. Nitrous oxide production has recently been shown to be a significant product of dissimilatory nitrate-reducing bacteria (12; J. M. Tiedje, N. V. Caskey, M. S. Smith, B. H. Bleakley, and R. B. Firestone, Agron. Abstr., 1979, p. 165).

The above ^{15}N experiments indicate the presence of bacteria able to perform the dissimilation of NO_3^- to NH_4^+. These bacteria reside in Baltic Sea water in or just below the halocline. Judging from the ease of obtaining strains that have the capacity of reducing NO_3^- to NH_4^+, this process may be common in nature under suitable environmental conditions.

The water bodies where the organisms were isolated are recognized as areas with a large NO_3^- anomaly; concentrations of NO_3^- are 30 to 40 μmol/liter below values calculated from Richards decomposition model (11). These water bodies also contain high N_2O concentrations (unpublished data) although this could also be from denitrification. The bottles containing Baltic Sea water which showed high NH_4^+ production also had a high N_2 formation, showing that

both processes occurred concurrently and competed for NO_3^- as an electron acceptor. The main difference to the ecosystem between the denitrifiers and the NH_4^+ producers is that the latter conserve nitrogen, whereas denitrifiers cause a nitrogen loss. However, even dissimilatory nitrate reduction to NH_4^+ may cause some nitrogen loss, since there is evidence (12) that N_2O production is also associated with this process. How large this flow is and its ecological significance should be investigated in the future.

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