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Bacteria Obtained from a Sequencing Batch Reactor That Are Capable of Growth on Dehydroabietic Acid

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Eleven isolates capable of growth on the resin acid dehydroabietic acid (DhA) were obtained from a sequencing batch reactor designed to treat a high-strength process stream from a paper mill. The isolates belonged to two groups, represented by strains DhA-33 and DhA-35, which were characterized. In the bioreactor, bacteria like DhA-35 were more abundant than those like DhA-33. The population in the bioreactor of organisms capable of growth on DhA was estimated to be 1.1×10^6 propagules per ml, based on a most-probable-number determination. Analysis of small-subunit rRNA partial sequences indicated that DhA-33 was most closely related to *Sphingomonas yanoikuyae* ($S_{ab} = 0.875$) and that DhA-35 was most closely related to *Zoogloea ramigera* ($S_{ab} = 0.849$). Both isolates additionally grew on other abietanes, i.e., abietic and palustric acids, but not on the pimaranes, pimaric and isopimaric acids. For DhA-33 and DhA-35 with DhA as the sole organic substrate, doubling times were 2.7 and 2.2 h, respectively, and growth yields were 0.30 and 0.25 g of protein per g of DhA, respectively. Glucose as a cosubstrate stimulated growth of DhA-33 on DhA and stimulated DhA degradation by the culture. Pyruvate as a cosubstrate did not stimulate growth of DhA-35 on DhA and reduced the specific rate of DhA degradation of the culture. DhA induced DhA and abietic acid degradation activities in both strains, and these activities were heat labile. Cell suspensions of both strains consumed DhA at a rate of 6 μmol mg of protein^{-1} h^{-1}. On the basis of this consumption rate, the estimated population of organisms capable of growth on DhA is at the lower limit required to account for DhA removal in the bioreactor. Both isolates have very similar resin acid degradation activities, which, because of their specificities for abietanes, can only partly account for resin acid removal in the bioreactor from which the strains were isolated.

Resin acids, including dehydroabietic acid (DhA), are a minor component of wood. Commonly occurring resin acids are tricyclic diterpenes, which are divided into two classes, abietanes, having an isopropyl substituent at C-13, and pimaranes, having both methyl and vinyl substituents at C-13 (Fig. 1). Resin acids have antimicrobial activities (reviewed in reference 5) and may protect plants from infection. These naturally occurring compounds are believed to cause much of the toxicity of pulp mill effluents to fish (11, 18). In addition, resin acids are a component of pitch, which interferes in the paper-making process. Such interference will tend to worsen as paper mills are designed to minimize wastewater discharge by increasing recycling of process streams. For the above reasons, it is important that biological treatment systems for process streams and for effluents of pulp and paper mills effectively degrade resin acids.

Despite the significance of resin acids, very little is known about their biodegradation. Decades ago, four aerobic, gram-negative bacteria which use DhA as the sole carbon source and electron donor were described (2, 12). DhA metabolites from those bacteria were identified, suggesting various degradation pathways, all beginning with oxidation of DhA to alcohol and ketone derivatives, which were further degraded (1a, 2, 3). The fungus *Mortierella isabellina* also oxidizes resin acids (9), but the resulting alcohols are not degraded.

Consistent with the ability of certain bacteria to use DhA as the sole organic substrate, experience indicates that aerobic biological treatment systems are capable of effectively removing resin acids. However, such systems are prone to occasional performance failures, which can be costly and environmentally damaging. Further, the physiological limits (e.g., temperature and pH) of resin acid biodegradation have not been tested, so that ranges of operating parameters for treatment systems are not known. It would be desirable to better understand at a fundamental level the organisms in biological treatment systems responsible for resin acid degradation. Biochemical, physiological, and ecological understanding of these organisms would provide a rational basis for maximizing the efficiency and stability of treatment systems. The objectives of this study were (i) to isolate microorganisms that are likely to be important for resin acid removal in a biological treatment system, (ii) to characterize resin acid biodegradation by those isolates, and (iii) to generally characterize those isolates physiologically and phylogenetically.

MATERIALS AND METHODS

Enrichment and isolation. Bacteria capable of growth on DhA were isolated from a laboratory scale (10-liter) sequencing batch reactor (SBR) used to treat a high-strength process stream from a paper mill. This influent contained both plug screw pressate from a thermomechanical pulp mill (containing resin acids) and evaporator bottoms from a closed-cycle chemithermomechanical pulp mill (containing high levels of organic matter). The total resin acid concentration in the influent was typically 30 mg/liter, including 10 mg of DhA per liter, 6 mg of abietic acid (AbA) per liter, and 5 mg of isopimaric acid (IpA) per liter. The other major "extractives" in the influent were the fatty acids linoleic acid and palmitic acid, typically at 7 and 3 mg/liter, respectively. Samples from the SBR were kindly donated by E. Hall, Department of Civil Engineering, University of British Columbia. The SBR was operated at 30°C on a 24-h cycle with a hydraulic retention time of 48 h and a solids retention time of approximately 20 days. The SBR demonstrated a high efficiency of removal of all resin acids.

Isolates were obtained both by selective enrichment and by end point dilution

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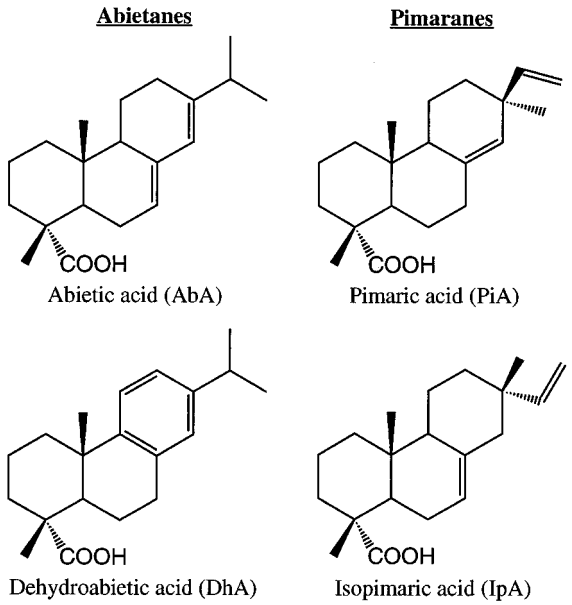

FIG. 1. Resin acids used in this study and their abbreviations.

on medium with 30 mg of DhA per liter as the sole organic substrate. Prior to use as an inoculum, bioreactor samples were shaken vigorously to disperse microorganisms; however, some flocs did resist this treatment. For selective enrichment, three serial liquid enrichment cultures were grown from 1% inocula, and the final liquid culture was repeatedly streaked on solid medium. For end point dilution, a series of 10-fold dilutions were made in the above liquid medium. The greatest dilution exhibiting both growth and DhA disappearance was repeatedly streaked on solid medium. The dilutions were performed with five replicates to allow determination of the most probable number (MPN) of microorganisms capable of growth on DhA as the sole organic substrate. MPN determination was performed by the method of Hartman et al. (8). Isolates were grown on liquid medium to verify their ability to grow on DhA as the sole organic substrate (in the absence of agar) and to remove DhA from the culture medium. Liquid enrichment cultures were performed with 2.5-ml volumes and were incubated stationary. All incubations in this study were carried out at 30°C, unless otherwise specified.

The basal medium used for enrichment and isolation cultures was formulated to partially resemble the above wastewater and contained the following final concentrations (per liter): 0.33 g of K_2HPO_4, 0.16 g of KH_2PO_4, 0.54 g of NH_4Cl, 0.10 g of Na_2SO_4, 0.030 g of $MgCl_2 \cdot 6H_2O$, and 0.003 g of $CaCl_2 \cdot 2H_2O$. The first four compounds were dissolved in 90% of the final volume, and the pH of that solution was adjusted to 7.0 with HCl and NaOH. The last two compounds and a trace-element solution (13) were dissolved in 10% of the final volume. The two solutions were autoclaved separately and mixed after cooling. Vitamins and 10 mg of $FeSO_4 \cdot 7H_2O$ per liter were added from filter-sterilized stock solutions. The vitamin solution, when diluted 1,000-fold, yielded the following final concentrations (per liter): 20 μg of biotin, 20 μg of folic acid, 60 μg of lipoic acid, 50 μg of thiamine, 50 μg of riboflavin, 50 μg of nicotinic acid, 100 μg of pyridoxal · HCl, 50 μg of pantothenic acid, 50 μg of cyanocobalamin, 50 μg of *p*-aminobenzoic acid, and 200 μg of naphthoquinone. Solid media included 1% select agar (BBL, Cockeysville, Md.). Resin acids were obtained from Helix Biotechnologies, Richmond, Canada, and ranged in purity from 90 to 99%. Resin acids were dissolved in NaOH solutions, filter sterilized, and added to autoclaved media. Resin acids were used at concentrations far above their aqueous solubilities and formed visible suspensions in the culture medium, particularly in the case of IpA.

Physiological tests. The above basal medium was used for subsequent cultures of the isolates, except that the phosphate buffer concentration was increased to 1.10 g of K_2HPO_4 per liter and 0.53 g of KH_2PO_4 per liter (10 mM phosphate). Organic substrates were added to culture media from filter-sterilized stock solutions. The presence or absence of growth on resin and fatty acids was evaluated by microscopy, since the insoluble substrates interfered with optical density (OD) measurement. Growth on other substrates was evaluated by an increase in OD_{610} relative to uninoculated controls. Growth curves were based on OD_{610}. Protein was quantified by the bicinchoninic acid method (15). Protein quantification was not affected by the presence of up to 150 mg of DhA per liter. Dry weights could not be used to determine yields because of interference by resin acid precipitates. Anaerobic cultures were performed with 10-ml volumes with a headspace of N_2 and were incubated stationary in 26-ml tubes sealed with serum bottle stoppers. Concentrations of substrates in anaerobic cultures were as follows (per liter): glucose, 0.90 g; pyruvate, 1.10 g; and sodium nitrate, 1.70 g. Oxidation of a battery of substrates was tested with GN MicroPlates (Biolog, Hayward, Calif.). *Zoogloea ramigera* ATCC 19544 was obtained from the American Type Culture Collection.

Cell suspensions. Cells for suspensions were grown to late exponential phase in 50-ml cultures incubated on a shaker. Cultures were harvested by centrifugation, and the cells were washed in an equal volume and suspended in a smaller volume of basal medium without trace elements or vitamins and with 50 μg of chloramphenicol per ml. Cell suspensions had an OD_{610} of 0.3 (14 to 20 mg of protein per ml). Killed cell suspensions were boiled for 10 min. Each treatment was repeated at least once in a replicate experiment, with results essentially the same as those shown.

Resin acid analysis. Resin acids were quantified by gas chromatography. Samples of 500 μl were taken from vortexed cultures. An internal standard of 12,14-dichloroabeitic acid was added to samples, which were then extracted twice with equal volumes of ethyl acetate. Resin acids were derivatized with diazomethane. The extracts were dried over anhydrous $NaSO_4$. For analysis, 1.0 μl of each extract was injected on a Hewlett-Packard 5890 gas chromatograph equipped with an autoinjector, an HP-5 column (25 m long by 0.32 mm in diameter with a 0.17-μm-thick solid phase), and a flame ionization detector. The injector and detector were set at 260 and 300°C, respectively. The temperature program was 2 min at 70°C, 30°C/min to 195°C, 0.6°C/min to 200°C, 20°C/min to 280°C, and 2 min at 280°C. The carrier was H_2 at a constant flow rate of 2.4 ml/min. Extraction efficiencies were 90 to 104%, based on at least triplicate determinations of each resin acid.

rRNA sequences. Nucleic acids were isolated from late-exponential-phase cultures by the lysis and extraction procedures of Giovannoni (6). Most of the small-subunit (ssu) rRNA gene was amplified from the extract by PCR, using the reagents and procedure of Gibco BRL Life Technologies, Inc., Gaithersburg, Md. The primers surrounded bases 27 to 1518 (6) and were synthesized by the Oligonucleotide Synthesis Laboratory, University of British Columbia. The PCR products were purified on spin columns (Qiagen, Inc., Chatsworth, Calif.). Partial sequences of the ssu rRNA genes were determined by the DNA Sequencing Laboratory, University of British Columbia, using AmpliTaq DyeDeoxy Terminator Cycle Sequencing and a model 373A automated DNA sequencer (Applied Biosystems). Primers for the sequencing reaction were the same as those used for PCR, so there were two sequences for each rRNA gene. Sequences were ranked for similarity (S_{ab}) with 2,251 other prokaryotic ssu rRNA sequences by using the electronic mail server of the Ribosomal Database Project (10).

Nucleotide sequence accession numbers. Partial sequences of the DhA-33 SSU rRNA gene were deposited in the GenBank database under accession numbers U22537 and U22538. Partial sequences of the DhA-35 SSU rRNA gene were deposited in the GenBank database under accession numbers U22534 and U22536.

RESULTS

Isolation of DhA degraders. With DhA as the sole organic substrate, 11 strains of aerobic, gram-negative bacteria were isolated from the SBR. These isolates were in two groups, distinguishable by colony morphology, cell morphology, and substrate range. Strains within each group were indistinguishable, with one exception noted below in the discussion of strain DhA-35. The two groups are represented by strains DhA-33 (three isolates) and DhA-35 (eight isolates). Both groups were obtained in serial enrichment cultures (three isolates of each group). Only the latter group was obtained by end point dilution of biomass from the SBR (five isolates), suggesting that it was the more abundant of the two groups. MPN analysis indicated that the total population in the SBR of organisms capable of growing on DhA was 1.1×10^6 propagules per ml (at the 95% confidence level, 0.4×10^6 to 3.0×10^6 propagules per ml).

Strain DhA-33. Cells of strain DhA-33 were motile and 1.0 to 2.4 μm by 0.7 μm (Fig. 2A). Colonies of DhA-33 were pale yellow, smooth, circular, and convex. In addition to using DhA, strain DhA-33 was able to use a variety of substrates likely to be present in the SBR influent, including wood sugars, fermentation products of such sugars, fatty acids, and other resin acids (Table 1). DhA-33 did not grow anaerobically on glucose, either fermentatively or with nitrate as an electron acceptor. DhA-33 grew at 30°C but not at 37°C. A 733-base partial sequence of the ssu rRNA sequence of DhA-33 was determined, including bases 30 to 448 and 1175 to 1521 (*E. coli*

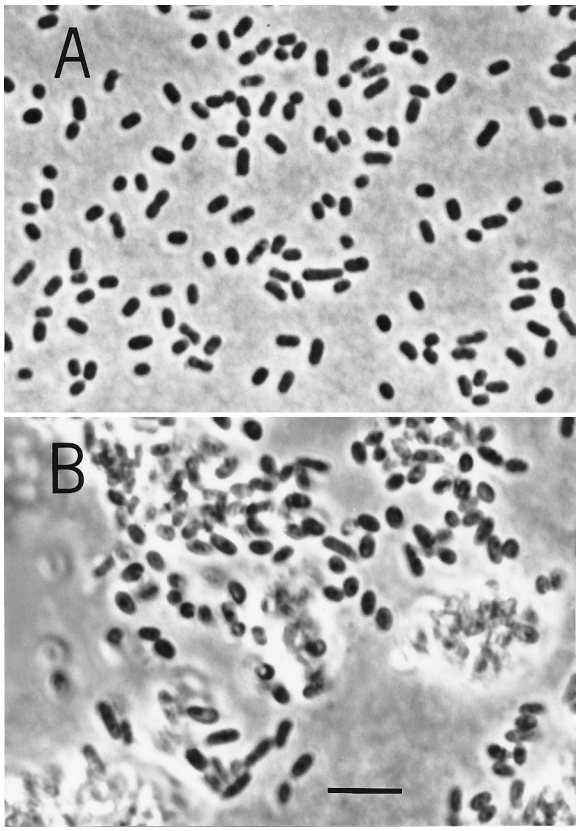

FIG. 2. Photomicrographs of strains DhA-33 (A) and DhA-35 (B) grown on 0.060 g of DhA per liter in 2.5 ml of liquid medium with stationary incubation. Bar, 5.0 μm.

TABLE 1. Substrate use by DhA degraders

Substrate	Concn (g/liter)	Substrate use by[a]:	
		DhA-33	DhA-35
Wood sugars			
Arabinose		O	NO
Cellobiose		O	NO
Galactose		O	NO
Glucose	1.0	G	NG
Glucuronate		NO	O
Xylose	1.0	G	(G)
Resin acids			
Abietic	0.060	G	G
Dehydroabietic	0.060	G	G
Isopimaric	0.060	NG	NG
Palustric	0.060	G	G
Pimaric	0.060	NG	NG
Fatty acids			
Linoleic	0.20	G	NG
Palmitic	0.20	G	G
Others			
Acetate	1.0	G	G
Benzoate	0.24	NG	G
Ethanol	1.0	G	G
Glycerol	1.0	NG	NG
Methanol	0.10	NG	NG
Pyruvate	1.0	(G)	G

[a] G, supports growth as sole organic substrate in 2.5-ml culture with stationary incubation; (G), supports poor growth; NG, does not support growth; O, oxidized in Biolog assay; NO, not oxidized.

numbering). This partial sequence was most similar to those of three *Sphingomonas* strains, i.e., *Sphingomonas* sp. strain SS86 (S_{ab} = 0.889), *S. yanoikuyae* GIFU 9882 (S_{ab} = 0.875), and *S. yanoikuyae* JCM 7371 (S_{ab} = 0.795).

Strain DhA-35. Cells of DhA-35 were motile and large, 1.5 to 2.8 μm by 1 μm (Fig. 2B). These cells grew in clumps bound by a profusion of exopolymer. Colonies of DhA-35 were white, smooth, punctiform, and convex, and they tenaciously stuck to the agar. Compared with DhA-33, DhA-35 oxidized or grew on fewer of the wood sugars and fatty acids likely to be present in the SBR influent (Table 1). DhA-35 grew anaerobically on pyruvate with nitrate as an electron acceptor; however, it did not grow fermentatively on pyruvate. DhA-35 grew on benzoate, turning the medium a pea-green color, suggesting aromatic degradation via the *meta*-cleavage pathway. DhA-35 grew at 30 and 37°C but not at 40°C. A 854-base partial sequence of the ssu rRNA sequence of DhA-33 was determined, including bases 28 to 429 and 1061 to 1519 (*E. coli* numbering). This partial sequence was most similar to those of three strains of *Z. ramigera*, ATCC 19544 (S_{ab} = 0.849), ATCC 19324 (S_{ab} = 0.808), and IAM 12136 (S_{ab} = 0.800). Strain ATCC 19544 is the type strain of *Zoogloea ramigera*. Unlike DhA-35, ATCC 19544 did not use any of the five resin acids listed in Table 1 as the sole organic substrate (it did grow on pyruvate in the same medium).

Not all of the isolates in the group represented by DhA-35 were identical, an example being strain DhA-39. DhA-39 resembled DhA-35 in morphology and in most physiological tests. The partial ssu rRNA sequences of the two strains were identical. However, DhA-39 was much less prone than DhA-35 to form clumps of cells during growth on liquid media. Also, unlike DhA-35, DhA-39 did not grow on benzoate.

Growth on DhA and cosubstrates. All 11 strains isolated on DhA were able to grow on the same range of resin acids. This specificity is exemplified by strains DhA-33 and DhA-35, which used AbA and palustric acid but not IpA or pimaric acid (Table 1). The growth yields of strains DhA-33 and DhA-35 were 0.30 and 0.25 g of protein per g of DhA, respectively (based on duplicate cell yields from at least four DhA concentrations). Assuming that the cells are 55% protein, these growth yields correspond to approximately 0.54 and 0.46 g of cell dry weight per g of DhA. Assuming that the cells are 60% carbon, the proportions of substrate carbon incorporated into biomass were 40 and 34%. The growth rates of DhA-33 and DhA-35 on DhA were similar (Fig. 3).

DhA-33 was grown on DhA, on DhA plus glucose, and on glucose (Fig. 3A). Cultures with DhA added had higher initial OD_{610} values because the insoluble DhA was in suspension. Growth of DhA-33 was faster on DhA (t_d, 2.7 h) than on glucose (t_d, 4.9 h). Together, the two substrates had a synergistic effect on DhA-33. Growth was fastest on both substrates (t_d, 1.8 h), and both substrates appeared to be used simultaneously. Accordingly, the presence of glucose as a cosubstrate increased the rate of DhA consumption by the culture (Fig. 3A).

DhA-35 was grown on DhA, on DhA plus pyruvate, and on pyruvate (Fig. 3B). The two strains were not tested with the same combination of cosubstrates because of their different substrate ranges. DhA-35 grew more slowly on DhA (t_d, 2.2 h) than on pyruvate (t_d, 0.96 h). Together, the two substrates had an antagonistic effect on DhA-35. Growth was slower on both substrates (t_d, 2.3 h) than on pyruvate alone; thus, the presence of DhA inhibited growth on pyruvate. Both substrates appeared to be used simultaneously, despite a slight discontinuity

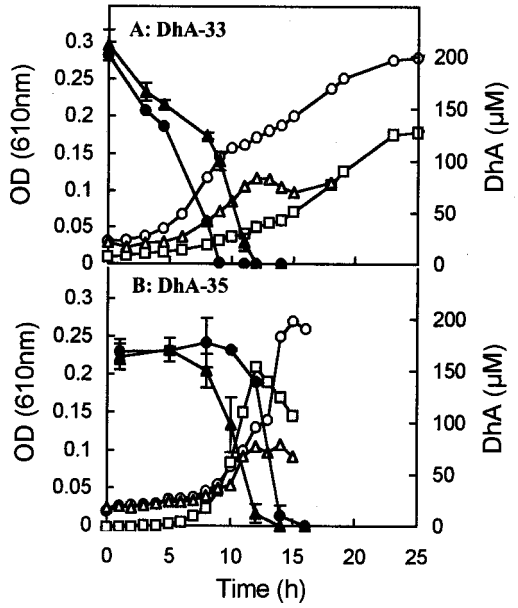

FIG. 3. Effects of cometabolites on growth and DhA removal in 14-ml cultures incubated on a tube roller. Open symbols, OD; solid symbols, DhA removal with standard deviation bars ($n = 3$). For strain DhA-33 (A), the inoculum was grown on glucose. Triangles, cultures with 0.060 g of DhA per liter only; squares, cultures with 0.30 g of glucose per liter only; circles, cultures with 0.060 g of DhA per liter and 0.30 g of glucose per liter. For strain DhA-35 (B), the inoculum was grown on pyruvate. Triangles, cultures with 0.060 g of DhA per liter only; squares, cultures with 0.30 g of pyruvate per liter only; circles, cultures with 0.060 g of DhA per liter and 0.30 g of pyruvate per liter.

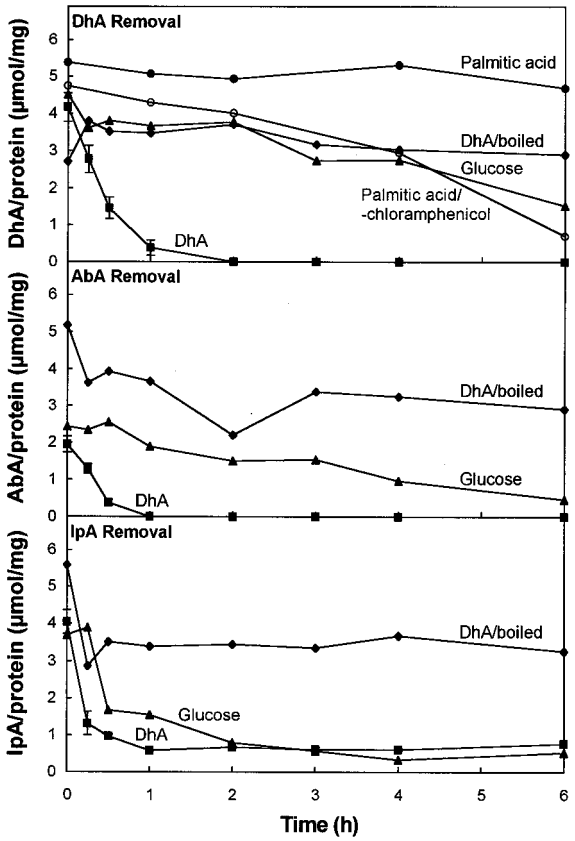

FIG. 4. Removal of three resin acids by cell suspensions of DhA-33 grown on 0.12 g of DhA per liter, 1.0 g of glucose per liter, or 0.20 g of palmitic acid per liter; 5- to 14-ml cell suspensions were incubated on a tube roller; $n = 1$ or, for DhA-grown cells, $n = 3$ (standard deviation bars are shown).

in the growth curve. The presence of pyruvate as a cosubstrate decreased the rate of DhA consumption by the culture (Fig. 3B). In this case (and not in the above case of DhA-33), it appears that the specific rate of DhA consumption was affected, since the higher consumption rate occurred in cultures with the lower biomass (measured as OD).

Resin acid degradation. DhA degradation was inducible and heat labile in both DhA-33 and DhA-35. Suspended cells of both strains, grown on DhA, had initially linear rates of DhA and AbA removal (Fig. 4 and 5). The DhA removal rate for both strains was 6 μmol mg of protein^{-1} h^{-1}. DhA-35 consumed AbA at the same rate, while DhA-33 removed AbA at 3 μmol mg of protein^{-1} h^{-1}. Resin acid removal was inhibited by boiling the cells, although these controls usually exhibited some initial removal. This initial removal was possibly due to sorption of resin acids to the culture tubes or irreversible sorption to the cells. Suspended cells of both strains grown on substrates other than DhA (glucose, pyruvate, or palmitic acid) were not induced for DhA or AbA degradation. In the absence of chloramphenicol, suspended cells of both strains, grown on palmitic acid, degraded DhA after a lag of approximately 2 h, indicating induction of DhA degradation activity during that period. The only degradation intermediate detected was 7-oxo-AbA, which appeared transiently in minor amounts when cell suspensions of either strain degraded DhA or AbA. The lack of biotransformation products and the relatively high growth yields of both strains on DhA (above) strongly suggest that DhA is mineralized by both strains.

In contrast to DhA and AbA, IpA was not completely removed by cell suspensions of DhA-33 or DhA-35 (Fig. 4 and 5). However, both strains partially removed IpA. No transformation products of IpA were observed by gas-liquid chromatography. Removal of IpA by DhA-33 was similar whether cells were grown on DhA or glucose but was reduced if the cells were boiled. These trends were less clear with DhA-35, despite repeated experiments. During growth on a cosubstrate (glucose or pyruvate), neither strain significantly removed IpA (data not shown). These results and the inability of the strains to grow on IpA are most consistent with a nondegradative mechanism of IpA removal in cell suspensions, such as sorption to the culture tubes or irreversible sorption to the cells.

DISCUSSION

The physiological characteristics of the two strains described above are generally consistent with the limited phylogenetic analysis of those strains. DhA-33 is most closely related to members of the genus *Sphingomonas*, which is in the alpha-4 subclass of the *Proteobacteria* (16). The physiological characteristics of DhA-33 determined in this study are consistent with those of *S. yanoikuyae* (19). It is not reported whether members of this genus can use abietanes as a substrate. DhA-35 is most closely related to *Z. ramigera*. Presently, *Z. ramigera* includes diverse strains in at least three distinct groups within both the alpha and beta subclasses of the *Proteobacteria* (14). DhA-35 is most closely related to the type strain, *Z. ramigera* ATCC 19544, and two other strains, all of which constitute one group in the beta subclass. The physiological characteristics of DhA-35 determined in this study are consistent with those of *Z. ramigera* ATCC 19544 (17). However, DhA-35 does differ from ATCC 19544 in its ability to use resin acids as organic

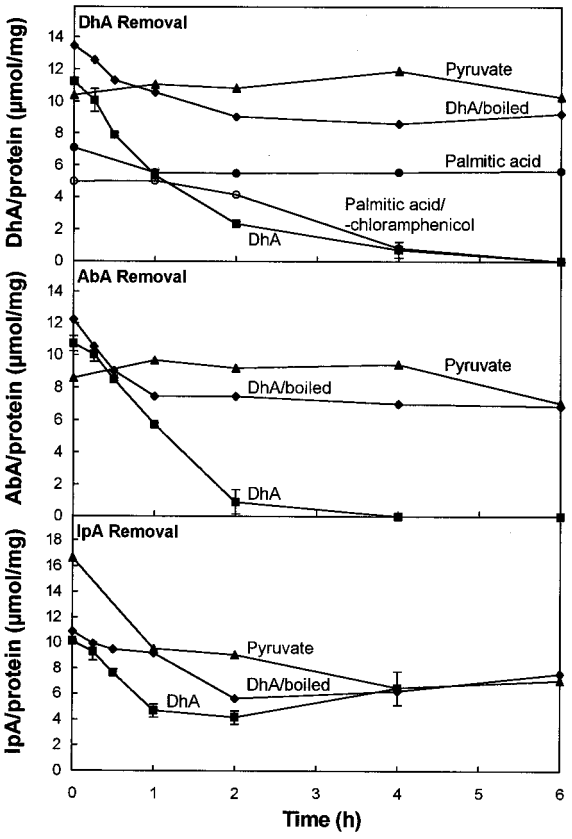

FIG. 5. Removal of three resin acids by cell suspensions of DhA-35 grown on 0.12 g of DhA per liter, 1.0 g of pyruvate per liter, or 0.20 g of palmitic acid per liter; 5- to 14-ml cell suspensions were incubated on a tube roller; $n = 1$ or, for DhA-grown cells, $n = 3$ (standard deviation bars are shown).

substrates. It will require further phylogenetic analysis to determine if either of the two strains described in this study constitute a new species.

Bacteria represented by strain DhA-35 are relatively abundant in the SBR and may account for DhA removal in the SBR. These were the only organisms detected in the most dilute positive tubes of the MPN estimate of organisms capable of growth on DhA. Bacteria represented by strain DhA-33 may also have a significant role in DhA removal; it is known only that this population is smaller than the former. Finally, one cannot rule out the possibility that other DhA-degrading organisms were not detected either because they cannot use DhA as a sole organic substrate (i.e., they require a cosubstrate) or because they were not selected by the isolation methods used. On the basis of measured DhA removal rates (Fig. 4 and 5), one can estimate the potential DhA degradation activity of the population detected by MPN analysis at approximately 3.5 μmol liter^{-1} h^{-1}, which is at the lower limit of that required to account for DhA removal observed in the SBR. This estimate assumes a cell mass of 10^{-11} g and a cell composition of 5% (wet weight) protein. This activity estimate is probably low, since the MPN analysis would tend to underestimate the population of floc-forming organisms such as DhA-35. Further evidence is required to confirm the role of the described bacteria in DhA removal in the SBR.

All isolates in this study were very similar with respect to resin acid degradation. The 11 isolates had the same specificity for resin acids, namely, abietanes and not pimaranes were used (Table 1; Fig. 4 and 5). This suggests that the substituents at C-13 of pimaranes (Fig. 1) inhibit degradative enzymes or prevent their induction. Previous reports of DhA-degrading organisms do not indicate the range of resin acids used; however, recent isolates from other habitats appear to have the same specificity for abietanes found in this study (1). This specificity suggests that in the SBR, at least two populations are responsible for resin acid removal. The apparent mineralization of DhA found in this study is consistent with the detection of ring cleavage products of DhA and other resin acids in previous studies with bacteria (1a, 2, 4) and is in contrast to results of studies with fungi (9).

Resin acid-degrading bacteria may also play a role in fatty acid degradation. The two groups of isolates in this study differed in the ability to use the major fatty acids in the influent of the SBR. Both used palmitic acid, but only the group represented by DhA-33 used linoleic acid (Table 1). Perhaps surprisingly, palmitic acid, but not linoleic acid, accumulated in the SBR (7). Thus, it appears that some factor in the SBR prevents degradation of palmitic acid by organisms capable of that process.

This study suggests that the complex organic composition of biological treatment system influents probably affects resin acid removal, not only by population selection but also by more direct effects on resin acid metabolism. The two strains described above have different substrate ranges. In addition to resin and fatty acids, DhA-33 may have used wood sugars likely to be present in the SBR influent, while DhA-35 could not have done so (Table 1). On the basis of the effect of glucose on DhA degradation by DhA-33 (Fig. 3A), it is likely that any wood sugars present in the SBR influent stimulated DhA degradation via an increase in the population of DhA-33. In contrast, DhA degradation by DhA-35 was inhibited by a cosubstrate, pyruvate (Fig. 3B). The relevance of this inhibition is difficult to interpret, because pyruvate would not be likely to be present in significant amounts in the SBR influent. The narrow substrate range of DhA-35 would tend to limit similar inhibition by other substrates. To better understand and control resin acid degradation in biological treatment systems, it would be useful to know more about the effects of complex substrate mixtures on resin acid metabolism.

The numerical dominance of organisms like DhA-35 in the SBR, relative to those like DhA-33, suggests that the former have a competitive advantage in that habitat. One possible explanation is the higher growth rate of DhA-35 on DhA. However, this advantage would tend to be offset by the higher growth yield of DhA-33 on DhA and the ability of DhA-33 to simultaneously use more substrates likely to be present in the SBR influent. Another factor possibly contributing to the competitiveness of DhA-35 is its ability to form flocs. Floc formation would be a selective advantage in an SBR system, since biomass must settle in order to be retained in the system. Suspended biomass would tend to have been lost, because the effluent was decanted and replaced with influent at the end of each 24-h operating cycle. Floc formation may make organisms like DhA-35 desirable from an engineering perspective, because many treatment systems require settling of biomass.

This study has characterized what appear to be the major DhA-degrading organisms in a biological treatment system. Ecological studies of this system and others are now required to determine how broadly the above conclusions can be applied. It would be of value to know whether the above resin acid degradation rates and specificities and the effects of cosubstrates on resin acid degradation are common to organisms in other systems. It would also be of value to know whether

these data can be used to predict the performance of the SBR used in this study and of other biological treatment systems.

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