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Aerobic Biodegradation of *N*-Nitrosodimethylamine by the Propanotroph *Rhodococcus ruber* ENV425[▽]

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The propanotroph *Rhodococcus ruber* ENV425 was observed to rapidly biodegrade *N*-nitrosodimethylamine (NDMA) after growth on propane, tryptic soy broth, or glucose. The key degradation intermediates were methylamine, nitric oxide, nitrite, nitrate, and formate. Small quantities of formaldehyde and dimethylamine were also detected. A denitrosation reaction, initiated by hydrogen atom abstraction from one of the two methyl groups, is hypothesized to result in the formation of *n*-methylformaldimine and nitric oxide, the former of which decomposes in water to methylamine and formaldehyde and the latter of which is then oxidized further to nitrite and then nitrate. Although the strain mineralized more than 60% of the carbon in [^{14}C]NDMA to $^{14}CO_2$, growth of strain ENV425 on NDMA as a sole carbon and energy source could not be confirmed. The bacterium was capable of utilizing NDMA, as well as the degradation intermediates methylamine and nitrate, as sources of nitrogen during growth on propane. In addition, ENV425 reduced environmentally relevant microgram/liter concentrations of NDMA to <2 ng/liter in batch cultures, suggesting that the bacterium may have applications for groundwater remediation.

N-Nitrosodimethylamine (NDMA) is a potent carcinogen that has recently been detected in groundwater, wastewater, and drinking water (1, 2, 17, 18). It forms as a disinfection byproduct in wastewater and drinking water treated with chloramine and other disinfectants (17, 18, 43). NDMA has also been found to be present in aquifers at several military sites that have used 1,1-dimethylhydrazine, a component of liquid rocket propellant that contained NDMA as an impurity (6, 9). Although there is presently no federal maximum contaminant level for NDMA in drinking water, a risk assessment conducted by the U.S. Environmental Protection Agency suggested that concentrations as low as 0.7 ng/liter can increase lifetime cancer risk by 1×10^{-6} (34). In addition, California currently has a 10 ng/liter notification level for NDMA concentrations in drinking water and has recently recommended an even lower public health goal of 3 ng/liter (3, 20). Thus, the presence of even trace concentrations of this chemical in drinking water represents a potential public health concern.

The rates and extents of NDMA biodegradation in natural environments, including surface water, sludges, and soils, are highly variable. In some studies, the compound has been reported to be recalcitrant or only partially biodegraded (16, 30, 31); in others, fairly rapid and extensive biodegradation has been previously observed (2, 13, 22, 40). Few studies have been conducted to examine NDMA biodegradation in groundwater. However, the persistence of NDMA derived originally from 1,1-dimethylhydrazine-based rocket fuel over decades in some groundwater aquifers (e.g., Rocky Mountain Arsenal, CO; former Air Force Plant PJKS, CO; and Aerojet Superfund Site, CA) suggests that this molecule can be very recalcitrant (8, 9, 35). At sites where biodegradation has been observed, the organisms responsible and the microbial degradation pathways are largely unknown.

The metabolism of NDMA and other nitrosamines by mammals has received extensive study. NDMA requires metabolic activation to the methyldiazonium ion (a strong alkylating agent) to exert its genotoxic effects (1, 19, 34). This activation reaction is catalyzed by specific isozymes of the cytochrome P-450-dependent mixed-function oxidase system and proceeds through an initial α-hydroxylation reaction. Alternately, NDMA can be oxidized by the P-450 system via a denitrosation route, which does not result in the formation of a highly carcinogenic intermediate (11, 28, 37).

The bacterial transformation of NDMA has not been studied in significant detail. Several bacteria expressing broad-specificity monooxygenase enzymes have been reported to degrade NDMA via cometabolism. These include the propanotrophs *Rhodococcus* sp. strain RHA1 (25, 26) and *Rhodococcus ruber* ENV425 (29) as well as *Mycobacterium vaccae* JOB5 (25), the methanotroph *Methylosinus trichosporium* OB3b (42), and the toluene oxidizer *Pseudomonas mendocina* KR1 (7). We recently characterized the pathway of NDMA transformation used by *P. mendocina* KR1, a bacterium that utilizes the enzyme toluene-4-monooxygenase (T4MO) to cometabolically degrade NDMA and other anthropogenic pollutants (7, 38). The pathway of NDMA transformation by KR1 differs from the two pathways described for mammals. A majority of the NDMA metabolized by T4MO in this strain is oxidized to *N*-nitrodimethylamine (NTDMA) and then further to *N*-nitromethylamine (NTMA), which accumulates as a terminal product (7).

In this report, we describe the pathway used by the pro-

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panotroph *R. ruber* ENV425 to catabolize NDMA. This strain was originally isolated from turf soil, where propane was used as the sole carbon source, and was previously reported to oxidize methyl tertiary-butyl ether and other gasoline oxygenates (27). Our data show that the pathway of NDMA degradation mediated by strain ENV425 differs from that mediated by *P. mendocina* KR1. Rather, the pathway used for transformation of NDMA by ENV425 appears to be similar to the denitrosation pathway catalyzed by various P-450 isozymes in mammals, resulting in the production of nitric oxide (NO), nitrite, nitrate, formaldehyde, formate, and methylamine (MA) (11, 12, 28, 39). A significant fraction of the carbon in the NDMA molecule was released as CO_2 by strain ENV425, although growth on NDMA could not be confirmed. However, the bacterium was observed to utilize NDMA as well as the NDMA-degradation intermediates MA and nitrate as sources of nitrogen during growth on propane as a sole carbon and energy source.

MATERIALS AND METHODS

Chemicals. [^{14}C]NDMA (>97% purity; specific activity of 55 mci/mmol) was from American Radiolabeled Chemicals, Inc. (St. Louis, MO). NTDMA and NTMA were gifts from Ron Spanggord (SRI International, Menlo Park, CA). All other chemicals used in this study were from Sigma-Aldrich and were of reagent grade or higher purity.

Analytical methods. NDMA, NTDMA ($NO_2NCH_3CH_3$), NTMA (NO_2NHCH_3), nitrate (NO_3^-), nitrite (NO_2^-), formate ($HCOO^-$), formaldehyde (HCHO), methanol (CH_3OH), MA (CH_3NH_2), and dimethylamine (DMA; CH_3CH_3NH) were analyzed as previously reported (7). The capture and quantification of $^{14}CO_2$ liberated from radiolabeled [^{14}C]NDMA were performed as described previously (7). NO was analyzed using an Apollo 4000 free radical analyzer (WPI, Saratosa, FL) and an ISO-NOP (2.0-mm-diameter) NO sensor. The sensitivity of the probe was 1.06 pA/nM. The concentration of NO was determined by directly inserting the sensor into the reaction mixture as it was agitated with a magnetic stirrer. The current (in picoamperes) generated by the NO present in the sample was recorded. The method detection limit for NO was 4 nM.

Growth and assay conditions for metabolic studies. *R. ruber* ENV425 was grown in tryptic soy broth (TSB; BD, Franklin Lakes, NJ), basal salts medium (BSM; 25) with propane (~ 25% of headspace gas), or BSM with glucose (20 mM) and/or NDMA (270 μM). The cells were incubated at 30°C under aerobic conditions with air (or oxygen gas when propane was the substrate) and agitated on a rotary shaker at 175 rpm. After the culture was grown to the late logarithmic phase (optical density at 600 nm [OD_{600}] of ~3.5), the cells were harvested and washed twice in BSM without nitrogen or carbon. Cells were then added (OD_{600} of ~ 3.0) to 6-ml vials containing BSM and NDMA or NTDMA (135 μM or as specified). In some experiments, ammonium phosphate (2 mM) was added to the salts solution prior to conducting assays to prevent cell uptake and/or utilization of organic and inorganic N metabolites (5). The BSM used in the metabolic studies contained $NaNO_3$ (12 mM) rather than NH_4^+ as a source of inorganic nitrogen. Two sets of controls were prepared, the first containing NDMA without bacterial cells and the second containing bacteria without NDMA. The vials, covered in aluminum foil to prevent photolysis of NDMA, were incubated at 30°C with shaking at 175 rpm.

Evaluation of NDMA as a growth substrate or nitrogen source. Strain ENV425 was tested for growth on NDMA as a sole source of carbon and energy. Duplicate sterile 160-ml serum bottles were prepared in which ENV425 was inoculated into minimal salts medium (MSM) (10) supplemented with either 0.3 mM NDMA or 0.3 mM glucose. MSM contains NH_4Cl (37 mM) as a source of inorganic nitrogen. Controls consisted of ENV425 in MSM with no carbon source added. Every 2 to 3 days, the bottles were opened and a small aliquot was removed for OD measurement. Additional 0.3 mM glucose or NDMA was added to the appropriate bottles at select sampling times.

The ability of strain ENV425 to use NDMA, MA, and NO_3^- as sole sources of N was tested. The bacterium was initially grown to the late logarithmic phase (OD_{600} of ~3.5) in MSM with propane (~25% of headspace gas) at 28°C. The cells were then washed twice in N-free MSM (i.e., without NH_4Cl added) and added at a low density (OD_{600} of ~0.008) in 10-ml volumes to sterile serum vials containing N-free MSM with NDMA, MA, NO_3^-, or NH_4^+ as the sole source of N. The quantity of each compound was equalized on a molar basis with respect to total N (0.7 mM as N). In addition to these treatments, bottles were prepared with complete MSM (with ~10 mM N from NH_4Cl) as a positive control for cell growth and with complete MSM plus NDMA (0.7 mM) to confirm that strain ENV425 was not capable of using NDMA as a carbon and energy source. Propane (10% of headspace) was added to each bottle (except for the bottle with NDMA only) as a carbon and energy source, and oxygen (90% of headspace) was added as the electron acceptor. The bottles were incubated in the dark at room temperature (~22°C) on a rotary shaker, and the OD_{600} was measured over time to assess growth.

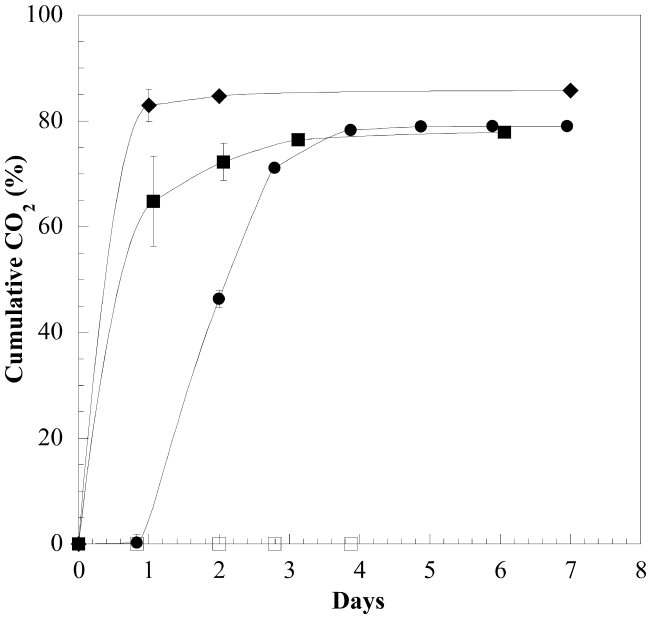

FIG. 1. Mineralization of [^{14}C]NDMA into $^{14}CO_2$ by washed cells of *R. ruber* ENV425 initially cultivated in TSB (●), BSM with glucose (■), or BSM with glucose and NDMA (◆). Values from samples with TSB only (no cells) are also shown (□). Values represent means and standard deviations of the results obtained using triplicate samples.

Extent of NDMA biodegradation by strain ENV425. An experiment was performed to determine whether ENV425 was capable of degrading NDMA from typical (μg/liter) to low (ng/liter) groundwater concentrations. The culture was grown in the dark in a 9-liter glass bottle containing 5 liters of MSM. A 12-liter Tedlar bag containing 25% propane and 75% oxygen was attached to the bottle. After 12 days, when the culture density reached an OD_{600} of 1.0, the cell suspension was amended with NDMA to achieve a final concentration of ~8 μg/liter (~0.1 μM). After 18, 72, and 166 h, a 1-liter volume of the culture was filtered through a Corning 0.22-um-pore-size disposable cellulose acetate filter (Corning Inc., Corning, NY) unit to remove cells and stop activity; then, the sample was sent to Maxxam Analytics, Inc. (Burlington, Ontario, Canada). The samples were analyzed by high-resolution mass spectrometry according to method 607 (modification 1625) of the Environmental Protection Agency (33). The method detection limit is 0.5 ng/liter, and the reporting limit is 2.0 ng/liter. In preliminary studies, NDMA was quantitatively recovered after membrane filtration and after incubation with killed cells of strain ENV425 (>93% recovery), and no loss was observed after more than 1 month of storage in the filter jar in the dark.

RESULTS

Metabolism of NDMA by *R. ruber* ENV425. Initial studies using [^{14}C]NDMA revealed that strain ENV425 mineralized significant quantities of NDMA to CO_2 after growth on TSB, glucose, or glucose plus NDMA (Fig. 1). More than 75% of the added radiolabeled fraction was recovered as $^{14}CO_2$ in these experiments. In a separate experiment, the strain was

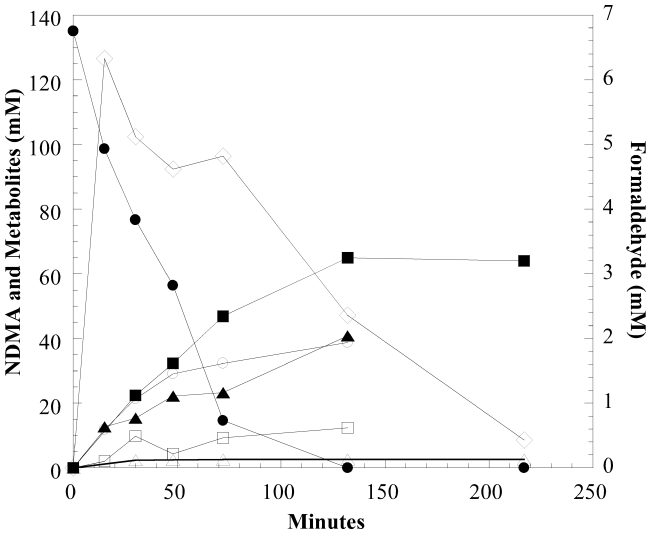

FIG. 2. Products observed during the transformation of NDMA by *R. ruber* ENV425 after growth on propane. Symbols: NDMA (●), formate (▲), formaldehyde (◇), MA (■), DMA (Δ), nitrate (○), and nitrite (□). NTMA or NTDMA were not detected.

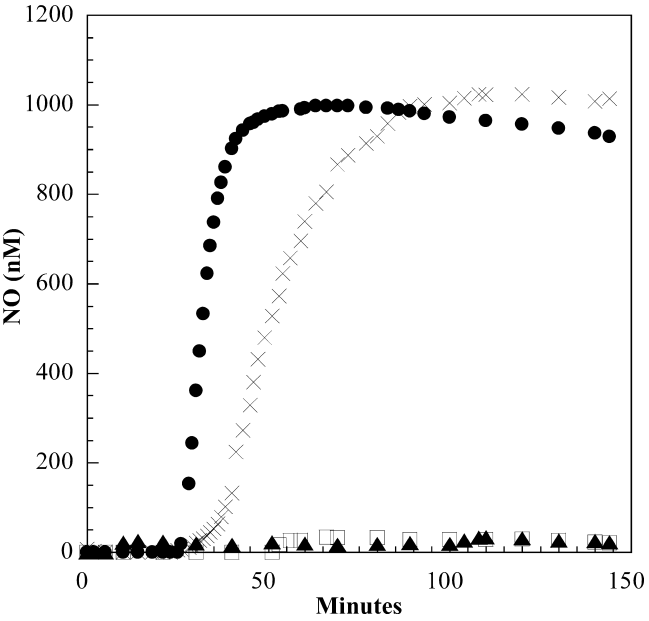

FIG. 3. Liberation of NO from NDMA by *R. ruber* ENV425 after growth on glucose and NDMA (●) or propane (×). NO was not detected when cells grown on glucose and NDMA were washed and then incubated with NTDMA (▲) or NO_2^- (□).

also observed to mineralize more than 60% of [^{14}C]NDMA to $^{14}CO_2$ after growth on propane (data not shown). Despite the rapid mineralization of NDMA after growth on the aforementioned substrates, the cell density of ENV425 did not increase measurably when NDMA was provided as the sole energy and carbon source for growth. Cell growth was observed when an equivalent molar concentration of glucose was provided.

Based on the initial data revealing that strain ENV425 mineralized NDMA, experiments were conducted to establish the degradative pathway(s). Washed cells that were initially grown on propane as a sole carbon source degraded NDMA from an initial concentration of 135 μM to below detection in ~2.2 h (Fig. 2). The primary metabolites detected were MA (65 μM), NO_3^- (39 μM), NO_2^- (12 μM), and formate (43 μM). Formaldehyde and DMA were also observed in trace concentrations, with the former compound reaching a peak concentration of ~6 μM after 15 min that declined thereafter. The concentration of DMA remained below 3 μM. NTDMA and NTMA were not detected.

MA and NO_2^- are expected to be produced when NDMA undergoes an initial denitrosation reaction (13). However, based on studies of mammalian systems, the initial production of NO would also be expected via the denitrosation but not the α-hydroxylation reaction (11, 37). Rapid production of NO from NDMA was observed for cells grown on propane or on glucose (plus NDMA as an inducer) (Fig. 3). NO was not observed when cells were grown on glucose (plus NDMA) and then washed and incubated with NTDMA or NO_2^- but with no NDMA.

In an initial experiment performed with glucose-grown ENV425 bacteria, formate, formaldehyde, and NO_2^- were detected as primary metabolites, and there was a transient production of methanol. MA and DMA were not observed. However, NH_4^+ was not added to the reaction mixture in this experiment, and it is likely that the aforementioned metabolites (MA and DMA) were utilized by the bacterium as a source of N (5). The ability of the strain to utilize MA as an N source was subsequently confirmed. When NH_4^+ was added, both MA and DMA were detected in quantities similar to those observed for propane-grown cells (Fig. 4). In addition, formaldehyde, MA, NO_3^-, and NO_2^- were detected during catabolism of NDMA by cells cultivated on TSB, suggesting a similar primary reaction mechanism.

Strain ENV425 was also observed to biodegrade NTDMA, which is a suspected carcinogen (24), and an initial degradation product produced during NDMA oxidation by *P. mendocina*

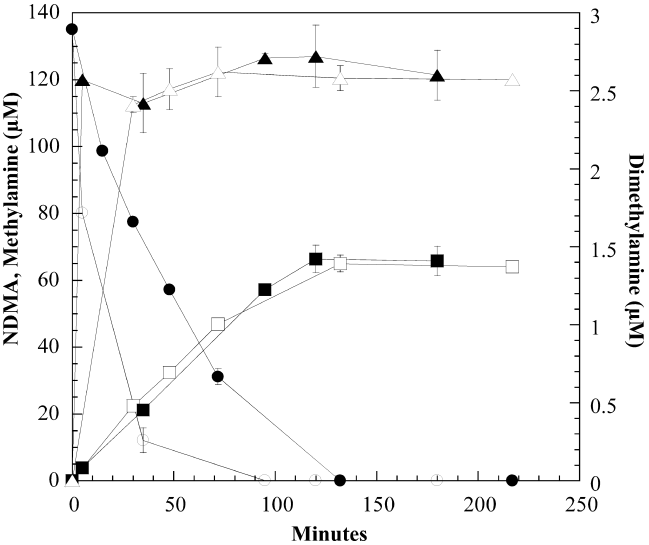

FIG. 4. Liberation of MA and DMA from NDMA by *R. ruber* ENV425 after growth on glucose and NDMA (closed symbols) or propane (open symbols). Symbols: NDMA (●, ○); MA (■, □); DMA (▲, Δ).

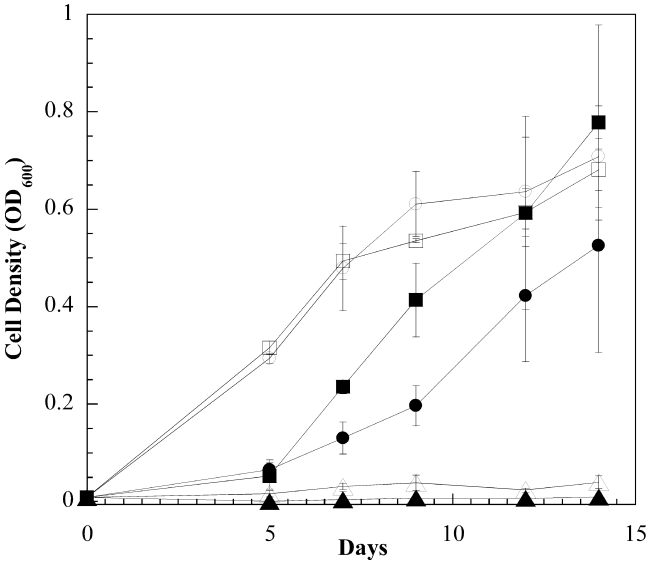

FIG. 5. Growth of strain ENV425 on propane with ammonium (□), NDMA (●), MA (■), or nitrate (○) as the sole N source (N, 0.7 mM). One set of samples received propane but no added N (Δ), and one set received NDMA only without propane (▲). Error bars represent standard deviations of the results obtained using duplicate samples.

KR1 (7). The degradation of NTDMA resulted in the formation of formaldehyde (transient) and NTMA (data not shown). NO was not detected (Fig. 3).

Evaluation of NDMA as a growth substrate or nitrogen source. ENV425 grew from an initial OD_{600} of 0.008 to a density of 0.7 ± 0.04 with NH_4^+ and 0.7 ± 0.1 with NO_3^- as the sole N source (0.7 mM each as N) after 14 days (Fig. 5). With MA and NDMA as the sole sources of N, cell densities were 0.8 ± 0.2 and 0.5 ± 0.2, respectively, after 14 days. In the absence of an exogenous N source, cell density was 0.05 ± 0.04 after 14 days, and with NDMA only (i.e., no propane added), cell density was 0.03 ± 0.01. Thus, the data show that strain ENV425 is capable of using NO_3^- and MA (N metabolites of NDMA degradation during cell growth on propane) as well as NDMA as sole sources of N. Cell growth with NDMA and MA was initially slower than when NO_3^- or NH_4^+ was provided, but significant growth was observed with each compound compared to the results seen with controls without N. Cell growth was not observed with NDMA provided as the sole source of carbon and energy in this study, which confirms our previous data.

Extent of NDMA biodegradation by strain ENV425. NDMA was biodegraded by ENV425 from an initial concentration of 8.3 μg/liter to <2 ng/liter after 18 h in batch culture (data not shown). NDMA remained at a concentration of <2 ng/liter in culture samples collected after 72 h and 166 h.

DISCUSSION

The propanotroph ENV425 is capable of rapidly biodegrading NDMA after growth on several different substrates, including propane, TSB, and glucose (plus NDMA). The bacterium oxidizes significant quantities of NDMA to CO_2 but, based on cell density measurements, does not appear to utilize the nitrosamine as a sole source of carbon and energy. The metabolites produced during NDMA degradation by ENV425 after growth on propane include NO, MA, DMA, NO_3^-, NO_2^-, formaldehyde, and formate. NTDMA and NTMA, two of the primary metabolites produced during NDMA oxidation by *P. mendocina* KR1 (T4MO), were not detected, indicating that different pathways are utilized by the two bacteria.

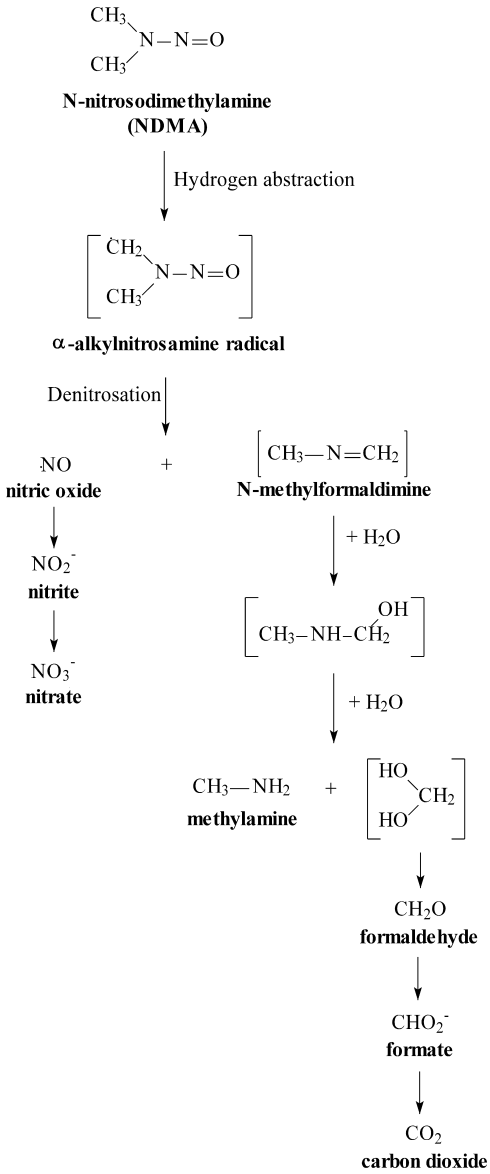

FIG. 6. Proposed denitrosation pathway of NDMA degradation by strain ENV425 (modified from mammalian pathways; see references 11, 28, and 37).

Based on the metabolites detected and on the rapid production of NO during NDMA catabolism, strain ENV425 is hypothesized to biotransform NDMA primarily via the denitrosation pathway shown in Fig. 6. The key metabolites observed during NDMA metabolism by ENV425 are the same as those described as having been observed during denitrosation of NDMA via P-450 isozymes in mammals (11, 12, 14, 32, 37). The denitrosation pathway is thought to

proceed through an initial hydrogen atom abstraction from one of the two methyl groups of NDMA, which results in the formation of an α-alkylnitrosamine radical, $^{\bullet}CH_2(NNO)CH_3$, followed by loss of NO to form an unstable imine, *n*-methylformaldimine ($CH_2{=}N{-}CH_3$) (Fig. 6). Subsequent abiotic hydrolysis of $CH_2{=}N{-}CH_3$ leads to production of MA and formaldehyde (11, 14), the latter of which can oxidize further to formate and then CO_2, as shown in Fig. 6. Oxidation of NO is expected to produce NO_2^- and then NO_3^-. Previous studies with P-450 isozymes (11, 14, 37) support the idea of an initial formation of the α-alkylnitrosamine radical during NDMA degradation. Hydrogen atom abstraction has also been proposed as a key step in the catalysis of various substrates by soluble methane monoxygenase, one of the most widely studied bacterial monooxygenase enzymes (4, 36).

In mammalian systems, the α-alkylnitrosamine radical can also undergo an α-hydroxylation of one of the methyl groups to form *N*-nitrosohydroxymethylmethylamine [$HOCH_2(NNO)CH_3$], which is unstable and subsequently decomposes to *N*-nitrosomethylamine (CH_3NHNO) (and formaldehyde) and then rapidly to the methyldiazonium ion, which is a strong alkylating agent (24, 32, 39). The same P-450 enzymes appear to catalyze both pathways. The only metabolite that is readily detected during α-hydroxylation of NDMA is formaldehyde, which was detected at low concentrations in this study, but formaldehyde can be generated by both the denitrosation and α-hydroxylation pathways in mammals (14, 37), so its presence does not provide conclusive evidence for the use of either route. A small amount of methanol, which can potentially be produced via hydrolysis of the methyldiazonium ion generated from α-hydroxylation (7), was observed in initial studies with strain ENV425 during growth on glucose and TSB. Thus, it is possible that α-hydroxylation represents a minor pathway of NDMA degradation by ENV425, as was previously proposed for *P. mendocina* KR1 based on generation of small quantities of methanol (7). However, the primary metabolites detected during NDMA catabolism by ENV425, including NO, NO_2^-, NO_3^-, and MA, are expected only during NDMA catabolism via the denitrosation pathway (11, 12, 14, 37).

DMA, the presence of which was observed at low concentrations during NDMA catabolism by strain ENV425, has also been observed as a minor metabolite in the denitrosation pathway catalyzed by P-450 in mammals (14, 37) and during photocatalytic degradation of NDMA (15). However, the pathway(s) of DMA formation is less clear than is the case for the other degradation intermediates. In a previous study conducted with liver microsomes, DMA was observed during NDMA catabolism but was shown not to be an intermediate in the formation of MA or produced through methylation of MA (14). No alternate route of DMA generation was proposed. Potential routes of formation of DMA include direct denitrosation of NDMA to form NO and the dimethylaminyl radical ($^{\bullet}NCH_3CH_3$), the latter of which can then form DMA either through hydrogenation or through a disproportionation reaction. The disproportionation reaction is expected to also produce $CH_2{=}N{-}CH_3$, which would subsequently decompose in water to MA and formaldehyde, as shown in Fig. 6. Additional studies would be required to evaluate routes of DMA formation in ENV425. It should be noted, however, that the molar quantities of DMA observed in studies with NDMA were generally <5% of the quantities of MA observed, so the proposed pathway is not likely to be a major route of NDMA biotransformation.

The identity of the enzyme responsible for NDMA oxidation in the propanotroph ENV425 has not been determined, but it is suspected to be a propane monooxygenase (PMO) belonging to the larger family of soluble diiron monooxygenases. Preliminary molecular studies with ENV425 revealed that the bacterium contains representatives of at least three distinct classes of monooxygenase, including a putative PMO, a P-450, and a membrane-bound AlkB-type monooxygenase. However, when grown with propane or glucose plus NDMA (but not glucose alone), the PMO genes, but not the P-450 or AlkB genes, were induced, suggesting that a PMO is involved in NDMA oxidation by this strain (unpublished data). These data support the recent studies of Sharp et al. (26), who showed that a PMO was responsible for NDMA oxidation in *Rhodococcus* sp. strain RHA1, a bacterium that appears to have traits similar to those of strain ENV425. However, additional studies with ENV425 are required to confirm these preliminary results.

The catabolism of NDMA by strain ENV425 differs significantly from that described for the toluene oxidizer *P. mendocina* KR1, which degrades the nitrosamine to NTMA and formaldehyde, via the enzyme T4MO (7), and the methanotroph *Methylosinus trichosporium* OB3b, which is hypothesized to degrade NDMA via an initial α-hydroxylation reaction, although this mechanism has yet to be confirmed (41, 42). The degradation of NDMA by ENV425 also differs from the route proposed for a variety of different intestinal isolates under anoxic conditions (23). Many of these strains converted NDMA to NO_2^- and DMA, without subsequent production of either formate or CO_2. Thus, although NDMA is a small molecule, its degradation by bacteria can potentially follow a number of different routes.

To our knowledge, no bacterial strains that utilize NDMA as a sole source of carbon and energy for growth have yet been described. Strain ENV425 appears to be no exception, even though it can utilize NDMA, MA, and NO_3^- as sole sources of nitrogen and produces formaldehyde on its way to converting >60% of the NDMA-derived carbon to CO_2. Formaldehyde can serve as a source of reducing equivalents via dehydrogenase enzymes and as fixable carbon via the ribulose monophosphate pathway, which is known to occur in many *Rhodococcus* spp., including the NDMA-degrading organism RHA1 (21), although the presence of this pathway in ENV425 has not been examined. It is possible that the net energy yield of NDMA catabolism via the pathway(s) utilized by ENV425 and other bacteria is not sufficient to support cell growth or that cell yields are so low during growth that the process cannot be readily measured via OD determinations. In similarity to the results seen with NDMA, ENV425 was previously shown to oxidize >60% of the carbon in methyl tertiary-butyl ether to CO_2 but not to grow on the oxygenate (27).

Although ENV425 did not appear to grow on NDMA, the bacterium was capable of reducing microgram/liter concentrations of the nitrosamine to <2 ng/liter during growth on propane as a primary substrate. The ability of this or any other bacterium to achieve low nanogram/liter concentrations of NDMA is important if bioremediation is to be considered as a potential remedial option for NDMA in the future. The current action level for NDMA in California is 10 ng/liter, and the Cali-

fornia Office of Environmental Health Hazard Assessment recently recommended 3 ng/liter as a public health goal based on risk assessment (3, 20). Bioremediation is not generally considered to achieve the reduction of contaminants from microgram/liter to nanogram/liter concentrations in groundwater. However, this study reveals that, with propane as a primary growth substrate, ENV425 is capable of reducing NDMA to low nanogram/liter concentrations. Recent bioreactor studies conducted in our laboratory confirmed this result and revealed that biodegradation of NDMA to these low concentrations is sustainable (unpublished data). Thus, the data suggest that in situ and ex situ bioremediation with ENV425 and possibly other propanotrophs may have applications for NDMA treatment.

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