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Metabolism of Carbaryl via 1,2-Dihydroxynaphthalene by Soil Isolates *Pseudomonas* sp. Strains C4, C5, and C6

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***Pseudomonas* sp. strains C4, C5, and C6 utilize carbaryl as the sole source of carbon and energy. Identification of 1-naphthol, salicylate, and gentisate in the spent media; whole-cell O_2 uptake on 1-naphthol, 1,2-dihydroxynaphthalene, salicylaldehyde, salicylate, and gentisate; and detection of key enzymes, viz, carbaryl hydrolase, 1-naphthol hydroxylase, 1,2-dihydroxynaphthalene dioxygenase, and gentisate dioxygenase, in the cell extract suggest that carbaryl is metabolized via 1-naphthol, 1,2-dihydroxynaphthalene, and gentisate. Here, we demonstrate 1-naphthol hydroxylase and 1,2-dihydroxynaphthalene dioxygenase activities in the cell extracts of carbaryl-grown cells. 1-Naphthol hydroxylase is present in the membrane-free cytosolic fraction, requires NAD(P)H and flavin adenine dinucleotide, and has optimum activity in the pH range 7.5 to 8.0. Carbaryl-degrading enzymes are inducible, and maximum induction was observed with carbaryl. Based on these results, the proposed metabolic pathway is carbaryl → 1-naphthol → 1,2-dihydroxynaphthalene → salicylaldehyde → salicylate → gentisate → maleylpyruvate.**

Carbamate insecticides, such as carbaryl (1-naphthyl-*N*-methylcarbamate), are highly toxic, have a wide range of activity, and comprise a major portion of pesticides used in the agriculture industry. Widespread and repeated use leads to pollution of soil and groundwater (5). The ester bond between *N*-methylcarbamic acid and 1-naphthol is responsible for carbaryl toxicity. Carbamates are competitive inhibitors of neuronal nicotinic acetylcholine receptors and acetylcholinesterase (28). *N*-Nitrosocarbamates and the 1-naphthol that they generate are potent mutagens and are more toxic and recalcitrant than carbaryl itself (8, 22, 26, 27, 32). Carbamate pesticides generally do not persist in the environment for a long time. In aqueous solutions, carbaryl hydrolyzes to 1-naphthol, methylamine, and CO_2 (30). Bacteria capable of degrading carbamate pesticides have been isolated from the soil (3, 6, 7, 13, 16, 25). The first step in degradation is the hydrolysis of carbaryl to 1-naphthol by carbaryl hydrolase, which has been purified and characterized from various organisms (3, 5, 10, 11, 20, 24). Depending on the strain, 1-naphthol is metabolized via salicylate to either gentisate or catechol (4, 7, 9, 16, 17). It has been proposed that prior to ring cleavage, 1-naphthol is hydroxylated either to 4-hydroxy-1-tetralone (1), 3,4-dihydro-dihydroxy-1(2H)-naphthalenone (31), or 1,4-naphthoquinone (25). Though 1-naphthol, salicylate, and gentisate are well-established intermediates in carbaryl degradation, the steps and enzymes responsible for the conversion of 1-naphthol to salicylate have not been demonstrated so far.

In this study, we report isolation of three soil bacterium *Pseudomonas* sp. strains, C4, C5, and C6, utilizing carbaryl as the carbon source via 1-naphthol, salicylate, and gentisate. The aim of this study is to investigate the catabolic pathway and its regulation, with emphasis on the enzymes involved in the conversion of 1-naphthol to salicylate. Here, we report 1-naphthol hydroxylase and 1,2-dihydroxynaphthalene (1,2-DHN) dioxygenase activities in the cell extracts and induction patterns of key enzymes. Based on the results, we propose 1,2-dihydroxynaphthalene as a metabolic intermediate in carbaryl degradation.

MATERIALS AND METHODS

Chemicals. Salicylate, gentisate, catechol, 1,2-dihydroxynaphthalene, flavin adenine dinucleotide (FAD), NADH, NADPH, and NAD were purchased from Sigma-Aldrich. 1- and 2-naphthol were purchased from Merck. Carbaryl was a gift from Bayer Ltd. (India). Analysis of the carbaryl gave a single spot by thin-layer chromatography (TLC) and a single peak by gas chromatography (GC). All other chemicals were of analytical grade and purchased locally.

Bacterial strains and culture conditions. Using enrichment culture techniques, the three bacterial strains C4, C5, and C6 were isolated from soil contaminated with carbaryl. The cultures were grown in 150 ml minimal salt medium (MSM) in 500-ml baffled Erlenmeyer flasks at 30°C on a rotary shaker (200 rpm). For strains C4 and C5, the components of MSM per liter of distilled water were as follows: K_2HPO_4, 6 g; KH_2PO_4, 4.1 g; NH_4NO_3, 1 g; $MgSO_4 \cdot 7H_2O$, 100 mg; $MnSO_4$, 1 mg; $CuSO_4 \cdot 5H_2O$, 1 mg; $FeSO_4 \cdot 7H_2O$, 5 mg; H_3BO_3, 1 mg; $CaCl_2 \cdot 2H_2O$, 5 mg; $ZnSO_4$, 1 mg; and $NaMoO_4$, 1 mg (pH 6.8). For strain C6, MSM composition was the same except that 8 g of K_2HPO_4 and 1 g of KH_2PO_4 (pH 7.4) were used. The medium was supplemented aseptically with appropriate hydrocarbon (0.1%) or glucose (0.25%) as the carbon source. Growth was monitored spectrophotometrically at 540 nm.

Isolation and identification of metabolites. To isolate and identify the metabolites, spent medium was acidified to pH 2 with 2 N HCl and extracted with an equal volume of ethyl acetate. The organic phase was dried over anhydrous sodium sulfate, concentrated, and analyzed by TLC using hexane:chloroform:acetic acid 8:2:1 (vol/vol/vol) as a solvent system. Metabolites were identified by comparing R_f and UV fluorescence properties with authentic compounds. Metabolites were purified using preparative TLC and subjected to UV–visible-light spectroscopy (Perkin-Elmer Lambda UV35). GC-mass spectrometry (MS) analysis was carried out on a Hewlett-Packard G1800A mass spectrometer attached to a gas chromatograph. GC analysis was carried out using an HP1 column (30-m length; inlet temperature, 100°C, increased to 280°C at the rate of 10°C/min) and using helium (20 ml/min) as a carrier gas. The conditions for mass analysis were ionization at 70 eV, a mass range of 10 to 425 *m/z*, and a chamber temperature of 280°C.

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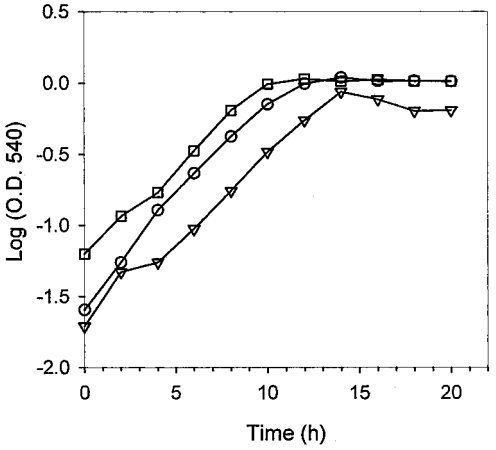

FIG. 1. Growth profile of *Pseudomonas* sp. strains C4 (○), C5 (▽), and C6 (□) on carbaryl (0.1%). O.D. 540, optical density at 540 nm.

Biotransformation and oxygen uptake studies. Late-log-phase cells grown on respective hydrocarbons were harvested by centrifugation (12,000 × *g*), washed twice, suspended in MSM plus hydrocarbon (0.1%), and incubated at 30°C on a shaker for 3 to 4 h. The products were extracted from spent media and analyzed by TLC as described above. For O_2 uptake studies, washed cells were suspended at 100 mg (wet weight) per ml of phosphate buffer (50 mM, pH 7.5). The respiration rates were measured at 30°C using an oxygraph (Hansatech, United Kingdom) fitted with Clark's type O_2 electrode. The reaction mixture (2 ml) contained cells (4 mg, wet weight), a substrate (50 μM), and phosphate buffer (50 mM, pH 7.5). The rates were corrected for endogenous cell respiration and expressed as nmol O_2 consumed · min^{-1} · mg^{-1} of cells.

Preparation of cell extract. Late-log-phase cells (1 g) were suspended in 4 ml ice-cold phosphate buffer (50 mM, pH 7.5, K-PO_4 buffer) and disrupted by sonication (Ultrasonic processor, model GE130) at 4°C with four cycles of 15 pulses each and an output at 11 W with a 3- to 4-min interval. Cell homogenate was centrifuged at 40,000 × *g* for 30 min at 4°C. The clear membrane-free supernatant obtained was referred as cell extract and used as the enzyme source.

Enzyme assays and bulk enzyme reaction. Carbaryl hydrolase (CH) was monitored spectrophotometrically (Perkin Elmer; model Lambda 35) by measuring the rate of increase in absorbance at 322 nm due to the formation of 1-naphthol. The reaction mixture (1 ml) contained the substrate (100 μM), an appropriate amount of enzyme, and K-PO_4 (50 mM, pH 7.5) buffer. The activity was calculated by using the molar extinction coefficient at 322 nm (ε_{322}) of 1-naphthol of 2,200 (M^{-1} cm^{-1}) in K-PO_4 buffer. 1-Naphthol hydroxylase (1-NH) was monitored by two methods. In an oxygraph assay, the reaction mixture (2 ml) contained K-PO_4 buffer (50 mM, pH 7.5), the substrate (100 μM), FAD (6.25 μM), and NADH (100 μM). The reaction was started by the addition of enzyme. In the spectrophotometric assay, the disappearance of NADH at 340 nm was monitored. The assay mixture (1 ml) contained K-PO_4 buffer, the substrate (100 μM), FAD (6.25 μM), NADH (100 μM), and enzyme. The enzyme activity was calculated using the molar ε_{340} of NADH of 6,220 (M^{-1} cm^{-1}). 1,2-Dihydroxynaphthalene dioxygenase (1,2-DHNO) (19, 23) was monitored using an oxygraph. The reaction mixture (2 ml) contained K-PO_4 buffer (50 mM, pH 7.5), the substrate (100 μM), and an appropriate amount of enzyme. Gentisate dioxygenase (GDO) (29) and catechol dioxygenase (14, 21) were monitored spectrophotometrically in K-PO_4 buffer (50 mM, pH 7.5). The reaction mixture contained buffer, the substrate (100 μM), and enzyme. Catechol dioxygenase was also monitored by an oxygraph under similar conditions. A time-dependent increase in the product formation was monitored spectrophotometrically for CH and GDO at defined intervals from 200 to 400 nm. All enzyme activities are expressed either as nmol of the substrate that disappeared, the product that appeared, or oxygen consumed · min^{-1}. Specific activities are reported as nmol · min^{-1} · mg^{-1} of protein. Protein estimation was carried out as described by Lowry et al. (18) using bovine serum albumin as a standard.

Bulk enzyme reactions (10 ml) were performed for CH, 1-NH, 1,2-DHNO, and salicylaldehyde dehydrogenase. Depending on the enzyme, the substrate (25 μM) and cofactor(s), namely, FAD (3.75 μM), NADH (50 μM), or NAD (50 μM), were added intermittently at 15, 30, and 60 min. The salicylaldehyde dehydrogenase reaction was carried out in glycine–KOH buffer (50 mM, pH 9.6) and NAD. Reaction mixtures were incubated at 30°C for 90 min, and products were extracted and analyzed as described earlier. To confirm that 1-NH belongs to an oxygenase group, a reaction was performed in an O_2-free environment for 1 h at 30°C in a Thunberg tube. All reaction components were degassed, and anaerobic conditions were maintained by applying vacuum. The reaction was started by tipping NADH solution from the side arm of the tube into the main reaction chamber. The products were identified as described above.

RESULTS

Isolation and characterization of bacterial strains. Bacterial strains capable of utilizing carbaryl as the sole source of carbon and energy were isolated by a culture enrichment technique from three garden soil samples (top 1 to 2 cm of the soil layer; nursery, IIT B Campus, Mumbai, India) contaminated with carbaryl. Soils (2 g) were inoculated in MSM plus carbaryl (0.1%). Cultures were allowed to develop for the first 4 days and were then transferred subsequently every 48 h. Pure cultures were raised by inoculating a single colony from the 2YT plate onto MSM plus carbaryl (0.1%). The three pure bacterial cultures obtained were designated strains C4, C5, and C6. All strains were gram-negative, motile, aerobic rods and catalase, oxidase, urease, and citrate positive. Based on biochemical properties, isolates were tentatively identified as *Pseudomonas* sp. (12) and referred to as strains C4, C5, and C6. Besides carbaryl, all strains utilize 1-naphthol, salicylate, and 4-hydroxybenzoate; however, they failed to grow on naphthalene and 3-hydroxybenzoate. The growth pattern of the strains on 0.1% carbaryl is shown in Fig. 1.

Metabolism of carbaryl by strains C4, C5, and C6. TLC analysis of the metabolites of early- to mid-log-phase (6 to 12 h) cultures showed four major spots with R_f and UV fluorescence properties similar to those of authentic carbaryl, 1-naphthol, salicylate, and gentisate (Table 1). Metabolites corresponding to 1,4-naphthoquinone and catechol could not

TABLE 1. Identification of carbaryl metabolites from the spent media of C4 cultures[a]

Identified metabolite	TLC		UV-visible spectral properties (nm)[b]	MS analysis {*m/z* (% relative intensity) [molecular ion]}
	R_f	UV fluorescence		
Carbaryl	0.60	Dark blue	280, 312, 318	201 (4) [M^+], 144 (100), 127 (3), 115 (52), 89 (10), 77 (2)
1-Naphthol	0.71	Brown black	298, 308, 323	144 (100) [M^+], 115 (77), 88 (10), 77 (2)
Salicylate	0.78	Sky blue	306	ND[c]
Gentisate	0.18	Blue-green	338	154 (37) [M^+], 136 (100), 108 (10), 77 (1)

[a] Similar results were obtained with strains C5 and C6.
[b] Ethyl acetate was the solvent.
[c] ND, not detected by GC-MS.

TABLE 2. Biotransformation and bulk enzyme reactions for strain C4[a]

Metabolite identified[b]	Biotransformation result with:			Bulk enzyme reaction result with:			
	Carb	1,4-NQ	SAld	Carb	1-NL	1,2-DHN	SAld
Carb	+	−	−	+	−	−	−
1-NL	+	−	−	+	+	−	−
SAld	−	−	−	+	+	+	+
Salicylate	+	−	+	+	+	+	+
Gentisate	+	−	+	−	−	−	−
1,4-NQ	−	−	−	−	−	−	−
Catechol	−	−	−	−	−	−	−

[a] Similar results were obtained with strains C5 and C6. Carb, carbaryl; 1,4 NQ, 1-4,naphthoquinone; SAld, salicylaldehyde; 1-NL, 1-naphthol.
[b] Metabolites identified by TLC and GC-MS in biotransformation and bulk enzyme reactions with various substrates.

TABLE 3. Whole-cell oxygen uptake for strain C4[a]

Metabolite identified	O_2 uptake (nmol of O_2 consumed $min^{-1} \cdot mg^{-1}$ cells)[b] grown on:		
	Carbaryl	Salicylate	Glucose
Carbaryl	2.9	TR	TR
1-Naphthol	7.3	TR	TR
1,2-DHN	1.7	TR	TR
1,4-Naphthoquinone	tr	TR	—
Salicylaldehyde	5.6	1.0	TR
Salicylate	2.2	1.3	TR
Gentisic acid	2.3	1.6	TR
Catechol	0.9	TR	TR

[a] Similar results/trends were observed with strains C5 and C6.
[b] Values are corrected for endogenous O_2 uptake. TR, O_2 uptake rates less than 0.3 nmol are reported as traces; —, not done.

be detected. Purified metabolites were subjected to UV-visible spectroscopy and mass analysis. Spectral properties and fragmentation patterns of the metabolites were identical to those of the authentic compounds (Table 1), confirmi ng the metabolites as carbaryl, 1-naphthol, salicylate, and gentisate. The stationary-phase (18- to 24-h) spent media showed a metabolite (R_f of 0.79, sky-blue fluorescence) corresponding to salicylate. To elucidate the metabolic sequence, biotransformation experiments were performed, and results are summarized in Table 2. When supplemented with carbaryl, metabolites with R_f and fluorescence properties similar to those of authentic carbaryl, 1-naphthol, salicylate, and gentisate were detected. With salicylaldehyde, spots corresponding to salicylate and gentisate were observed; however, the cells failed to transform 1,4-naphthoquinone (Table 2).

Various metabolic studies with strains C4, C5, and C6 showed similar results; hence, data pertaining only to strain C4 are presented. To elucidate the steps involved in the conversion of 1-naphthol to salicylaldehyde, whole-cell O_2 uptake rates, enzyme activities, and products of bulk enzyme reactions were monitored. Carbaryl-grown C4 cells showed good O_2 uptake on carbaryl, 1-naphthol, 1,2-DHN, salicylaldehyde, salicylate, and gentisate; however, they failed to respire on 1,4-naphthoquinone (Table 3). Salicylate-grown cells showed comparable levels of O_2 uptake on salicylate and gentisate but significantly low respiration on other probable intermediates. Glucose-grown cells showed very low respiration (<0.1 nmol). Carbaryl-grown cells showed CH, 1-NH, 1,2-DHNO, and GDO activities (Table 4). Salicylate-grown cells showed comparable activities of GDO but significantly low activities of CH and 1-NH. The activity of 1,2-DHNO was two- to threefold lower than that of carbaryl-grown cells. We failed to detect salicylate-5-hydroxyalse activity. Enzyme activities from glucose-grown cells were significantly low (Table 4). Growth versus specific activity showed the maximum activity of CH, 1-NH, and GDO during the late-log phase of growth (Fig. 2). Conversion of the substrate to product was monitored by time-dependent spectral changes during the enzyme reaction and identifying the products of bulk enzyme reactions using carbaryl, 1-naphthol, 1,2-DHN, and salicylaldehyde as substrates. Time-dependent spectral changes for CH showed a decrease in carbaryl at 280 nm, with a concomitant appearance of 1-naphthol at 322 nm and an isobestic point at 287 nm (Fig. 3). GDO showed an increase in absorbance at 330 nm due to the conversion of gentisate (320 nm) to maleylpyruvate (330 nm) (Fig. 3B). The bulk enzyme reaction with carbaryl gave 1-naphthol, salicylaldehyde, and salicylate (Table 2). With either 1-naphthol, 1,2-DHN, or salicylaldehyde as the substrate, salicylaldehyde and salicylate were detected as reaction products. None of the bulk enzyme reactions showed spots corresponding to gentisate or catechol formation (Table 2).

We demonstrated the presence of 1-NH and 1,2-DHNO activities in the cell extracts of carbaryl-degrading strains. 1,2-DHNO is purified and characterized from naphthalene-degrading organisms (15, 19, 23); however, 1-NH has not been characterized. We studied 1-NH for its cofactor and O_2 requirement. The enzyme showed good activity with FAD and NADH. When NADPH and FAD were used as cofactors, a 20 to 30% increase in activity was observed (Table 5). 1-NH showed maximum activity in the pH range 7.5 to 8.0. Heat denaturation (enzyme in a boiling water bath for 10 min) or trichloroacetic acid precipitation failed to show activity and product formation by TLC (Table 5). Under aerobic conditions in the presence of NADH and FAD, 1-NH showed conversion of 1-naphthol to salicylate (Table 2). However, under anaerobic conditions, we failed to detect salicylate by TLC (Table 5), suggesting that the enzyme requires O_2 as one of the substrates. The enzyme was inducible and showed maximum activity from carbaryl compared to its activity in salicylate- or glucose-grown cells (Table 4).

TABLE 4. Enzyme activities from the cell extract of strain C4[a]

Enzyme	Sp act (nmol $\cdot$ $min^{-1} \cdot mg^{-1}$ of protein) from cells grown on:		
	Carbaryl	Salicylate	Glucose
CH	31	1.5	ND[b]
1-NH	260	6	2
1,2-DHNO	98	37	5
GDO	484	880	0.2
CO	88	45	35

[a] Similar values were obtained with strains C5 and C6.
[b] ND, activity not detectable.

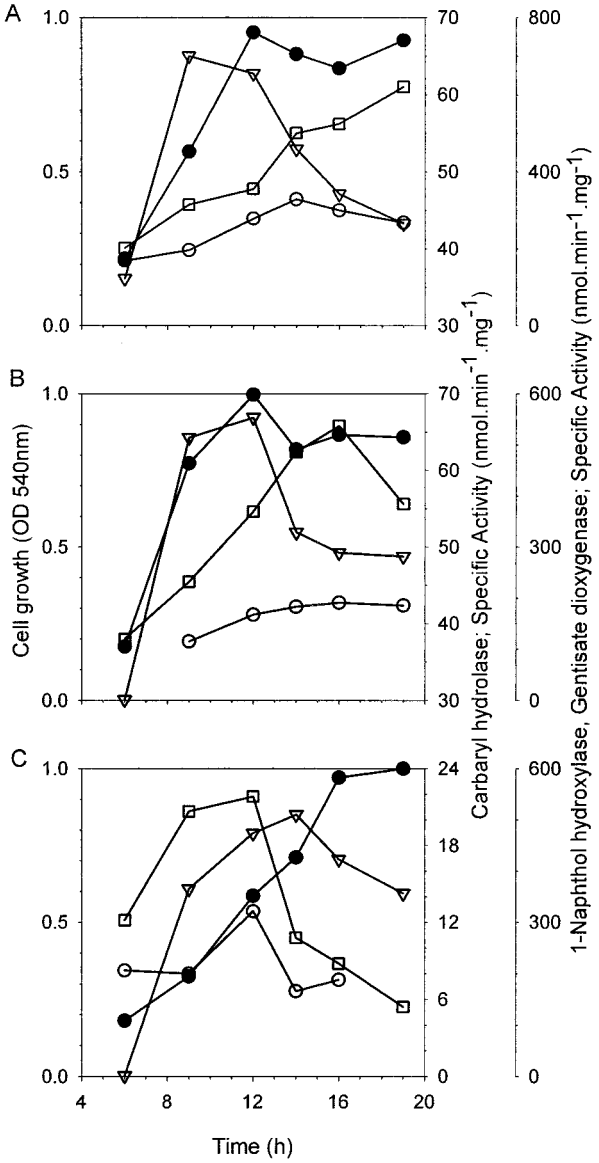

FIG. 2. Growth (●) versus specific activities of carbaryl hydrolase (□), 1-naphthol hydroxylase (○), and gentisate dioxygenase (▽) from *Pseudomonas* sp. strains (A) C4, (B) C5, and (C) C6. Experiments were performed twice with the enzyme activities in triplicate for each enzyme. Similar trends were observed, and the best for each strain is depicted in the figure. OD 540nm, optical density at 540 nm.

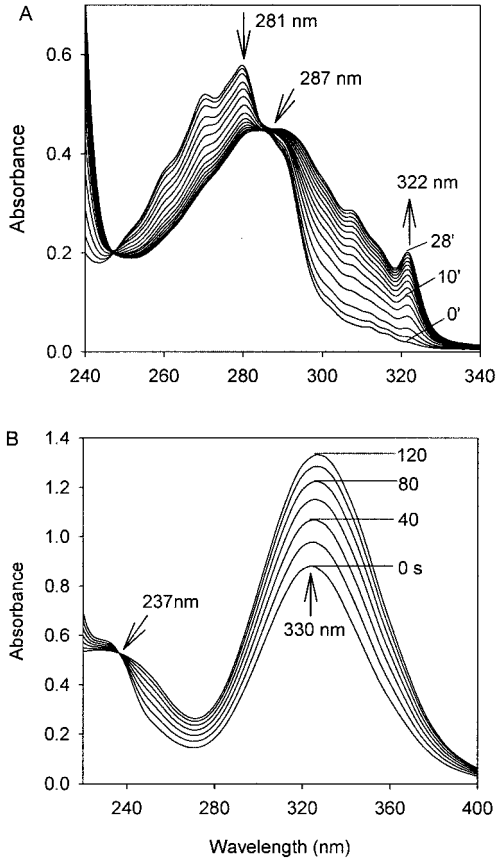

FIG. 3. Time-dependent spectral changes for (A) carbaryl hydrolase and (B) gentisate dioxygenase reactions. The up arrow indicates the appearance of the product, while the down arrow indicates the disappearance of the substrate. The crossover point indicates the isobestic point for the conversion of the substrate into the product. For carbaryl hydrolase and gentisate dioxygenase, spectral changes were recorded every 2 min and 20 s, respectively.

DISCUSSION

Pseudomonas sp. strains C4, C5, and C6 utilize carbaryl. Compared to reported organisms, isolated strains degrade carbaryl at very high concentrations (1% so far tested). Based on various metabolic studies (Tables 1 to 4), the proposed metabolic pathway for carbaryl degradation is shown in Fig. 4. Degradation of carbaryl via 1-naphthol, salicylate, and gentisate is well documented (4, 7, 9); however, the metabolic steps and enzymes involved in the conversion of 1-naphthol to salicylate have not been reported so far. A few 1-naphthol-degrading microorganisms have been reported to hydroxylate 1-naphthol to 4-hydroxy-1-tetralone (1), 3,4-dihydro-dihydroxy-1(2H)-naphthalenone (31), or 1,4-naphthoquinone (25). However, further metabolism of these hydroxylated intermediates is not clearly understood. Conversion of carbaryl, 1-naphthol, and 1,2-DHN to salicylaldehyde and salicylate (Ta-

TABLE 5. Properties of 1-naphthol hydroxylase enzyme for strains C4, C5, and C6

Conditions	1-Naphthol hydroxylase activity (%)[a]		
	C4	C5	C6
Cofactors			
None	—	—	—
NADH	90	82	80
NADH + FAD	100	100	100
NADPH	100	110	110
NADPH + FAD	115	135	130
Optimum pH	8.0	7.5	7.5
Boiled enzyme	—	—	—
Anaerobic reaction	—	—	—

[a] Activity with NADH and FAD is taken as 100%. —, activity was not detected, or there was no product formation under anaerobic condition as monitored by TLC.

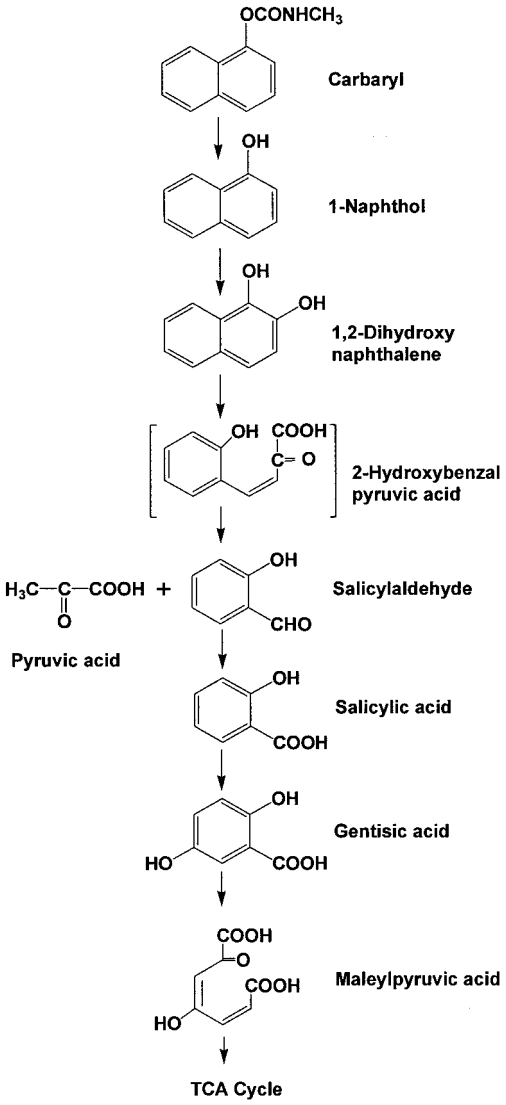

FIG. 4. Proposed pathway for the metabolism of carbaryl in *Pseudomonas* sp. strains C4, C5, and C6. The metabolite shown in brackets, 2-hydroxybenzalpyruvic acid, is a probable intermediate based on the detection of 1,2-DHNO activity.

ble 2), O_2 uptake on 1,2-DHN (Table 3), detection of 1-NH and 1,2-DHNO (Table 4), and the inability to transform 1,4-naphthoquinone strongly support 1,2-DHN being an intermediate in the carbaryl metabolic pathway. We could detect salicylate but failed to observe 1,2-DHN as an intermediate in bulk enzyme reactions with carbaryl and 1-naphthol (Table 2). This could be due to the presence of enzymes, viz, 1,2-DHNO, 2-hydroxybenzalpyruvate aldolase, and SalDH, responsible for the conversion of 1,2-DHN to salicylate. Similar enzyme reactions are reported for naphthalene degradation (33). Interestingly, none of the three carbaryl-degrading strains utilize naphthalene as the carbon source. This could be due to the lack of a ring hydroxylting naphthalene dioxygenase, the first enzyme of the pathway. 1,2-DHNO is an extradiol ring-cleaving enzyme. Besides 1,2-DHN, it catalyzes a reaction with 4-methylcatechol, yielding 2-hydroxy 5-methyl *cis*,*cis*-mucconic semialdehyde (382 nm) (2, 19, 23). Cell extracts of the three isolates showed increases in the absorption at 382 nm with 4-methylcatechol as a substrate, suggesting the presence of an extradiol ring-cleaving 1,2-DHNO enzyme. The cells metabolize salicylate to gentisate, as evident from biotransformation and whole-cell O_2 uptake studies (Table 2 and 3); however, we failed to monitor salicylate-5-hydroxylase activity in the cell extracts. This could be due to the complex and unstable nature of the enzyme, as reported for several other hydroxylases.

Involvement of CH and GDO in the carbaryl degradation is well documented (4, 7, 9). However, to the best of our knowledge, the activity of 1-naphthol oxygenase has been demonstrated at the whole-cell level by O_2 uptake studies with the carbaryl-degrading organisms *Pseudomonas* sp., *Rhodococcus* sp. (16), and *Micrococcus* sp. (7). The cofactor requirement and oxygenase nature of the enzyme have not been investigated so far. Here, we demonstrate for the first time the activity of 1-NH in the cell extract of carbaryl-degrading strains. Initial characterization indicates that 1-NH is oxygenase and requires FAD and a reducing cofactor (NADH or NADPH) (Table 5), suggesting that the enzyme belongs to a flavin monooxygenase group of an oxidoreductase class of enzymes. The enzymes of the carbaryl metabolic pathway are inducible, as carbaryl-grown cells showed maximum activities compared to those of glucose-grown cells. Carbon source-dependent O_2 uptake and enzyme activity studies (Tables 3 and 4) suggest that the carbaryl pathway is probably organized into upper and lower pathways. The upper pathway metabolizes carbaryl to salicylate, while the lower pathway converts salicylate to tricarboxylic acid cycle intermediates through gentisate. We propose that carbaryl induces both upper and lower pathway enzymes while salicylate induces lower pathway enzymes only.

In conclusion, *Pseudomonas* sp. strains C4, C5, and C6 utilize carbaryl via 1-naphthol, 1,2-DHN, salicylate, and gentisate. The detection of 1-NH and 1,2-DHNO confirms the carbaryl metabolic pathway via 1,2-DHN. The ability to degrade high concentrations of carbaryl makes these strains ideal candidates for their application in pesticide degradation/remediation at the contaminated sites.

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