Hydrolysis of 4-Hydroxybenzoic Acid Esters (Parabens) and Their Aerobic Transformation into Phenol by the Resistant *Enterobacter cloacae* Strain EM

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Enterobacter cloacae strain EM was isolated from a commercial dietary mineral supplement stabilized by a mixture of methylparaben and propylparaben. It harbored a high-molecular-weight plasmid and was resistant to high concentrations of parabens. Strain EM was able to grow in liquid media containing similar amounts of parabens as found in the mineral supplement (1,700 and 180 mg of methyl and propylparaben, respectively, per liter or 11.2 and 1.0 mM) and in very high concentrations of methylparaben (3,000 mg liter⁻¹, or 19.7 mM). This strain was able to hydrolyze approximately 500 mg of methyl-, ethyl-, or propylparaben liter⁻¹ (3 mM) in less than 2 h in liquid culture, and the supernatant of a sonicated culture, after a 30-fold dilution, was able to hydrolyze 1,000 mg of methylparaben liter⁻¹ (6.6 mM) in 15 min. The first step of paraben degradation was the hydrolysis of the ester bond to produce 4-hydroxybenzoic acid, followed by a decarboxylation step to produce phenol under aerobic conditions. The transformation of 4-hydroxybenzoic acid into phenol was stoichiometric. The conversion of approximately 500 mg of parabens liter⁻¹ (3 mM) to phenol in liquid culture was completed within 5 h without significant hindrance to the growth of strain EM, while higher concentrations of parabens partially inhibited its growth.

The esters of 4-hydroxybenzoic acid, also called parabens, are widely used as antimicrobial agents in a large variety of food, pharmaceutical, and cosmetic products (20) due to their excellent antimicrobial activities and low toxicity (11). They are stable, effective over a wide pH range, and active against a broad spectrum of microorganisms. However, their mode of action is not well understood. They are postulated to act by disrupting membrane transport processes (7) or by inhibiting synthesis of DNA and RNA (17) or of some key enzymes, such as ATPases and phosphotransferases, in some bacterial species (15). Propylparaben is considered more active against most bacteria than methylparaben. However, because the latter is more soluble in water, they are often used as a mixture in commercial preparations.

Batches of a dietary mineral supplement normally well stabilized with a mixture of methylparaben and propylparaben have shown signs of microbial contamination in conjunction with the disappearance of the parabens. Very little has been established regarding microbial resistance and degradation pathways with respect to parabens. There are few cases in the scientific literature of microbial growth in paraben-stabilized products, although resistance to parabens by strains of *Pseudomonas aeruginosa*, *Burkholderia cepacia*, and *Cladosporium resinae* has been reported (4, 27, 29, 31). However, as parabens are prominent antimicrobial agents in a variety of industries, the economic and medical impact of their degradation by microbial contaminants can be significant. Here, we report the

characterization of a highly resistant strain of the ubiquitous bacterium *Enterobacter cloacae* isolated from a paraben-stabilized mineral supplement and the identity of the main degradation products generated by this strain.

MATERIALS AND METHODS

Materials. Chemicals were obtained as follows: 4-hydroxybenzoic acid esters, 4-hydroxybenzoic acid, protocatechuic acid, and bovine albumin from Sigma-Aldrich (St. Louis, Mo.); phenol from Fluka (Buchs, Switzerland); bis(trimethylsilyl)trifluoroacetamide from Pierce (Rockford, Ill.); ethyl acetate and acetonitrile from EM Science (Gibbstown, N.J.); acetic acid from Mallinckrodt (Pointe-Claire, Quebec, Canada); ultrapure sucrose from GibcoBRL (Life Technologies, Inc., Gaithersburg, Md.); tryptic soy broth, tryptic soy agar, and all other growth media from Difco Laboratories (Detroit, Mich.); and all other chemicals from Anachemia (Ville Saint-Pierre, Quebec, Canada). Antibiotics were purchased as follows: streptomycin, penicillin, trimethoprim, polymyxin B, and norfloxacin from Sigma; rifampin and chloramphenicol from Boehringer Mannheim (Mannheim, Germany); tetracycline, erythromycin, ampicillin, and gentamicin from ICN Biomedicals (Aurora, Ohio); and kanamycin from Fisher (Fair Lawn, N.J.). Restriction endonucleases were purchased from Pharmacia Biotech (Baie d'Urfé, Quebec, Canada), and molecular weight markers were purchased from MBI Fermentas (Vilnius, Lithuania).

Growth conditions. Solid medium containing paraben crystals was prepared by autoclaving tryptic soy agar, and while still hot, methylparaben (5 g liter⁻¹) or propylparaben (1 g liter⁻¹) was added. Immediately after pouring, the petri dishes were cooled at 4°C, which caused the parabens to form small, but clearly visible, crystals within the agar (4). The plates were incubated at 30°C overnight, and disappearance of the crystals around growing bacteria was monitored as an indication of paraben degradation. Liquid cultures of tryptic soy broth with parabens were prepared by autoclaving media already containing parabens. Paraben stability during autoclaving was verified by high-pressure liquid chromatography (HPLC). Determination of the optimum growth temperature was performed in tryptic soy broth with and without a mixture of methyl- and propylparabens at concentrations similar to those of the mineral supplement. Cell growth in liquid media was monitored by optical density (OD) readings at 600 nm, which were always measured below an OD of 0.3 after dilution, and where a reading of 1.0 OD corresponded to 6×10^8 cells/ml. All cultures for

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subsequent assays were grown at 30°C in a rotary agitator at 250 rpm under aerobic conditions.

Plasmid extraction. *E. cloacae* strains EM and E were cultured in tryptic soy broth in the absence of parabens. The alkaline lysis protocol for plasmid extraction was followed as described by Sambrook et al. (25), and plasmid DNA was purified by CsCl gradient centrifugation (25). Plasmid transfer experiments were attempted by transformation of competent cells of strain E treated with calcium chloride according to the method of Sambrook et al. (25), by transformation of commercially prepared competent cells of *Escherichia coli* XL1-Blue (Stratagene, La Jolla, Calif.), and by electroporation of strain E according to the method of Smith and Igleski (26), using a Gene Pulser apparatus (Bio-Rad Laboratories, Richmond, Calif.).

Paraben sensitivity. MICs were determined in tryptic soy broth by the method of Eklund (6), with the following modifications: a small aliquot of a culture grown overnight in tryptic soy broth was diluted into tryptic soy broth medium that had been autoclaved with the appropriate concentrations of parabens such that the starting OD of the culture was approximately 0.05 at 600 nm. The density of the bacterial suspensions was measured immediately after dilution and after 24 and 48 h of incubation at 30°C with shaking. The MICs were defined as the amounts of preservative added to the media that prevented an increase in the OD of the cell suspension after 48 h relative to the density immediately after inoculation.

Characterization of hydrolytic activity. Liquid cultures were prepared from EM cells grown in tryptic soy broth overnight, centrifuged, and resuspended in fresh medium before being inoculated into tryptic soy broth containing the appropriate paraben. The OD of all cultures was monitored in the same manner as for the MICs. The cells were incubated at 30°C with shaking; at timed intervals, 1-ml aliquots were removed for HPLC analysis and heated immediately to 80°C for 10 min to prevent further enzymatic degradation of the parabens. Cell lysates of strain EM grown in tryptic soy broth without parabens were prepared with an ultrasonicator probe (Heat Systems, Inc., Farmingdale, N.Y.) using three 20-s pulses at 143 W, followed by centrifugation at $16,000 \times g$ for 15 min. A 30-fold dilution of the cell lysate was subsequently made in tryptic soy broth containing 1,000 mg of methylparaben liter⁻¹ (6.6 mM) and incubated for 2 h at 30°C with shaking. Sampling was performed at timed intervals as described above, and a control was prepared in the same manner from an EM culture that had not been sonicated. Total protein concentration was measured in the cell extracts of sonicated and nonsonicated cultures with the Bio-Rad protein assay (Bio-Rad Laboratories, Richmond, Calif.) by the method of Bradford (3), using bovine albumin as a standard.

Identification of paraben degradation products. The metabolites of the parabens were identified both by HPLC and gas chromatography (GC)-mass spectrometry (MS). The HPLC analyses were performed on an HP 1100 (Agilent Technologies, Kirkland, Quebec, Canada) equipped with a 150- by 4-mm C₁₈ reverse-phase Hypersil ODS column (5 µm; Agilent Technologies) and a variable-wavelength UV detector. A water-acetonitrile gradient containing a constant amount of 0.1% acetic acid was used, starting with 90% water and ending with 90% acetonitrile after 5 min at a flow rate of 2 ml/min. The GC was a Varian 3500 (Varian Canada, St-Laurent, Quebec, Canada) equipped with a DB-5 column (30 m; 320-µm inside diameter; film thickness, 0.25 µm). The carrier gas was helium at a flow rate of 2.2 ml/min. The temperature gradient started at 70°C, rising to 250°C at 10°C/min and to 310°C at 25°C/min. The MS was a Finnigan Ion Trap 800 (Thermo Quest, Schaumburg, Ill.), and the scan range was from 70 to 440 Da.

The samples were prepared for HPLC analysis by centrifugation of the cell suspensions to remove cell debris and adding acetonitrile containing 1% acetic acid to the supernatants to a final concentration of 10%. The parabens, 4-hydroxybenzoic acid, and phenol were identified according to their retention times and quantified with the appropriate calibration curves. Samples for GC-MS analysis were prepared by homogenizing the cell cultures by vortexing, saturating with sodium chloride, and extracting with ethyl acetate. The organic layer was dried with anhydrous sodium sulfate, and the compounds retained in the organic layer were derivatized by direct addition of bistrimethylsilyltrifluoroacetamide and heating at 70°C before injection. The trimethylsilyl derivatives of 4-hydroxybenzoic acid and phenol were identified by comparison of their retention times and mass spectra to those of standards derivatized in the same manner.

RESULTS AND DISCUSSION

Characterization of bacterial strains. The paraben-resistant *E. cloacae* strain EM was isolated from batches of a dietary mineral supplement which was normally well stabilized with methyl- and propylparabens. This supplement contained vari-

ous mineral glycerophosphates, plant extracts, and methyl- and propylparabens at 1.7 and 0.18 g liter⁻¹, respectively. The contamination was observed simultaneously in batches manufactured at distant production plants, indicating that the origin of the contaminants was not environmental but that they were probably introduced from one of the ingredients of the mixture. The most obvious sign of bacterial growth was the inflation of the plastic bottles due to the production of gas. A facultatively anaerobic gram-negative short rod isolated from these bottles was identified as *E. cloacae* by API 20E galleries (bioMérieux, St-Laurent, Quebec, Canada) and named strain EM. The identification was further verified by comparing it to a collection strain *E. cloacae* LSPQ 3022 (Laboratoire de Santé Publique du Québec, St-Anne de Bellevue, Quebec, Canada), named strain E. Strain EM formed large smooth colorless colonies with an aspect and color identical to those of the reference strain E on tryptic soy agar, blood, and McConkey and Hektoën media. The identification was confirmed by sequencing a 591-bp PCR-amplified portion of the 16S rRNA gene using the universal eubacterial primers 5'AGAGTTTGATCCTGGCTCAG3' (nucleotides 8 to 27) and 5'AAGGAGGTGATCCAGCCGCA3' (nucleotides 1522 to 1541) for *E. coli* (GenBank accession no. J01695). A similarity of >99% compared to the sequence of the *E. cloacae* 16S rRNA gene (ATCC 13047T; GenBank accession no. AJ251469) was obtained. Strain EM grew in tryptic soy broth with the production of gas at temperatures between 25 and 37°C, and its optimal growth temperature was found to be 30°C with or without parabens (data not shown). Comparative antibiograms of the two strains demonstrated that, as previously reported for this species, both *E. cloacae* strains were resistant to high concentrations of penicillin and ampicillin (100 µg/ml) (21, 30) and were equally resistant to 100 µg of erythromycin per ml and to 50 µg of trimethoprim per ml. Both strains were found to be sensitive to 25 µg of streptomycin per ml, as well as to 10 µg of tetracycline or polymyxin B per ml and to 0.5 µg of norfloxacin per ml. Strain EM was found to differ from the reference strain E by being sensitive to 50 µg of rifampin per ml and to 10 µg of either chloramphenicol, gentamicin, or kanamycin per ml, although at 50 µg of the three latter compounds per ml, both strains became sensitive.

Plasmid characterization. Resistance to biocides is often associated with plasmids (24). A large plasmid was detected in strain EM, while no plasmid was found in the reference strain E. The plasmid in strain EM was extracted and purified by CsCl gradient centrifugation, and its molecular size was estimated at 100 ± 20 kb. High-molecular-weight plasmids were previously found in *E. cloacae* isolates from nosocomial infections that had a high level of multiresistance (14, 22). Transfer of the plasmid from strain EM by transformation of strains *E. cloacae* E and of *E. coli* XL1-Blue did not yield transformants able to hydrolyze crystallized parabens on tryptic soy agar. Further attempts to transfer the plasmid to strain E by electroporation did not yield transformants able to grow on plates containing 2,500 mg of crystallized ethylparaben liter⁻¹ (15.0 mM), suggesting that the large size of the plasmid may not permit it to cross the bacterial membrane. Strain EM was also not cured of its plasmid by growing cultures at 42°C or in ethidium bromide. Interestingly, strain EM was able to grow in the presence of concentrations of ethidium bromide as high as

FIG. 1. (A) Growth of strains EM (● and ○) and E (■ and □) in tryptic soy broth (—) and growth in tryptic soy broth containing methyl- and propylparabens (1,400 and 150 mg, respectively, liter⁻¹, or 9.2 and 0.83 mM) (.....). (B) Growth of strain E in the presence of 400 mg each of methylparaben (2.6 mM) (○) and propylparaben (2.2 mM) (□) liter⁻¹ and 500 mg of ethylparaben liter⁻¹ (3.0 mM) (◇). (C) Growth of strains EM (○) and E (□) after 24 h in tryptic soy broth with increasingly high concentrations of methylparaben.

1,000 mg liter⁻¹ (2.5 mM). Such resistance has been associated with plasmid-mediated efflux in *Staphylococcus aureus* and has been cloned into *E. coli* (23).

Resistance to parabens. Both strains E and EM grew to very high cell densities in tryptic soy broth alone (Fig. 1A), with the reference strain E reaching slightly higher densities than strain EM. However, strain EM grew in the presence of methyl- and propylparabens at concentrations similar to those present in the mineral supplement (1,400 and 150 mg liter⁻¹, respectively, or 9.2 and 0.83 mM), while at these concentrations, the growth of strain E was suppressed (Fig. 1A). Due to limitations in the solubility of propylparaben, the effects of similar concentrations of the series methyl-, ethyl-, and propylparabens could only be studied at approximately 500 mg liter⁻¹ (3 mM). Growth of the reference strain E was considerably hindered by methyl- and ethylparaben concentrations of 400 and 500 mg liter⁻¹, respectively, as it reached ODs of only 4.5 and 2.4 after

24 h (Fig. 1B) and was suppressed by 400 mg of propylparaben liter⁻¹. The growth of strain EM at these paraben concentrations was comparable to that obtained in tryptic soy broth alone, reaching ODs of 3 after 5 h and 9 after 24 h for all three parabens (results not shown). Furthermore, strain EM survived in very high concentrations of methylparaben (3,000 mg liter⁻¹, or 19.7 mM) after 24 h and was further able to grow, while the growth of strain E was suppressed at concentrations of 2,000 mg liter⁻¹ (13.1 mM) and remained significantly below that of strain EM at lower paraben concentrations (Fig. 1C).

The differences in the antimicrobial activities of methyl-, ethyl-, and propylparabens are clearly demonstrated by the effects of similar concentrations on the growth of the paraben-sensitive strain E (Fig. 1B). Propylparaben was the most effective of the three, as 400 mg liter⁻¹ (2.2 mM) suppressed the growth of strain E, while similar amounts of ethyl- or methylparaben did not inhibit cell growth entirely. The stronger antibacterial action of propylparaben may be due to its greater solubility in the bacterial membrane, which may allow it to reach cytoplasmic targets in greater concentrations. However, since a majority of the studies on the mechanism of action of parabens suggest that their antibacterial action is linked to the membrane (6, 7), it is possible that its greater lipid solubility disrupts the lipid bilayer, thereby interfering with membrane transport processes and perhaps causing the leakage of intracellular constituents.

Paraben resistance of strain EM relative to strain E is also reflected in the MICs of the four most commonly used parabens. The methylparaben concentration required to suppress the growth of strain EM was 4,000 mg liter⁻¹ (26.3 mM). Due to limitations in their solubilities, the concentrations of ethyl-, propyl-, and butylparabens required to prevent growth of strain EM could be determined only as higher than 1,600, 600, and 200 mg liter⁻¹ (9.6, 3.3, and 1.0 mM), respectively, as this strain was able to reach very high ODs (>7 at 600 nm) at these concentrations. In comparison, the MICs for strain E of methyl-, ethyl-, and propylparabens were 2,000, 800, and 600 mg liter⁻¹ (13.1, 4.8, and 3.3 mM), respectively, while butylparaben limited the growth of strain E considerably without completely suppressing it at 200 mg liter⁻¹ (1.0 mM). The MICs reported in the literature of these four parabens for *E. cloacae* ATCC 23355 are 1,000, 1,000, 500, and 250 mg liter⁻¹ (6.6, 6.0, 2.8, and 1.3 mM) (11), respectively, showing the remarkable resistance of strain EM toward these compounds. Due to the high resistance of strain EM to all four parabens, as evidenced by the MICs, the minimum bactericidal concentrations were not determined.

Degradation of parabens by strain EM. When a 10-μl/ali-quot of an overnight culture of EM grown in tryptic soy broth without parabens was deposited in the middle of a tryptic soy agar plate containing 1, 5, or 10 g of crystallized propylparaben liter⁻¹, a time-dependent clearance zone around the growing bacteria was observed within a few hours. After 8 days of incubation, the diameter of this zone on 1 g of propylparaben liter⁻¹ had increased to the extent that the paraben crystals over the entire area of a standard 100-mm-diameter petri dish had disappeared, although the diameter of the bacterial spot where the cells were initially deposited did not increase significantly (results not shown). Furthermore, when a small area of

FIG. 2. Conversion of 1,000 mg of methylparaben liter⁻¹ (6.6 mM) (● and ○) into 4-hydroxybenzoic acid (■ and □) by the supernatants of sonicated (—) and nonsonicated (·····) cultures of strain EM, yielding 4-hydroxybenzoic acid concentrations of 950 and 900 mg liter⁻¹ (6.9 and 6.5 mM), respectively. The appearance of the degradation product was monitored by HPLC.

agar cleared of crystallized parabens by strain EM, but outside the diameter of bacterial growth, was transferred to liquid medium containing 1,000 mg of methylparaben liter⁻¹ (6.6 mM), only 7% of the original amount of paraben remained after 24 h and 0.02% of the paraben was found in the medium after 48 h. No evidence of cell growth was detected at these time points, demonstrating that the factor responsible for paraben degradation diffused through agar outward from the perimeter of bacterial growth (results not shown). Additionally, when a culture filtrate (0.2 μm) of strain EM was placed on tryptic soy agar containing crystallized propylparaben, a 2.5-cm clearance zone was observed after a 24 h of incubation. No clearance zone was observed with a EM filtrate heated to boiling, indicating that the factor was heat sensitive and probably enzymatic in nature. Additionally, no clearance zone was observed with a filtrate of strain E (results not shown). Hence, the considerable growth of strain EM in methyl-, ethyl-, and propylparabens above their reported MICs (11) and in comparison with the control strain E can be explained in terms of the enzymatic degradation of these antibacterial agents.

Conversion of parabens to 4-hydroxybenzoic acid. When strain EM was incubated at 30°C overnight in tryptic soy broth containing concentrations of methyl- and propylparabens similar to those in the mineral supplement (1,400 and 150 mg liter⁻¹, respectively, or 9.2 and 0.83 mM), both parabens disappeared within 24 h of incubation at 30°C, while no significant change occurred in the concentrations of parabens present in a parallel culture of strain E (results not shown). Additionally, when the supernatant of a culture of strain EM grown without parabens was diluted 1:30 and inoculated into tryptic soy broth containing 1,000 mg of methylparaben liter⁻¹ (6.6 mM), only 6% (0.4 mM) of the initial paraben remained in the medium after 2 h (Fig. 2). The disappearance of the paraben was accompanied by a corresponding increase in the amounts of 4-hydroxybenzoic acid, which reached 900 mg liter⁻¹ (6.5 mM)

FIG. 3. Complete transformation of methylparaben by strain EM in tryptic soy broth. The paraben (●) at a concentration of 400 mg liter⁻¹ (2.6 mM) is rapidly hydrolyzed into 4-hydroxybenzoic acid (■), which is stoichiometrically decarboxylated into phenol (△). The appearance of the degradation products was monitored by HPLC.

after 2 h. To assess if the enzyme responsible for paraben hydrolysis was extra- or intracellular, the same EM culture was sonicated, and the supernatant was equally diluted and placed in 1,000 mg of methylparaben liter⁻¹ (6.6 mM) (Fig. 2). It was found that only 0.06% of the methylparaben (0.004 mM) remained in the medium after 15 min of incubation, while 80% of the paraben still remained in the supernatant of the non-sonicated culture after the same time, resulting in a hydrolysis rate difference greater than 800-fold, while the amount of protein released after sonication was only 69-fold greater (1.1 mg/ml). This extremely rapid hydrolysis by the sonicated cell suspension was paralleled by the rapid appearance of 950 mg of 4-hydroxybenzoic acid liter⁻¹ (6.8 mM). The nature of the degradation product and the differences between its rate of formation by the sonicated and the nonsonicated cultures indicate that the enzyme may be of intracellular nature or that it may be targeted to the periplasm.

Transformation of 4-hydroxybenzoic acid into phenol. Strain EM grown without parabens was inoculated into tryptic soy broth containing 400 mg of methylparaben liter⁻¹ (2.6 mM) and after 60 min transformed more than 99% of the paraben into 4-hydroxybenzoic acid (Fig. 3). However, the acid produced accumulated in the medium only transiently, as more than 99.9% was transformed into phenol after 5 h of incubation (Fig. 3). The kinetics of paraben hydrolysis and phenol formation were nearly identical for ethyl- and propylparabens at similar concentrations (results not shown). No cell lysis, indicated by decreases in OD, was observed in cultures of EM in media containing approximately 500 mg of either of the three parabens liter⁻¹ (3 mM) after 5 h of incubation, at which point phenol reached its maximal concentration (Fig. 3). Further confirmation of the origin of phenol was obtained from the 1:1 stoichiometric conversion of 4-hydroxybenzoic acid to phenol obtained by incubating strain EM with 700, 1,500, 2,300, 2,400, and 3,000 mg of methylparaben liter⁻¹ (4.6, 9.9, 15.1, 15.8, and 19.7 mM). A linear relationship with a slope of

FIG. 4. Degradation pathway of esters of 4-hydroxybenzoic acid into phenol by strain EM. The initial hydrolysis of methylparaben produces 4-hydroxybenzoic acid and methyl alcohol. Further degradation of 4-hydroxybenzoic acid does not follow the protocatechuate pathway. Instead, the 4-hydroxybenzoic acid produced is stoichiometrically converted into phenol by a decarboxylase-type enzyme operating under aerobic conditions.

1.0 was established between the amount of 4-hydroxybenzoic acid produced from the parabens and the amount of phenol present after the complete disappearance of the acid (results not shown). It is reported in the literature that 800 mg of phenol liter⁻¹ (8.5 mM) induced a lag phase in *Enterobacter aerogenes*, a species closely related to *E. cloacae* (28), during which the cells nonetheless remained viable (5). This concentration is close to the 900 mg of phenol liter⁻¹ (9.6 mM) which accumulated in the media when strain EM was grown in a mixture of methylparaben (1,400 mg liter⁻¹ [9.2 mM]) and propylparaben (150 mg liter⁻¹ [0.83 mM]). The growth of strain EM at these concentrations of parabens was considerably reduced after 24 h in comparison with EM grown in tryptic soy broth alone (Fig. 1A). The main difference with this latter experiment, aside from the presence of rapidly degraded parabens, is the transient accumulation of 4-hydroxybenzoic acid and subsequently of phenol (results not shown). This suggests that these compounds may be responsible for the reduced growth over 24 h of strain EM initially cultivated in the presence of parabens.

The decarboxylation of 4-hydroxybenzoic acid into phenol by aerobic bacteria has been reported only once, with a strain of *E. aerogenes*, (28), under both aerobic (19) and anaerobic (10) conditions. The usual degradation pathway of 4-hydroxybenzoic acid by aerobic bacteria is through the β -ketoadipate pathway, resulting in the formation of protocatechuic acid instead of phenol (Fig. 4). This pathway and the enzymes necessary for the degradation reactions, encoded by the *pca* operon, are highly conserved and have been extensively characterized in several prokaryotes, including *Acinetobacter calcoaceticus*, *Pseudomonas putida*, and *Agrobacterium tumefaciens* (12, 18). The metabolism of the 4-hydroxybenzoic acid generated from parabens by a resistant *P. aeruginosa* strain was

also found to proceed through the formation of protocatechuic acid (31). In the present study, no protocatechuic acid was detected by HPLC or GC-MS during paraben degradation by strain EM. Instead, the decarboxylation of 4-hydroxybenzoic acid to phenol proceeded stoichiometrically (Fig. 4). It has been documented that under anaerobic conditions, 4-hydroxybenzoic acid can be decarboxylated into phenol. This pathway has been found in a number of anaerobic consortia isolated from the environment (1, 8, 33) as well as in *Clostridium hydroxybenzoicum* and *Moorella* (basonym, *Clostridium*) *thermoacetica* (13, 32). However, the aerobic transformation of 4-hydroxybenzoic acid into phenol is a rarely documented pathway and raises questions about the ability of other ubiquitous *Enterobacteriaceae* to carry out these reactions.

The utilization of parabens as growth substrates by various bacterial genera has been observed by Beveridge and Hart (2). Close and Nielsen have reported hydrolysis of the parabens and their utilization as sole carbon source (4), while Suemitsu et al. reported the formation of 4-hydroxybenzoic acid as a degradation product (29), both by strains of *Pseudomonas cepacia*. However, in these studies, low concentrations of parabens (less than 100 mg liter⁻¹) were used, and more than 2 to 4 weeks were required to achieve complete degradation. Similarly, a *P. aeruginosa* strain isolated by Zedan and Serry required 5 days to completely hydrolyze 100 mg of propylparaben liter⁻¹ (31), while the *Cladosporium* strain isolated by Sokolski et al. was capable of hydrolyzing 70% of a 2,000-mg liter⁻¹ paraben solution in 5 days (27). In contrast, strain EM was capable of completely hydrolyzing approximately 500 mg of methyl-, ethyl-, or propylparaben liter⁻¹ in less than 2 h and a mixture of methyl- and propylparabens (1,400 and 150 mg, respectively, liter⁻¹), similar to amounts used in commercial preparations, in less than 4 h, demonstrating the remarkable activity of this strain toward parabens. To our knowledge, this is the first report of a strain of *E. cloacae* that can inactivate high amounts of parabens in the early stages of growth and continue to grow in high concentrations of 4-hydroxybenzoic acid and phenol. The introduction of *Enterobacter* species as clinical pathogens has previously been reported for strains of *E. cloacae* present in contaminated dextrose infusion fluid or from sources as varied as formulated oral feeds, hydrotherapy tanks, or liner caps of intravenous fluid bottles (9, 16). The resistance of strain EM to such common preservatives as parabens can engender health-related concerns, as they are used in a number of pharmaceutical products, which might create the potential for the spread of paraben-resistant bacteria as nosocomial pathogens.

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