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Isolation and Identification of Intestinal Steroid-Desulfating Bacteria from Rats and Humans

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We isolated 12 strictly anaerobic steroid-3-sulfate–desulfating strains from the intestinal floras of rats and humans. Two strains (S1 and S2) of the same atypical *Clostridium* species and an atypical *Lactobacillus* strain (termed R9) were obtained from rats. The human isolates were identified as *Eubacterium cylindroides* (two strains, H1 and H2), *Peptococcus niger* (two strains, H4 and H89), and *Clostridium clostridiiforme*. We also isolated, from different human fecal samples, four strains of phenotypically similar asaccharolytic *Bacteroides* strains, H6.2a, H6.2b, H65, and H175. Aryl steroid sulfatase activity for estrogen sulfates was present in all isolates. Alkyl steroid sulfatase activity for both 3α- and 3β-sulfates was found only in *P. niger* H4. The same *P. niger* strain and *Clostridium* strains S1 and S2 also possessed bile acid sulfatase activity.

Steroids excreted in the bile are to a large extent sulfo- or glucurono-conjugated. In the large intestine these conjugated steroids are almost completely deconjugated. Steroid-deglucuronidating activity is of both bacterial and mucosal origin (11); steroid sulfatase activity is exclusively of bacterial origin (1, 11). Deconjugation of these steroids decreases their water solubility and increases their lipid solubility, thus promoting enteric reabsorption and enterohepatic recirculation. It has been shown that modification of the intestinal microflora in humans and rats influences the enterohepatic circulation and in consequence the levels of certain steroid hormones in plasma (2, 4, 16, 20). The failure of contraceptive treatment in women receiving antibiotics was attributed to the suppression of the intestinal desulfating flora and the resulting interruption of the normal enterohepatic circulation (10, 17). At present, few data exist about the intestinal bacterial species that desulfate steroid sulfates. We have already described bile acid sulfatase activity in *Clostridium* strains S1 and S2 isolated from rats (15, 19) and steroid sulfatase activity in *Peptococcus niger* H4 isolated from the human intestinal flora (22). We now describe the steroid and bile acid sulfatase activities of these and several other recently isolated intestinal strains. However, we do not exclude the possibility that steroid-desulfating species other than those described here are present in the intestines.

Steroid-desulfating enzymes in humans and mollusks have been studied (7, 9). In humans they fulfill important roles in the intermediary metabolism and activation of steroid hormones (13). Deficiencies in steroid sulfatase activity lead to important pathological syndromes (21). The possibility of studying bacterial steroid sulfatase enzymes offered another incentive for the isolation of steroid sulfatase-producing bacteria.

MATERIALS AND METHODS

Isolation and identification procedures. Approximately 0.5 g of freshly voided human or rat fecal material or 0.5 ml of rat cecal content was transferred to an anaerobic glove box, suspended in tubes with 5 ml of different culture media supplemented with 50 μg of steroid sulfates or synthetic arylsulfates or with 20 μg of bile acid sulfates per ml, and incubated for 3 days.

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These mixed cultures were purified by sequential plating of sulfatase-positive cultures on solid media and isolation of different types of colonies. This procedure was repeated until pure cultures of sulfatase-positive strains were obtained. Identification was performed by the methods of Holdeman et al. (14).

Study of in vitro transformation. To study desulfation of steroid sulfate esters, we mixed 3 ml of liquid culture medium with 3 ml of a 20% NaCl solution and 50 μg of 5α-cholestane as an internal standard. This mixture was extracted three times with 5 ml of diethyl ether. The ether extracts were evaporated to dryness, and the reaction products were analyzed by gas-liquid chromatography on packed columns of 3% OV-1, 3% OV-17, or 1% QF-1 (Applied Science Europe BV, The Netherlands) at 240°C. The reaction products were acetylated by addition of 0.6 ml of dry pyridine-acetic anhydride (1:1, vol/vol). After 3 h at room temperature the resulting acetate esters were also analyzed by gas-liquid chromatography. The retention times of the nonacetylated and acetylated reaction products were compared with those of reference compounds, and mass spectrometry was performed to confirm identification. Mass spectra were recorded on a single-focusing AEI MS-12 mass spectrometer (Associated Electrical Industries, Ltd., Manchester, United Kingdom) coupled to a Pye-Unicam series 304 chromatograph.

To study bile acid sulfate hydrolysis, we extracted 3 ml of grown culture twice with 8 ml of diethyl ether after the culture had been mixed with 50 μg of 23-nordeoxycholic acid and acidified to pH 4 with 4 ml of buffered salt solution (10% NaCl plus 10% KH_2PO_4 in H_2O). The extract was washed with 7 ml of H_2O and evaporated, the resulting bile acids being converted to their methyl ester acetates for identification and quantification by gas-liquid chromatography (19). Identification was carried out by comparing relative retention times of the derivatized bile acids with those of reference compounds on columns of 3% OV-1 (268°C), 3% OV-17 (268°C), or 1% QF-1 (240°C).

To determine the desulfation of *p*-nitrophenyl sulfate, *p*-nitrocatechol sulfate, and phenolphthalein disulfate, we centrifuged 5 ml of grown culture for 15 min at 3,000 × *g* and combined 3 ml of supernatant with 3 ml of 1 N NaOH. Absorption was measured spectrophotometrically at 420 nm

for *p*-nitrophenol, 515 nm for *p*-nitrocatechol, and 560 nm for phenolphthalein.

Media and products used. All experiments were carried out at 37°C in an anaerobic glove box of the type described by Aranki et al. (3). The atmosphere consisted of a mixture of 80% N_2, 10% H_2, and 10% CO_2. Most experiments were carried out on tryptic soy broth (Bio-Mérieux, France) supplemented with 0.1 or 0.2% taurine (2-aminoethanesulfonic acid [Fluka AG, Buchs SG, Switzerland]); on Columbia broth (GIBCO, Bio-Cult Ltd., Paisley, U.K.) supplemented with 0.025% $MgSO_4$, 0.001% hemin, 0.0001% vitamin K_1 (Konakion; Roche, Basel, Switzerland), and 0.1 or 0.2% taurine; or, finally, on brain heart infusion medium (BBL Microbiology Systems, Cockeysville, Md.) supplemented with 0.001% hemin, 0.0001% vitamin K_1, and 0.5% yeast extract. Unconjugated steroid reference compounds and steroid sulfates were obtained from Sigma Chemical Co., St. Louis, Mo., and Steraloids Inc., Wilton, N.H. *p*-Nitrophenyl sulfate, *p*-nitrocatechol sulfate, and phenolphthalein disulfate were from Aldrich Chemical Co., Inc., Milwaukee, Wis., and EGA-Chemie, Steinheim, Federal Republic of Germany. Bile acids and bile acid sulfates were prepared in our laboratory as described previously (19).

The identity of the reference substances was confirmed by gas-liquid chromatography–mass spectrometry. To check for purity and stability of the reference compounds and for absence of impurities interfering with the gas-liquid chromatography analysis, we incubated solutions of reference substances, uninoculated culture media containing the substrates under investigation, and cultures on substrate-free medium and analyzed them under the same conditions as for the experimental samples. No product or culture medium contained more than 10% of unidentified impurities or a peak with the retention time of the metabolites under investigation.

RESULTS

Isolation and identification. Three strictly anaerobic steroid-desulfating bacterial strains were isolated from rat cecal and fecal material. Two of these were isolated on media supplemented with bile acid-3-sulfates and were phenotypically similar strains of the same unnamed *Clostridium* species, termed *Clostridium* strain S1 and *Clostridium* strain S2 (15, 19). The atypical *Lactobacillus* strain R9 was isolated for its triiodothyronine-desulfating activity (strain kindly supplied by M. P. Hazenberg, Erasmus University, Rotterdam, The Netherlands) (18).

Nine strictly anaerobic steroid-desulfating bacterial strains were isolated from human fecal material. One strain was isolated on culture media supplemented with dehydroisoandrosterone sulfate and identified as *P. niger* H4. Four strains were isolated on media supplemented with estrone sulfate and identified as *Eubacterium cylindroides* H1 and H2, *P. niger* H89, and an asaccharolytic *Bacteroides* strain termed H65. Four strains were isolated for their phenolphthalein-desulfating activity: one *Clostridium clostridiiforme* strain and three asaccharolytic *Bacteroides* strains termed H6.2a, H6.2b, and H175. Although the asaccharolytic *Bacteroides* strains H65, H6.2a, H6.2b, and H175 all came from different fecal samples, were isolated on culture media supplemented with different sulfate esters, and had different sulfatase activities, no differences were found in their biochemical and morphological characteristics. Therefore, we consider these strains to belong to the same unnamed *Bacteroides* species. This also applies to *P. niger* H4 and H89 and the *P. niger* type strain, DSM 20475 or ATCC 27731. The most important morphological and biochemical characteristics of all these bacteria are summarized in Table 1.

On the basis of their biochemical characteristics, we could distinguish between two groups of steroid-desulfating strains. The first group (*Clostridium* strains S1 and S2; *P. niger* H4, H89, and DSM 20475; and *Bacteroides* strains H6.2a, H6.2b, H65, and H175) contained isolates that did not ferment sugars, whose growth was stimulated by taurine, and that produced large amounts of H_2S. The second group, (*E. cylindroides* H1 and H2 and *C. clostridiiforme*) contained strains that fermented sugars, that were not stimulated in their growth by taurine, and that did not produce H_2S or produced it only in small amounts. *Lactobacillus* strain R9 did not ferment sugars and was not stimulated by taurine, but it produced copious amounts of H_2S.

The growth-stimulating effect of taurine was a very specific characteristic. Growth of *Clostridium* strains S1 and S2 in liquid media was visible only after the addition of 8 mM taurine. For the other strains, e.g., *P. niger* H4, growth as measured by optical density and cell count increased at least threefold after the addition of 8 mM taurine. Moreover, the growth-stimulating effect was closely linked to the molecular structure of taurine. We checked the effects on bacterial growth of several molecules that structurally resemble taurine (i.e., cysteine amine, cysteine, cysteic acid, β-alanine, methionine, tyramine, arginine, serine, aminomethanesulfonic acid, aminosulfonic acid, methanesulfonic acid, ethanesulfonic acid, propanesulfonic acid, pentanesulfonic acid, ethanolamine, and ethylamine) and found that none of these molecules stimulated growth. We observed that the growth of all the taurine-stimulated strains was, albeit to a lesser degree, enhanced by inorganic sulfite, by steroid sulfate esters, and, for *Bacteroides* strains H65, H175, H6.2a, and H6.2b, also by thiosulfate; the addition of taurine, sulfite, thiosulfate, or steroid sulfates to the culture media of these strains led to copious production of H_2S (Table 2). Strains that were not stimulated by taurine produced either no H_2S at all (*E. cylindroides* H1 and H2) or either small or large quantities (*C. clostridiiforme* and *Lactobacillus* strain R9, respectively) from sulfite or thiosulfate but not from taurine. We presume, therefore, that the structure of taurine is such that its sulfonic acid group can very easily be metabolized by these taurine-stimulated bacteria, which use it as an electron acceptor. The observation that other molecules with a slightly different structure but also having a sulfonic acid group (cysteic acid, aminomethanesulfonic acid, aminosulfonic acid, methanesulfonic acid, ethanesulfonic acid, propanesulfonic acid, and pentanesulfonic acid) were totally without effect stresses the uniqueness of taurine.

Although these steroid-desulfating strains, except *E. cylindroides* H1 and H2, reduced inorganic sulfite or thiosulfate to H_2S, they were all unable to reduce inorganic sulfate. Therefore, they differ from the group of sulfate-reducing bacteria, but they are physiologically related to a small number of dissimilatory sulfite-reducing bacteria that have been described previously (5).

We also observed that these taurine-stimulated bacteria deconjugated taurine-conjugated bile acids. Dietary taurine, together with taurine from the bile acids, would thus provide a source of taurine for the microflora in the intestinal tract of the host.

Desulfating activity. Desulfation in growing cultures was measured after 3 days of incubation, and the results are summarized in Table 3. All strains possessed arylsulfatase

TABLE 1. Morphological and biochemical characteristics of steroid-desulfating strains

Strain[a]	Source[b]	Morphology[c]	Size (μm)	Gram stain	Motility	Spore formation[d]	Growth[e] on: PYG	PYG-bile	PYG-taurine	PYG-pyruvate	Fermentation[f] of: Arabinose	Cellobiose	Esculin (pH)	Fructose	Galactose	Glucose	Maltose	Mannose	Melibiose	Raffinose	Salicin	Sucrose	Trehalose	Xylose	Esculin hydrolase	Starch hydrolase	Gelatinase	Lecithinase	Urease	Catalase	Indole production	Nitrate reduction[g]	H_2S production	NH_3 production	Fatty acids from PYG or BHI[h]
Clostridium strain S1	RF	SR	3–6 by 0.5	–	–	+ (ST)	<0.01	0	0.35	0.01	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	+	+	+	A
Clostridium strain S2	RF	SR	1.5–2.5 by 0.5	–	–	+ (ST)	<0.01	0	0.25	0.02	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	+	+	+	A
Lactobacillus strain R9	RC	CR	3–6 by 0.5	+	–	–	0.13	0.24	0.15	0.02	–	w	a	a	–	a	–	a	–	–	–	–	–	–	+	–	±	–	–	–	–	–	+	–	a, l
E. cylindroides H1	HF	SR	2.5 by 0.5	+	–	–	0.63	0.54	0.66	0.29	–	w	a	a	–	a	–	a	–	w	a	a	–	–	+	–	–	–	–	–	–	–	–	–	b, l
E. cylindroides H2	HF	SR	2.5 by 0.5	+	–	–	0.75	1.05	0.76	0.42	–	w	a	a	–	a	–	a	–	a	a	a	–	–	+	–	–	–	–	–	–	–	–	–	b, l
P. niger H4	HF	C	1.5	+	–	–	0.13	0.14	0.66	0.20	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	+	–	–	+	+	A, iB, B, iV, c, p
P. niger H89	HF	C	1.5	+	–	–	0.07	0.08	0.41	0.29	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	+	–	+	+	+	A, iB, B, iV, c, p
Bacteroides strain H65	HF	CB	2–3 by 1	–	–	–	0.05	0.62	0.57	0.41	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	+	–	+ (NO_2^-)	+	+	AV
Bacteroides strain H6.2a	HF	CB	2–3 by 1	–	–	–	0.09	0.70	0.60	0.42	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	+	–	+ (NO_2^-)	+	+	A
Bacteroides strain H6.2b	HF	CB	2–3 by 1	–	–	–	0.06	0.70	0.60	0.42	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	+	–	+ (NO_2^-)	+	+	A
Bacteroides strain H175	HF	CB	2–3 by 1	–	–	–	0.05	0.68	0.58	0.40	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	+	–	+ (NO_2^-)	+	+	A
C. clostridiiforme	HF	SR	5–10 by 1	–	+	+ (ST)	0.95	>1.5	0.90	>1.2	w	–	–	a	a	a	a	a	w	–	a	a	w	w	+	–	–	–	–	–	+	+ (NO_2^-)	±	+	A

[a] Strains were identified as described by Holdeman et al. (14).
[b] Abbreviations: RF, rat feces; RC, rat cecal content; HF, human feces.
[c] Abbreviations: SR, straight rod; CR, curved rod; C, coccus; CB, coccobacillus.
[d] ST, Subterminal location of spores.
[e] Growth measured as optical density at 520 nm. Abbreviations: PYG-bile, PYG plus 2% bile; PYG-taurine, PYG plus 8 mM taurine; PYG-pyruvate, PYG plus 10 mM pyruvate.
[f] Abbreviations: a, acid; w, weakly acid; –, none. The following were not fermented by any of the strains: adonitol, erythritol, glycogen, inositol, lactose, mannitol, melezitose, rhamnose, ribose, sorbitol, and starch (pH).
[g] + (NO_2^-), Reduction to nitrite only.
[h] Abbreviations: a, acetic acid; l, lactic acid; b, butyric acid; ib, isobutyric acid; iv, isovaleric acid; c, caproic acid; p, phenylacetic acid. Lowercase and capital letters indicate that small and large quantities, respectively, were produced.

TABLE 2. H_2S production by the steroid-desulfating strains after addition of sulfur-containing compounds

Compound (concn)	H_2S production[a] by following strains:												
	Clostridium		*Lactobacillus* strain R9	*E. cylindroides*		*P. niger*			*C. clostridiiforme*	*Bacteroides*			
	Strain S1	Strain S2		H1	H2	H4	DSM 20475	H89		Strain H6.2a	Strain H6.2b	Strain H65	Strain H175
Na_2SO_4 (20 mM)	−	−	−	−	−	−	−	−	−	−	−	−	−
Na_2SO_3 (3 mM)	++	++	++	−	−	++	++	++	−	++	++	++	++
$Na_2S_2O_3$ (3 mM)	−	−	++	−	−	−	−	−	+	++	++	++	++
Taurine (10 mM)	++	++	−	−	−	++	++	++	−	++	++	++	++
Cysteine (10 mM)	−	−	−	−	−	−	−	−	−	−	−	−	−
Methionine (10 mM)	−	−	−	−	−	−	−	−	−	−	−	−	−
Estrone sulfate (0.3 mM)	+	+	+	−	−	+	+	+	−	+	+	±	±

[a] Symbols: −, none; +, moderate; ++, abundant.

activity for estrogen sulfates. There were marked differences, however, in the hydrolysis of chromogenic arylsulfates by the different strains. Of the estrogen sulfates, estrone sulfate was always the most completely desulfated, estradiol-3-sulfate was less extensively desulfated, and only trace amounts of desulfated estriol were found. Estradiol-17β-sulfate was never hydrolyzed.

Three strains possessed steroid alkylsulfatase activity. *Clostridium* strain S1 desulfated 3α-sulfates of 5β-bile acids and 3β-sulfates of 5α- and 5β-bile acids, and *Clostridium* strain S2 desulfated 3α-sulfates of 5α- and 5β-bile acids. They did not hydrolyze androstane- or pregnane-3-sulfates.

P. niger H4 desulfated androstane- and pregnane-3-sulfates as well as bile acid-3-sulfates. Androstane- and pregnane-3-sulfate esters were desulfated to the greatest extent; bile acid-3-sulfates were less suitable as substrates, and 3α-sulfated bile acids with two and three hydroxyl groups were desulfated only when an androstane-3-sulfate with an identical configuration of the sulfate group and acting as an enzyme inducer was added to the culture medium. Bile acids sulfated at the 7- or 12-hydroxyl group and testosterone-17-sulfate were not desulfated.

The *P. niger* type strain (DSM 20475, ATCC 27731) also possessed sulfatase activity. The substrate specificity of its sulfatase activity was identical to that of *P. niger* H4, isolated from the human intestinal flora, except that the type strain did not desulfate any of the 3α-sulfate esters of androstanes or bile acids. *P. niger* H89, however, did not show alkylsulfatase activity and was active only on estrogen sulfates and phenolphthalein disulfate.

For the majority of steroid-desulfating strains (*Clostridium* strains S1 and S2; *P. niger* H4, H89, and DSM 20475; *Bacteroides* strains H6.2a and H6.2b; and *Lactobacillus* strain R9), desulfation of estrone sulfate clearly started during the exponential growth phase. Moreover, the desulfation rate was sufficiently high for the estrone sulfate to be completely desulfated within the exponential growth phase, except for strains H6.2a and H6.2b. The desulfation rate of these strains was lower, and desulfation continued well into the stationary growth phase. For strains H65 and H175 the desulfation rate was too low to observe whether desulfation had already started during the exponential growth phase.

E. cylindroides H1 and H2 and the *C. clostridiiforme* strain were different from the other strains in that desulfation

TABLE 3. Substrate specificity of steroid-desulfating strains

Substrate	Substrate specificity[a] of following strains:												
	Clostridium		*Lactobacillus* strain R9	*E. cylindroides*		*P. niger*			*C. clostridiiforme*	*Bacteroides*			
	Strain S1	Strain S2		H1	H2	H4	DSM 20475	H89		Strain H6.2a	Strain H6.2b	Strain H65	Strain H175
Arylsulfates													
Estrone-3-sulfate	+++	+++	+++	++	++	+++	+++	+++	++	+++	+	+	+
β-Estradiol-3-sulfate	+++	+++	+	+	+	+++	+++	+++	++	+	+	+	+
Estriol-3-sulfate	Tr	Tr	Tr	Tr	Tr	Tr	Tr	Tr	Tr	Tr	Tr	Tr	Tr
β-Estradiol-17-sulfate	−	−	−	−	−	−	−	−	−	−	−	−	−
p-Nitrocatechol sulfate	++	+++	+	++	Tr	++	++	−	−	−	−	−	−
p-Nitrophenyl sulfate	++	+++	−	+	+	++	++	−	−	−	−	−	−
Phenolphthalein disulfate	+	+++	−	+	Tr	++	++	++	++	+++	+	−	+
Alkylsulfates													
3α-Sulfate of 5α/5β-androstane	−	−	−	−	−	+++	−	−	−	−	−	−	−
3β-Sulfate of 5α,5β/Δ5-androsta(e)ne	−	−	−	−	−	+++	+++	−	−	−	−	−	−
3β-Sulfate of 5α/ 5-pregnane	−	−	−	−	−	+++	+++	−	−	−	−	−	−
3α-Sulfate of 5α-bile acids	−	+++	−	−	−	++	−	−	−	−	−	−	−
3α-Sulfate of 5β-bile acids	+++	+++	−	−	−	++	−	−	−	−	−	−	−
3β-Sulfate of 5α-bile acids	+++	−	−	−	−	++	++	−	−	−	−	−	−
3β-Sulfate of 5β-bile acids	++	−	−	−	−	++	++	−	−	−	−	−	−

[a] Percent desulfation after 3 days of incubation: +++, more than 75%; ++, more than 25%; +, less than 25%; Tr, less than 5%; −, none.

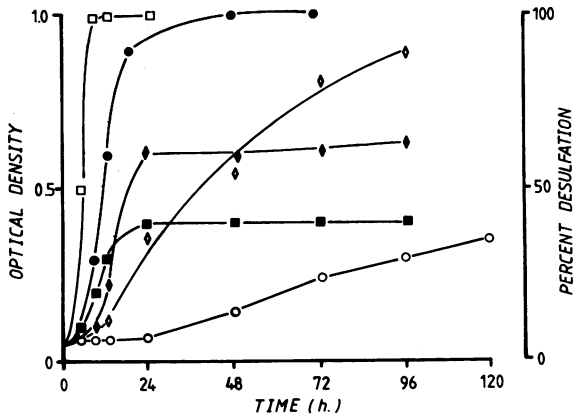

FIG. 1. Desulfation of estrone sulfate (50 μg of estrone sulfate per ml of culture medium) in relation to growth (measured as the optical density of cultures at 520 nm). Symbols: □, desulfation by *Clostridium* strain S2; ■, optical density of *Clostridium* strain S2 cultures; ○, desulfation by *E. cylindroides* H2; ●, optical density of *E. cylindroides* H2 cultures; ◇, desulfation by *Bacteroides* strain H6.2a; ◆, optical density of *Bacteroides* strain H6.2a cultures.

started during the stationary growth phase. The desulfation rate was comparable to that of strains H6.2a and H6.2b. Consequently, desulfation by strains H6.2a, H6.2b, H175, and H65, *E. cylindroides* H1 and H2, and *C. clostridiiforme* still continued after 3 days of incubation, and longer incubation times would therefore give higher desulfation values.

The alkylsteroid- and bile acid-desulfating activity of *P. niger* H4 and DSM 20475 and *Clostridium* strains S1 and S2 also started during the exponential growth phase. Desulfation rates were high, and desulfation of the added steroid or bile acid sulfate was complete within 20 h (Fig. 1).

DISCUSSION

The bacteria described in this paper are the first intestinal steroid-desulfating strains to be isolated. They were all strict anaerobes that belonged to the dominant or subdominant intestinal flora. The iodothyronine-desulfating strains isolated from the intestinal floras of rats and humans were also strict anaerobes (8, 18). Except for the *E. cylindroides* strains (data not shown), the presence of sulfate or cysteine in the culture medium did not influence sulfatase activity, although the presence of sulfur in the growth medium has been suggested as a possible cause of the lack of success in earlier isolation attempts (6).

The growth stimulation by taurine, inorganic sulfite, and sulfate esters, the copious production of H_2S from these molecules, and the lack of sulfatase-repressing activity of cysteine in the group of taurine-stimulated strains would suggest that the sulfatase enzymes provide reducible sulfur that can act as an electron acceptor in these strictly anaerobic strains. Although these bacteria reduce inorganic sulfite, they do not reduce inorganic sulfate and therefore do not belong to the group of sulfate-reducing bacteria. Consequently, if indeed the steroid sulfates act as electron sinks, it seems unlikely that the sulfatase activity of the steroid-desulfating strains would lead to the liberation of inorganic sulfate. So far, eight bacterial strains have been described that reduce inorganic sulfite but not inorganic sulfate in a dissimilatory way to H_2S (5). Whether these strains also metabolized taurine or produced sulfatase enzymes was not reported.

The lack of H_2S production, the sulfatase-repressing activity of cysteine, and the fact that the sulfatase activity is not linked to growth suggest that in the *E. cylindroides* strains that are not stimulated by taurine, the sulfatase enzymes provide sulfur for assimilatory pathways. Within the group of taurine-stimulated bacteria, the majority of the strains desulfated estrone sulfate quickly, completely, and during the exponential growth phase; the *E. cylindroides* strains, however, desulfated estrone sulfate slowly and during the stationary growth phase. This, too, may reflect a different metabolic fate for the liberated sulfur.

Until now, only steroid sulfatase enzymes from humans and mollusks have been studied. Our results indicate the presence of a wide variety of different bacterial sulfatase enzymes. Bacterial steroid sulfatase enzymes can apparently be divided into two major groups. Arylsulfatase enzymes desulfate steroids with an aromatic A ring, such as estrogen sulfates, and are, according to our results, more common in intestinal microorganisms than the group of alkylsulfatase enzymes, which desulfate steroids with a saturated A ring, such as androstanes, pregnanes, and bile acids. However, even within these two groups the different substrate specificies and relative activities suggest major differences. The absence of 3α-alkylsteroid-desulfating activity in the *P. niger* type strain as opposed to the 3α-alkylsteroid sulfatase activity in *P. niger* H4 suggests different 3α- and 3β-alkylsteroid-desulfating enzymes. These enzymes again seem to be different from the alkyl-desulfating enzymes of *Clostridium* strains S1 and S2, which are specific for bile acid sulfates.

The isolation of these steroid-desulfating strains offers the possibility of studying these steroid-desulfating enzymes and their role in bacterial metabolism more thoroughly. It also makes it possible, e.g., by the use of gnotobiotic rats, to determine more accurately their role in the enterohepatic circulation and overall metabolism of steroids (12, 23).

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LITERATURE CITED

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