

Identification and Functional Characterization of Arylamine *N*-Acetyltransferases in Eubacteria: Evidence for Highly Selective Acetylation of 5-Aminosalicylic Acid

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Arylamine *N*-acetyltransferase activity has been described in various bacterial species. Bacterial *N*-acetyltransferases, including those from bacteria of the gut flora, may be involved in the metabolism of xenobiotics, thereby exerting physiopathological effects. We characterized these enzymes further by steady-state kinetics, time-dependent inhibition, and DNA hybridization in 40 species, mostly from the human intestinal microflora. We report for the first time *N*-acetyltransferase activity in 11 species of *Proteobacteriaceae* from seven genera: *Citrobacter amalonaticus*, *Citrobacter farmeri*, *Citrobacter freundii*, *Klebsiella ozaenae*, *Klebsiella oxytoca*, *Klebsiella rhinoscleromatis*, *Morganella morganii*, *Serratia marcescens*, *Shigella flexneri*, *Plesiomonas shigelloides*, and *Vibrio cholerae*. We estimated apparent kinetic parameters and found that 5-aminosalicylic acid, a compound efficient in the treatment of inflammatory bowel diseases, was acetylated with a catalytic efficiency 27 to 645 times higher than that for its isomer, 4-aminosalicylic acid. In contrast, *para*-aminobenzoic acid, a folate precursor in bacteria, was poorly acetylated. Of the wild-type strains studied, *Pseudomonas aeruginosa* was the best acetylator in terms of both substrate spectrum and catalytic efficiency. DNA hybridization with a *Salmonella enterica* serovar Typhimurium-derived probe suggested the presence of this enzyme in eight proteobacterial and four gram-positive species. Molecular aspects together with the kinetic data suggest distinct functional features for this class of microbial enzymes.

The acetyl coenzyme A (AcCoA):arylamine *N*-acetyltransferases (NAT; EC 2.3.1.5.) catalyze the transfer of an acetyl group from AcCoA to the nitrogen or oxygen atom of primary arylamines, hydrazines, and their *N*-hydroxylated metabolites. They therefore play important roles in both the detoxification and metabolic activation of numerous xenobiotics. In the *Salmonella enterica* serovar Typhimurium mutation assays developed by Ames and coworkers (2), the carcinogenic aromatic amines are *N*-hydroxylated and subsequently *O*-acetylated before they can exert genotoxic properties. The *O*-acetylation step is mediated by the NAT of *S. enterica* serovar Typhimurium, which therefore governs the activity of many pro-mutagens through *N*-hydroxyarylamine *O*-acetyltransferase activity (26).

NAT isoforms (28) have been detected in several vertebrate species, including human (6), rabbit (53), rat (21), mouse (37), hamster (23), and chicken (47). Partial data are also available for amphibian (30), helminth (12, 16), and insect (66) species. In prokaryotes, NATs were first described in *S. enterica* serovar Typhimurium (65) and then in several species of facultative and obligate anaerobes of the dog and human intestinal microflora. NAT activity has been reported in *Escherichia coli*, *Bacteroides vulgatus*, *Clostridium sporogenes*, *Lactobacillus bifidus*, *Proteus vulgaris*, *Pseudomonas fluorescens*, *Enterococcus*

faecalis (48), *Staphylococcus aureus* (9), *Helicobacter pylori* (18), *Klebsiella pneumoniae* (32), *Aeromonas hydrophila* (15), *Enterobacter aerogenes* (61), *Pseudomonas aeruginosa* (33), *Lactobacillus acidophilus* (11), *Citrobacter koseri* (39), and *Shigella sonnei* (62). However, NAT genes have been cloned and their expression products characterized only for *S. enterica* serovar Typhimurium (57, 65), *Mycobacterium smegmatis*, and *Mycobacterium tuberculosis* (49).

The aminosalicilate isomers 5-aminosalicylic acid (5-ASA) and 4-aminosalicylic acid (4-ASA), both arylamine acceptor substrates of human NATs, are used to treat inflammatory bowel diseases such as ulcerative colitis and Crohn's disease (55, 59). 5-ASA is considered one of the most efficient therapies for inducing and maintaining remission in such diseases (50, 67). However, its use is associated with adverse side effects such as allergic rash, pancreatitis, nephropathy, and hepatitis (41). *N*-Acetylation activity for 5-ASA has been reported in both aerobic and anaerobic intestinal microflora in human (63). NATs from the bacteria of the intestinal microflora may therefore affect both the efficacy and side effects of this drug.

The catalytic residues of NATs from eukaryotic and prokaryotic species may be identical. Thus, the Cys⁶⁹ residue in the NAT sequence of *S. enterica* serovar Typhimurium has been shown to be crucial for enzyme activity (65), and the homologous Cys⁶⁸ in humans probably plays a similar role (22). The recent structure determination of *S. enterica* serovar Typhimurium NAT has revealed that the activation of the active-site cysteine requires the presence of a Cys⁶⁹-His¹⁰⁷-Asp¹²² catalytic triad (58). In addition, it has been suggested

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that basic residues highly conserved in all NAT sequences, at positions corresponding to Arg⁹ and Arg⁶⁴ in the human sequences, contribute to conformational stability and are involved in the enzyme-substrate interactions of the human isoenzymes, NAT1 and NAT2 (20). It has also been suggested that amino acids 125 and 127 are important determinants of NAT1-type and NAT2-type acceptor substrate selectivity (24). Extending the study of conserved residues to NATs from various bacterial species could therefore provide new insight into the functional specificities of this class of microbial enzymes.

As NATs in the bacteria of the gut microflora may be involved in the activation of carcinogens and the metabolism of drugs, we used functional and molecular approaches in an attempt to further identify and characterize this class of enzymes. We experimentally studied 40 different bacterial species, most of which were indigenous to the human gut. These species belonged to 30 genera from four different taxonomic groups: gram-positive bacteria with high and low G+C contents, *Bacteroides*, and *Proteobacteria*. The *Proteobacteria* studied included the *Enterobacteriaceae*, *Vibrionaceae*, and *Pasteurellaceae* families. We characterized bacterial NATs biochemically and compared them by determining their reaction spectra with arylamine substrates known to be specific for the human NAT1 or NAT2 isoform. We studied these enzymes kinetically using aminosalicylate substrates. We also found some features that were clearly common and specific to bacterial NATs, including a higher level of activity for 5-ASA than for its isomer, 4-ASA.

MATERIALS AND METHODS

Chemicals. 5-ASA was purchased from Acros Organics. 4-ASA, *p*-aminobenzoic acid (PABA), 2-aminofluorene (2-AF), sulfamethazine (SMZ), procainamide (PA), iodoacetamide, AcCoA, and the AcCoA-regenerating system D,L-acetylcarnitine and carnitine acetyltransferase were all obtained from Sigma Chemical Co. All other chemicals were of the highest purity commercially available.

Bacterial strains and growth conditions. Two types of strains were studied. First, six control strains were tested, including the recombinant *Escherichia coli* strains DMG100 and DMG200 (22), which overproduce the human NAT1 and NAT2 isoforms, respectively, and four *S. enterica* serovar Typhimurium tester strains originally constructed for genotoxicity quantitation assays. Of these strains, YG1024 (64) overexpresses multiple copies of the NAT gene cloned from the TA1538 strain (40), whereas the TA98 1,8/DNP₆ strain (43) produces no functionally active NAT. The TA98 strain (40) differs from TA1538 only in its greater sensitivity to mutagenesis. Second, the species listed in Table 1 were also studied. *H. pylori* was cultured for 3 days on selective or sheep blood agar plates (BioMérieux) in microaerobiosis conditions using an Oxoid Unipath jar with a Campyack Plus microaerophilic generator (Becton Dickinson). All other strains were cultured at 37°C until the exponential growth phase, using a two-step procedure: 100 ml of medium was inoculated using 10 ml of an 18-h culture and incubated for 3 h with shaking (obligate and facultative aerobes) or for 72 h without shaking (obligate anaerobes). Bacteria were grown in Trypticase-yeast-glucose-cysteine or Rosenow broth (*Bacteroides* sp.), from Sanofi Diagnostics Pasteur or in Luria-Bertani medium from Difco Laboratories (all other strains). The identity of the wild-type strains in each frozen glycerol stab was routinely checked using API20E and/or ID32GN (for *Enterobacteriaceae*, *Aeromonas hydrophila*, *Plesiomonas shigelloides*, *Vibrio cholerae*, and *P. aeruginosa*), API20NE (for *Alcaligenes xylosoxidans*), or Rapid ID32A (for *Bacteroides* sp.) identification kits, with the ATB automated expression system (BioMérieux). The purity of working cultures obtained from these stabs was checked in each experiment by examining the morphology of colonies from solid-medium cultures.

Enzyme assays. Bacterial cells were harvested by centrifugation (2,500 × g, 20 min, 4°C) and resuspended in 2 ml of Tris (pH 7.4)-EDTA-dithiothreitol-KCl buffer (22). The cells were lysed by sonication on ice twice for 30 s each, with a 30-s interval. The insoluble fraction was removed by centrifugation (20,000 × g, 5 min, 4°C), and the resulting supernatant was used for NAT activity assays

TABLE 1. Bacterial species studied

Species	Strain no.	Source ^a
<i>Proteobacteria</i>		
<i>Enterobacteriaceae</i>		
<i>Citrobacter amalonaticus</i>	82.89	CIP
<i>Citrobacter farmeri</i>	104553	CIP
<i>Citrobacter freundii</i>	57.32	CIP
<i>Citrobacter koseri</i>	82.94	CIP
<i>Escherichia coli</i>	54.8	CIP
<i>Escherichia coli</i>	K-12 MG1655	IJM
<i>Klebsiella oxytoca</i>	103434	CIP
<i>Klebsiella ozaenae</i>	52.211	CIP
<i>Klebsiella pneumoniae</i>	82.91	CIP
<i>Klebsiella rhinoscleromatis</i>	52.210	CIP
<i>Morganella morganii</i>	A.231	CIP
<i>Proteus vulgaris</i>	58.60	CIP
<i>Providentia alcalifaciens</i>	82.90	CIP
<i>Salmonella enterica</i>	60.62	CIP
serovar Typhimurium		
<i>Serratia marcescens</i>	103235	CIP
<i>Shigella flexneri</i>	82.48	CIP
<i>Vibrionaceae</i>		
<i>Aeromonas hydrophila</i>	76.14	CIP
<i>Helicobacter pylori</i>	43579	ATCC
<i>Plesiomonas shigelloides</i>	63.5	CIP
<i>Vibrio cholerae</i>	54.315	CIP
<i>Pasteurellaceae</i>		
<i>Haemophilus influenzae</i>	102514	CIP
<i>Pasteurella multocida</i>	103286	CIP
Others		
<i>Acinetobacter baumannii</i>	70.34	CIP
<i>Alcaligenes xylosoxidans</i>	71.32	CIP
<i>Bordetella bronchiseptica</i>	55.110	CIP
<i>Neisseria lactamica</i>	72.17	CIP
<i>Pseudomonas aeruginosa</i>	100720	CIP
<i>Bacteroides</i> sp.	100	CHU
High-G+C gram-positive		
<i>Corynebacterium urealyticum</i>	111	CHU
<i>Corynebacterium xerosis</i>	112	CHU
Low-G+C gram-positive		
<i>Bacillus</i> sp.	110	CHU
<i>Clostridium difficile</i>	101	CHU
<i>Enterococcus faecalis</i>	102	CHU
<i>Lactobacillus</i> sp.	113	CHU
<i>Listeria monocytogenes</i>	114	CHU
<i>Micrococcus</i> sp.	115	CHU
<i>Staphylococcus aureus</i>	116	CHU
<i>Staphylococcus epidermidis</i>	9901	CHU
<i>Streptococcus A</i>	117	CHU
<i>Streptococcus pneumoniae</i>	118	CHU
<i>Streptococcus mitis</i>	9902	CHU

^a CIP, Collection de l'Institut Pasteur, Paris, France; IJM, Institut Jacques Monod, Université Paris 7, Paris, France; ATCC, American Type Culture Collection; CHU, Centre Hospitalier Universitaire de Brest, Brest, France.

within 20 min of lysis. The soluble fraction of bacterial lysates contained 2 to 8 g of total proteins per liter, as assessed by a dye-binding assay (7). Lysates from DMG100 and DMG200 were prepared as previously described (22). NAT activity was also determined in human feces collected from five unrelated healthy Caucasian donors. Informed consent was obtained from all participants in accordance with local ethical committee guidelines. Samples (1 to 2 g) of feces that had been stored at -80°C were suspended in 10 ml of Tris (pH 8.0)-EDTA-dithiothreitol-KCl buffer (22) supplemented with lysozyme (0.5 g/liter) and treated by sonication on ice six times for 30 s each with 30-s intervals between pulses, to facilitate lysis of gram-positive cells. The insoluble fraction was sedimented by two centrifugation steps (2,500 × g, 5 min, 4°C, followed by 20,000 × g, 5 min, 4°C), and the final supernatant was used in an assay of NAT activity.

Unless otherwise stated, *N*-acetylation reactions were performed at 37°C in the

presence of 100 μ M AcCoA and an AcCoA-regenerating system (22). The NAT activity of bacterial lysates was tested in two or more 30-min reactions performed in duplicate with 200 μ M (for bacterial NATs) or lower half-saturating concentrations (for recombinant human NATs) of 2-AF, 5-ASA, 4-ASA, PABA, SMZ, or PA as the arylamine acceptor substrate. Arylamines and their acetylated products were quantified by reverse-phase high-pressure liquid chromatography as previously described (20, 25). 5-ASA and *N*-acetyl-5-ASA were eluted in a 7% (wt/wt) acetonitrile–20 mM sodium perchlorate (pH 2.5) mobile phase, with UV detection at 264 nm, resulting in retention times of 1.5 and 3.8 min, respectively. The apparent K_m and V_{max} (\pm standard error) values for 2-AF, 5-ASA, 4-ASA, PABA, and SMZ were determined using a nonlinear fit analysis. Steady-state kinetic parameters were determined after checking that rates of product formation were constant throughout the reaction. Kinetic studies were performed using arylamine substrate concentrations in the 0.1 to 10 K_m range unless substrate solubility was limited, as was the case for 2-AF. Maximum conversion rates of 10 to 20% of the initial substrate amount were obtained, if necessary by diluting the cell lysate in bovine serum albumin-containing buffer, as reported (20).

Inhibition studies. To achieve irreversible inhibition by iodoacetamide, undiluted cell extract was first incubated for 2 to 20 min at 25°C with various inhibitor concentrations. Aliquots were then withdrawn, and residual NAT activity was measured in 5-min assays performed at 25°C in the presence of 200 μ M 2-AF without the AcCoA-regenerating system. At the times indicated, the percent residual activity was calculated by comparison with a control assay with the same concentration of iodoacetamide but without prior incubation with inhibitor (set at 100%). For each inhibitor concentration, the logarithm of the percent residual activity was plotted against the length of time for which the cell extract was incubated with inhibitor.

Hybridization analysis. Southern blotting was carried out on genomic DNA from 22 of the wild-type *Proteobacteria* species studied. Genomic DNA was digested with *Eco*RI, size fractionated by electrophoresis in a 1% agarose gel, and transferred to a nylon membrane by capillary blotting. Genomic DNA from *Proteobacteria* and gram-positive strains was amplified by PCR using the BACT5 and BACT3 degenerate primers in the following thermocycling procedure: 5 min of denaturation at 94°C, 2 min of hybridization at 46 or 50°C, and then 40 cycles including 1 min of denaturation at 94°C, 30 s of hybridization at 46 or 50°C, and 2 min of extension at 72°C.

BACT5: 5'-CATGCTTACTTCACGAGA-3'
 T T C C
 G
 T

BACT3: 5'-CGCGGCCAGCTCCGCTC-3'
 A T
 G
 T

The $MgCl_2$ concentration of the PCR mixture was 4 mM for hybridization at 46°C and 3 mM for hybridization at 50°C. To test for the presence of NAT-hybridizing sequences in genomic and PCR-amplified bacterial DNA, a 184-bp probe including the region encoding the active site of the *S. enterica* serovar Typhimurium NAT (positions 124 to 307 from the initiation codon, GenBank accession number D90301) was generated by PCR. Primers SALM5 (5'-GATGTGCTACTGCCTCGTAA-3') and SALM3 (5'-GTAACTGGCGGGATGAGACA-3') were used in the presence of 1.5 mM $MgCl_2$ with the thermocycling procedure described above except that hybridization was performed at 60°C. The amplified DNA was labeled with [α - ^{32}P]dCTP using a random priming kit (Roche Diagnostics) and used in standard conditions to probe genomic or PCR-amplified DNA.

RESULTS

Comparison of substrate selectivity of bacterial NATs. 2-AF, which is efficiently acetylated by both human NAT1 and NAT2, was used for comparative screening of NAT activity levels in gram-negative species found in the human gut microflora, including 23 *Proteobacteria* and one *Bacteroides* species. The TA98, TA1538, and YG1024 (NAT-overproducing) *S. enterica* serovar Typhimurium strains were used as positive controls. Enzyme activity was also tested using the structurally related human NAT1-selective PABA, 4-ASA, and 5-ASA

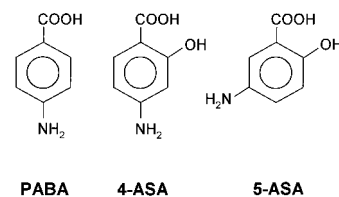


FIG. 1. Comparison of chemical structures of PABA, 4-ASA, and 5-ASA. 4-ASA is also called *p*-aminosalicylic acid in the literature.

(Fig. 1) and the human NAT2-selective SMZ and PA arylamine substrates (Table 2). The *E. coli* control strains DMG100 and DMG200, which overproduce the recombinant human NAT1 and NAT2 isoenzymes, respectively, displayed the expected level of activity with the substrates tested.

Significant NAT activity was identified for the first time in 11 *Proteobacteria* species from seven different genera: *C. amalonaticus*, *C. farmeri*, *C. freundii*, *K. ozaenae*, *K. oxytoca*, *K. rhinoscleromatis*, *M. morgani*, *S. marcescens*, *S. flexneri*, *P. shigelloides*, and *V. cholerae*. Fifteen *Proteobacteria* species had detectable NAT activity with 2-AF. With the exception of *M. morgani*, *H. pylori*, and *E. coli* K-12, the species or genera that acetylated 2-AF also acetylated 5-ASA, whereas the *Bacteroides* sp. acetylated 5-ASA but not 2-AF. Of the arylamines tested, 5-ASA and, to a lesser extent, 2-AF were the most highly acetylated by bacteria in our experimental conditions, with velocities of 0.006 (*S. marcescens*) to 2.20 (*P. aeruginosa*) and of 0.002 (*M. morgani*) to 1.06 (*P. aeruginosa*) nmol min⁻¹ (mg of protein)⁻¹, respectively. *P. aeruginosa*, *E. coli* 54.8, *C. freundii*, *C. koseri*, and *C. farmeri*, which acetylated both aminosalicylate isomers studied, had 8, 22, 50, 65, and 65 times higher levels of activity, respectively, with 5-ASA than with 4-ASA.

Only 4 of the 22 species tested for *N*-acetylation of PABA exhibited significant activity, from 0.002 (*A. hydrophila*) to 0.14 (*P. aeruginosa*) nmol min⁻¹ (mg of protein)⁻¹. *P. aeruginosa*, *C. amalonaticus*, and *S. enterica* serovar Typhimurium YG1024 acetylated 5-ASA 11, 21, and 1,480 times more efficiently than PABA, respectively, whereas *N*-acetylation velocities were of the same order of magnitude for both compounds in the *Bacteroides* sp. With the exception of *P. aeruginosa*, which showed similar levels of activity with PABA and 4-ASA, the PABA-acetyating species did not acetylate 4-ASA.

The bacterial species studied displayed a low level of *N*-acetylation activity with SMZ and PA in our experimental conditions (from 0.001 to 0.006 nmol min⁻¹ [mg of protein]⁻¹). PA was acetylated only by *C. amalonaticus*, and *P. shigelloides*, *P. vulgaris*, *P. alcalifaciens*, and *A. xylosoxidans* had no detectable NAT activity with the arylamine substrates studied here, nor did the NAT-defective *S. enterica* serovar Typhimurium TA98/1,8 DNP₆ strain used as a negative control. Overall, *P. aeruginosa* appeared from our comparative screening to be the most potent *N*-acetylase among the species studied, as it acetylated 2-AF and the three human NAT1-specific substrates with the highest velocity values.

NAT activity in human colonic content. We estimated the overall *N*-acetylation activity of the colonic microflora by performing enzyme assays with lysates of human feces and 5-ASA or 2-AF as the acceptor substrate. Samples collected from the

TABLE 2. *N*-Acetylation velocities of bacterial NATs for six arylamine substrates^a

Strain ^b	NAT activity (nmol min ⁻¹ [mg of protein] ⁻¹)					
	2-AF	5-ASA	4-ASA	PABA	SMZ	PA
Control strains						
DMG100 (human NAT1)	520	1,170	1,090	580	8.30	5.68
DMG200 (human NAT2)	0.99	1.89	0.075	0.002	2.41	0.06
<i>S. enterica</i> serovar Typhimurium YG1024	27.5	20.8	<0.001	0.014	<0.001	<0.001
<i>S. enterica</i> serovar Typhimurium TA98 1,8/DNP ₆	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
<i>S. enterica</i> serovar Typhimurium TA1538	0.04	ND	<0.001	<0.001	<0.001	<0.001
<i>S. enterica</i> serovar Typhimurium TA98	0.06	ND	<0.001	<0.002	<0.001	<0.001
Study strains						
<i>S. enterica</i> serovar Typhimurium	0.06	0.27	<0.001	0.005	<0.001	
<i>C. amalonaticus</i>	0.09	0.10	<0.001	0.005	<0.001	0.004
<i>C. farmeri</i>	0.10	0.33	0.006	<0.001	0.002	<0.001
<i>C. freundii</i>	0.14	0.16	0.005	<0.001	<0.001	<0.002
<i>C. koseri</i>	0.32	0.59	0.008	<0.001	0.003	<0.001
<i>E. coli</i> 54.8	0.05	0.09	0.004	<0.001	<0.003	<0.001
<i>E. coli</i> K-12	0.02	<0.001	0.01	<0.001	0.001	<0.001
<i>K. pneumoniae</i>	<0.001	<0.001	<0.001	<0.001	0.006	<0.001
<i>K. ozaenae</i>	0.007	ND	ND	ND	ND	ND
<i>K. oxytoca</i>	0.16	0.61	<0.001	<0.001	0.001	<0.001
<i>K. rhinoscleromatis</i>	0.15	ND	<0.002	<0.002	0.006	<0.002
<i>M. morgani</i>	0.002	<0.001	0.02	<0.001	<0.001	<0.001
<i>P. vulgaris</i>	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
<i>P. alcalifaciens</i>	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
<i>S. marcescens</i>	0.004	0.006	<0.001	<0.001	0.002	<0.001
<i>S. flexneri</i>	<0.001	<0.001	<0.001	<0.001	0.003	<0.006
<i>A. hydrophila</i>	<0.001	<0.001	<0.001	0.002	<0.001	<0.001
<i>H. pylori</i>	0.01	<0.002	<0.001	<0.001	<0.001	<0.001
<i>P. shigelloides</i>	<0.001	<0.002	<0.001	<0.001	<0.001	0.006
<i>V. cholerae</i>	0.001	<0.001	0.005	<0.001	<0.001	<0.001
<i>P. multocida</i>	<0.001	ND	ND	ND	ND	ND
<i>A. xylosoxidans</i>	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
<i>P. aeruginosa</i>	1.06	2.20	0.27	0.14	<0.001	<0.001
<i>Bacteroides</i> sp.	<0.001	0.02	<0.001	0.05	<0.001	<0.001

^a *N*-Acetylation activities were measured as described in Materials and Methods in the presence of 100 μ M AcCoA and, unless otherwise stated, 200 μ M arylamine substrate. Mean values from two or more experiments performed in duplicate are presented. ND, not determined. *N*-Acetylation activity was measured in the presence of a 20 μ M (human NAT1 with 2-AF), 2 μ M (human NAT2 with 2-AF), or 10 μ M (human NAT1 with 5-ASA, 4-ASA, and PABA) concentration of the arylamine substrate.

^b Underlined species also gave a positive signal in DNA hybridization experiments using the *S. enterica* serovar Typhimurium NAT probe and blots of PCR-amplified and/or whole genomic bacterial DNA (see Fig. 3). The strain numbers are given in Table 1.

various donors displayed NAT activity with 5-ASA (0.062 ± 0.011 , 0.063 ± 0.014 , 0.079 ± 0.007 , 0.113 ± 0.021 , and 0.639 ± 0.049 nmol min⁻¹ per g of initial sample). Conversely, the *N*-acetylation velocities by fecal lysates of 2-AF were lower than 0.015 nmol min⁻¹ per g in our experimental conditions. Given the estimated bacterial content in feces (4) and in our bacterial cultures (optical density at 600 nm), it can be roughly estimated that the feces lysates were made from 10 to 100 times less bacteria than the pure-culture lysates.

Comparative kinetics of bacterial NATs. The strains found to have the highest rates of enzyme activity in the reaction spectrum study were studied in more detail to characterize further and compare their *N*-acetylation activities. We determined apparent kinetic parameters for these strains (Tables 3 to 5). The five arylamine acceptor substrates studied can be assigned to three overlapping classes on the basis of their apparent K_m : (i) 5-ASA (26 to 350 μ M), 2-AF (44 to 405 μ M), and SMZ (59 to 550 μ M); (ii) 4-ASA (260 to 2,200 μ M); and (iii) PABA (870 to 51,600 μ M). Bacterial NATs had similar apparent affinities for 5-ASA, 2-AF, and SMZ, as shown by the weak differences between the corresponding K_m values for a given strain. Conversely, bacterial *N*-acetylation of the amin-

osalicylate isomers studied displayed a higher affinity for 5-ASA. The K_m of 4-ASA was 4 times higher than that of 5-ASA in *C. koseri*, 10 times higher in *P. aeruginosa*, 11 times higher in *E. coli* 54.8, 14 times higher in *C. freundii*, and 29 times higher in *C. farmeri*. Similarly, the K_m of human NAT2 for 4-ASA was 41 times higher than that for 5-ASA, whereas human NAT1 acetylated both isomers with similar Michaelis constants. The K_m values of bacterial NATs and human NAT2 were of the same order of magnitude for 5-ASA. Finally, of the arylamines studied, PABA was the substrate for which bacterial NATs had the lowest apparent affinity, as indicated in *P. aeruginosa* by K_m values 5, 27, and 47 times higher than those for 4-ASA, 2-AF, and 5-ASA, respectively.

In any given strain, the apparent V_{max} for 5-ASA acetylation by the bacterial NAT was higher than that for any other substrate (Table 4). Similarly, the highest catalytic efficiencies, V_{max}/K_m , were also measured for 5-ASA in bacteria (Table 5). In particular, V_{max}/K_m values were 27 (for *E. coli* 54.8) to 645 (for *C. farmeri*) times higher for 5-ASA than for 4-ASA, consistent with the K_m data. Human recombinant NAT2 also had a V_{max}/K_m for 5-ASA that was 32 times higher than that for 4-ASA, whereas NAT1 had similar catalytic efficiencies with

TABLE 3. K_m s of bacterial and human NATs^a

Strain	Mean K_m (μ M) \pm SD				
	2-AF	5-ASA	4-ASA	PABA	SMZ
Control strains					
DMG100 (human NAT1)	28 \pm 2.8 ^b	6.7 \pm 1.4	9.4 \pm 1.2 ^b	13 ^b	5,600 \pm 1,000 ^b
DMG200 (human NAT2)	2.25 \pm 0.12 ^b	184 \pm 32	7,590 \pm 1,350 ^b	— ^b	110 \pm 6.0 ^b
<i>S. enterica</i> serovar Typhimurium YG1024	365 \pm 46	ND	—	51,600 \pm 5,275 ^c	—
<i>S. enterica</i> serovar Typhimurium TA98	397 \pm 69	ND	—	—	—
<i>S. enterica</i> serovar Typhimurium TA1538	404 \pm 56	ND	—	—	—
Study strains					
<i>S. enterica</i> serovar Typhimurium 60.62	311 \pm 39 ^c	208 \pm 32 ^c	—	—	283 \pm 26 ^c
<i>E. coli</i> 54.8	127 \pm 13****	117 \pm 17**	1,240 \pm 173 ^c	—	59 \pm 8.1****
<i>E. coli</i> K-12	205 \pm 27*	—	1,160 \pm 150	—	121 \pm 3.3****
<i>P. aeruginosa</i>	44 \pm 5.1****	26 \pm 5.0****	258 \pm 63****	1,200 \pm 205****	—
<i>C. koseri</i>	96 \pm 7.8****	210 \pm 20	860 \pm 83*	—	115 \pm 16****
<i>C. farmeri</i>	64 \pm 3.8****	61 \pm 12****	1,790 \pm 259	—	70 \pm 10****
<i>C. freundii</i>	165 \pm 25***	158 \pm 22	2,200 \pm 365**	—	—
<i>C. amalonaticus</i>	82 \pm 11****	112 \pm 7.2***	—	ND	—
<i>K. rhinoscleromatis</i>	141 \pm 41***	ND	—	—	146 \pm 21****
<i>K. oxytoca</i>	158 \pm 27***	347 \pm 30***	—	—	—
<i>M. morgani</i>	117 \pm 18****	—	ND	—	—
<i>H. pylori</i>	106 \pm 11****	—	—	—	—
<i>S. marcescens</i>	79 \pm 12****	ND	—	—	550 \pm 58****
<i>Bacteroides</i> sp.	—	ND	—	870 \pm 150****	—

^a K_m and V_{max} were estimated for five arylamine substrates in two or more separate experiments. ND, not determined; —, no detectable activity.

^b Values determined in previous studies (18, 20). The values given for recombinant human NATs were not included in the statistical comparison.

^c Results obtained from *S. enterica* serovar Typhimurium or, by default (for the substrate 4-ASA), from *E. coli* and used as a reference for the statistical comparison with values obtained for the other bacterial NAT activities and for the same arylamine substrate, using Student's *t* test. ****, $P < 0.001$; ***, $P < 0.01$; **, $P < 0.02$; *, $P < 0.05$.

both isomers. Human recombinant NAT1 acetylated PABA with the highest V_{max}/K_m (22) (Table 5), whereas in *P. aeruginosa*, the catalytic efficiency of PABA acetylation was 1/100 that for 5-ASA and 1/30 that for 2-AF. In *S. enterica* serovar

Typhimurium YG1024, the catalytic efficiency was 1,500 times higher for 2-AF than for PABA. Conversely, *P. aeruginosa* displayed similar V_{max}/K_m values for PABA and 4-ASA. Finally, the catalytic efficiencies for the acetylation of SMZ by

TABLE 4. V_{max} s of bacterial and human NATs^a

Strain	Mean V_{max} (nmol min ⁻¹ [mg of protein] ⁻¹) \pm SD				
	2-AF	5-ASA	4-ASA	PABA	SMZ
Control strains					
DMG100 (human NAT1)	870 \pm 39 ^b	1,715 \pm 57	1,810 \pm 75 ^b	6,050 ^b	123 \pm 19 ^b
DMG200 (human NAT2)	2.34 \pm 0.04 ^b	2.98 \pm 0.18	3.90 \pm 0.14 ^b	— ^b	3.80 \pm 0.07 ^b
<i>S. enterica</i> serovar Typhimurium YG1024	60 \pm 4.8****	ND	<0.001	5.58 \pm 0.54 ^c	<0.001
<i>S. enterica</i> serovar Typhimurium TA98	0.18 \pm 0.02*	ND	<0.001	<0.002	<0.001
<i>S. enterica</i> serovar Typhimurium TA1538	0.12 \pm 0.01	ND	<0.001	<0.001	<0.001
Study strains					
<i>S. enterica</i> serovar Typhimurium 60.62	0.13 \pm 0.01 ^c	0.80 \pm 0.08 ^c	<0.001	<0.001	91 (\pm 5.4) $\times 10^{-4}$
<i>E. coli</i> 54.8	0.06 \pm 0.004****	0.09 \pm 0.005****	0.04 \pm 0.002 ^c	<0.001	30 (\pm 0.8) $\times 10^{-4}$ ****
<i>E. coli</i> K-12	0.04 \pm 0.003****	<0.001	0.05 \pm 0.004****	<0.001	13 (\pm 0.1) $\times 10^{-4}$ ****
<i>P. aeruginosa</i>	1.17 \pm 0.06****	2.44 \pm 0.13****	0.32 \pm 0.02****	1.09 \pm 0.06****	<0.001
<i>C. koseri</i>	0.48 \pm 0.02****	1.35 \pm 0.06****	0.03 \pm 0.001****	<0.001	8.3 (\pm 0.3) $\times 10^{-4}$ ****
<i>C. farmeri</i>	0.13 \pm 0.003	0.42 \pm 0.03****	0.02 \pm 0.001****	<0.001	18 (\pm 0.5) $\times 10^{-4}$ ****
<i>C. freundii</i>	0.23 \pm 0.02****	0.27 \pm 0.02****	0.08 \pm 0.01****	<0.001	<0.001
<i>C. amalonaticus</i>	0.14 \pm 0.01	0.16 \pm 0.004****	<0.001	ND	<0.001
<i>K. rhinoscleromatis</i>	0.20 \pm 0.03*	ND	<0.002	<0.002	85 (\pm 3.6) $\times 10^{-4}$
<i>K. oxytoca</i>	0.23 \pm 0.03****	1.73 \pm 0.08****	<0.001	<0.001	ND
<i>M. morgani</i>	0.003 \pm 0.0002****	<0.001	ND	<0.001	<0.001
<i>H. pylori</i>	0.03 \pm 0.0009****	<0.002	<0.001	<0.001	<0.001
<i>S. marcescens</i>	0.01 \pm 0.001****	ND	<0.001	<0.001	7.3 (\pm 0.4) $\times 10^{-4}$ ****
<i>Bacteroides</i> sp.	<0.001	ND	<0.001	0.27 \pm 0.02****	<0.001

^a See Table 3, footnotes a, b, and c.

TABLE 5. V_{\max}/K_m results for bacterial NATs^a

Strain	V_{\max}/K_m (% of control value)				
	2-AF	5-ASA	4-ASA	PABA	SMZ
Control strains					
DMG100 (human NAT1)	49 ^b	257	193 ^b	465 ^b	2.2 × 10 ^{-2b}
DMG200 (human NAT2)	1.04 ^b	1.6 × 10 ⁻²	5.1 × 10 ^{-4b}	— ^b	3.5 × 10 ^{-2b}
<i>S. enterica</i> serovar Typhimurium YG1024	1.6 × 10 ⁻¹ (39,480)	ND	—	1.1 × 10 ⁻⁴ (100)	—
<i>S. enterica</i> serovar Typhimurium TA98	4.7 × 10 ⁻⁴ (112)	ND	—	—	—
<i>S. enterica</i> serovar Typhimurium TA1538	2.9 × 10 ⁻⁴ (69)	ND	—	—	—
Study strains					
<i>S. enterica</i> serovar Typhimurium 60.62	4.1 × 10 ⁻⁴ (100)	3.8 × 10 ⁻³ (100)	—	—	3.2 × 10 ⁻⁵ (100)
<i>E. coli</i> 54.8	4.8 × 10 ⁻⁴ (115)	7.8 × 10 ⁻⁴ (20)	2.9 × 10 ⁻⁵ (100)	—	5.0 × 10 ⁻⁵ (155)
<i>E. coli</i> K-12	1.8 × 10 ⁻⁴ (42)	—	4.2 × 10 ⁻⁵ (140)	—	1.1 × 10 ⁻⁵ (33)
<i>P. aeruginosa</i>	2.6 × 10 ⁻² (6,375)	9.5 × 10 ⁻² (2,470)	1.2 × 10 ⁻³ (4,300)	9.1 × 10 ⁻⁴ (845)	—
<i>C. koseri</i>	5.0 × 10 ⁻³ (1,200)	6.4 × 10 ⁻³ (170)	3.2 × 10 ⁻⁵ (110)	—	7.2 × 10 ⁻⁶ (22)
<i>C. farmeri</i>	2.0 × 10 ⁻³ (480)	6.9 × 10 ⁻³ (180)	1.1 × 10 ⁻⁵ (37)	—	2.6 × 10 ⁻⁵ (81)
<i>C. freundii</i>	1.4 × 10 ⁻³ (330)	1.7 × 10 ⁻³ (44)	3.9 × 10 ⁻⁵ (130)	—	—
<i>C. amalonaticus</i>	1.7 × 10 ⁻³ (415)	1.4 × 10 ⁻³ (37)	—	ND	—
<i>K. rhinoscleromatis</i>	1.4 × 10 ⁻³ (335)	ND	—	—	5.8 × 10 ⁻⁵ (180)
<i>K. oxytoca</i>	1.5 × 10 ⁻³ (350)	5.0 × 10 ⁻³ (130)	—	—	—
<i>M. morgani</i>	2.5 × 10 ⁻⁵ (6)	—	ND	—	—
<i>H. pylori</i>	2.4 × 10 ⁻⁴ (59)	—	—	—	—
<i>S. marcescens</i>	1.3 × 10 ⁻⁴ (31)	ND	—	—	1.3 × 10 ⁻⁶ (4)
<i>Bacteroides</i> sp.	—	ND	—	3.1 × 10 ⁻⁴ (290)	—

^a See Table 3, footnotes a, b, and c.

bacterial NATs were of the same order of magnitude or lower than those for 4-ASA in a given species.

Consistent with the results obtained for the reaction spectra of bacterial NATs, with the exception of the recombinant NAT-overproducing strains, *P. aeruginosa* was the most efficient acetylator among the species studied for each arylamine substrate tested. It exhibited V_{\max}/K_m values 64, 25, and 8 times higher than those of *S. enterica* serovar Typhimurium for 2-AF, 5-ASA, and PABA, respectively. Its V_{\max}/K_m for 4-ASA was also 43 times higher than that of *E. coli* 54.8. In contrast *S. marcescens*, *H. pylori*, and *M. morgani* displayed the lowest catalytic efficiencies for 2-AF in our experimental conditions.

Inhibition of bacterial NAT activity. To obtain further insight into the nature of the putative active amino acid residue of bacterial NATs, we performed irreversible inhibition experiments with iodoacetamide. This compound irreversibly reacts with the thiol group of accessible Cys side chains in proteins to give a stable alkylated product (19). Bacterial lysates from *S. enterica* serovar Typhimurium YG1024, *C. koseri*, and *P. aeruginosa* were incubated with or without 100 to 800 mM iodoacetamide, corresponding to a 200 to 1,600-fold molar excess of the modifying agent over the estimated Cys residue content of bacterial lysates (45), and the residual NAT activity was measured in the presence of 2-AF. The inactivation of NAT depended on time and inhibitor concentration (Fig. 2). Apparent pseudo-first-order inactivation constants (K_{obs}) were obtained for each iodoacetamide concentration by linear regression of plots of the logarithm of the percentage of control activity versus time (38). Secondary plots of $\ln(K_{\text{obs}})$ versus \ln (iodoacetamide) were linear and had slopes of 0.81 for *S. enterica* serovar Typhimurium YG1024, 0.72 for *C. koseri*, and 1.07 for *P. aeruginosa*. Thus, the order of the inhibition reaction with iodoacetamide was probably similar for these three

bacterial NAT activities. In each case, we estimated that a single functional Cys reacted with the modifying agent.

Molecular hybridization of DNA from several bacterial species with an *S. enterica* serovar Typhimurium NAT probe. Having detected NAT activity in a number of bacterial species, we attempted to detect new NAT-encoding regions. Genomic DNA from various bacterial species was amplified by PCR at two different levels of stringency, using degenerate primers derived from the sequence of the *S. enterica* serovar Typhimurium NAT gene. The degenerate sense (BACT5) and anti-sense (BACT3) primers used hybridized to the coding sequence of the *S. enterica* serovar Typhimurium NAT gene at positions 16 to 33 and 797 to 804, respectively, resulting in a PCR product of 788 bp for *S. enterica* serovar Typhimurium. We checked the specificity of the amplified DNAs by Southern blotting of the PCR products, using a radiolabeled 184-bp DNA probe amplified by PCR from the putative active-site region of the *S. enterica* serovar Typhimurium NAT gene (i.e., spanning the Cys⁶⁹ residue) (Fig. 3). All positive strains gave the expected band of approximately 790 bp. An unidentified 500-bp band was also detected. In addition to *S. enterica* serovar Typhimurium, 8 of the 22 proteobacteria (5 members of the *Enterobacteriaceae* [*C. koseri*, *C. freundii*, *E. coli* 54.8, *K. ozaenae*, and *S. marcescens*] and 3 species from other families [*B. bronchiseptica*, *P. multocida*, and *P. aeruginosa*]) as well as 4 of the 12 gram-positive species studied (2 with a high G+C content [*Corynebacterium urealyticum* and *C. xerosis*] and 2 with a low G+C content [*S. aureus* and *S. mitis*]) produced a signal. A faint signal was obtained for *Streptococcus A* and *S. pneumoniae*. Genomic DNA blots (*EcoRI* digests) from all *Proteobacteria* strains studied except *E. coli* K-12 and *H. pylori* (Table 1) were also incubated with the probe (data not shown). The presence or absence of hybridization signals was recorded

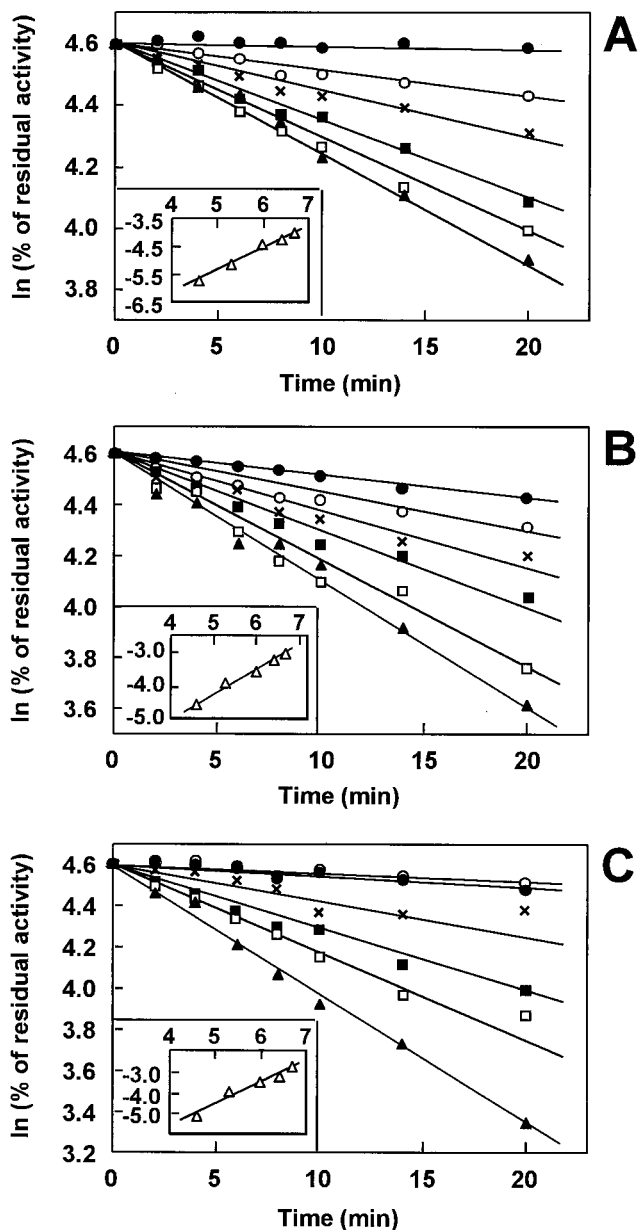


FIG. 2. Time- and concentration-dependent inactivation of bacterial NATs with iodoacetamide. Cell extracts from *S. enterica* serovar Typhimurium YG1024 (A), *C. koseri* (B), and *P. aeruginosa* (C) were incubated without (●) or with iodoacetamide at a final concentration of 100 (○), 200 (×), 400 (■), 600 (□), or 800 (▲) mM, and residual *N*-acetylation activity with 2-AF was measured. Results are the means of two experiments performed in duplicate. Insets show the double-logarithmic plots of the apparent first-order inactivation rate (k_{obs}) versus molar inhibitor concentration. A value of 0.81 ($r = 0.99$), 0.72 ($r = 0.99$) and 1.07 ($r = 0.98$) for the reaction order (n) with respect to iodoacetamide was calculated from the slopes for *S. enterica* serovar Typhimurium YG1024, *C. koseri*, and *P. aeruginosa*, respectively. The initial reaction rates without inhibitor were 11.23 ± 0.63 , 0.42 ± 0.02 , and 2.93 ± 0.03 nmol min⁻¹ (mg of protein)⁻¹ for *S. enterica* serovar Typhimurium YG1024, *C. koseri*, and *P. aeruginosa*, respectively.

with PCR-amplified as well as genomic DNA blots (Fig. 3). Of all the species studied only by genomic DNA hybridization, only *K. pneumoniae* and *S. flexneri* provided a faint hybridization signal.

DISCUSSION

In this study, we used molecular and functional approaches to identify and characterize bacterial NATs. Thirteen NAT sequences from nine eubacterial genera were retrieved from databases, using the *S. enterica* serovar Typhimurium NAT sequence as a query, and were multialigned (data not shown). These sequences showed a highly conserved Cys that aligned with Cys⁶⁹ in *S. enterica* serovar Typhimurium (65), which has been suggested to be the active-site residue in four bacterial NATs (49). We inhibited the NAT activity from *P. aeruginosa*, *C. koseri*, and *S. enterica* serovar Typhimurium YG1024 by a large excess of the Cys-modifying agent iodoacetamide. We found that this inhibition followed a 1:1 stoichiometry, suggesting that one essential Cys residue is accessible to the inhibitor in these three bacterial enzymes (Fig. 2). The essential nature of Cys⁶⁹ in *S. enterica* serovar Typhimurium has already been demonstrated by Watanabe and colleagues (65) from site-directed mutagenesis experiments. Thus, like vertebrate NATs (20), eubacterial isoforms seem to have a single Cys as the active-site residue. Our sequence alignment (data not shown) identified two strictly conserved residues aligning with His¹⁰⁷ and Asp¹²² of the *S. enterica* serovar Typhimurium NAT. These residues have recently been described as forming part of its catalytic triad (58). We also identified two highly conserved basic residues aligning with Arg⁹ and Arg⁶⁴ of human NATs. It has been suggested that the Arg⁹ and Arg⁶⁴ residues are essential for enzyme-substrate interactions and conformational stabilization in vertebrate NATs (20). The conservation of these residues (Arg¹¹ and Arg⁶⁵ of *S. enterica* serovar Typhimurium NAT) suggests that similar mechanisms may operate for all NATs. The structural study of *S. enterica* serovar Typhimurium NAT conducted by Sinclair et al. (58) supports that hypothesis. In particular, it was found that Arg⁶⁵ stabilizes the conformation of the Cys⁶⁹ residue through a salt bridge interaction with the highly conserved Glu³⁹.

We identified NAT activity for the first time in 11 proteobacterial species belonging to the *Citrobacter*, *Klebsiella*, *Morganella*, *Serratia*, *Shigella*, *Plesiomonas*, and *Vibrio* genera (Table 2). These enzyme activities were detected and some of them were kinetically characterized using one or several of six arylamine acceptor substrates chosen as NAT1-type (PABA, 4-ASA, and 5-ASA), NAT2-type (SMZ and PA), or mixed (2-AF) with regard to substrate selectivity of human NATs. The values obtained for the *S. enterica* serovar Typhimurium control strains showed that these activities were specific (Tables 3 to 5). It has to be noted that our K_m values for 2-AF in *P. aeruginosa*, *E. coli*, and *H. pylori* lysates are 3 to 35 times lower than those reported by Chung et al. (8, 10, 33). These discrepancies may be related to differences in experimental conditions, such as AcCoA concentration and waiting time between bacterial sonication and NAT assay. We observed that above a 20-min interval between cell lysis and enzymatic assay, the apparent K_m values could markedly increase, with loss in reproducibility. This might contribute to the variability in ki-

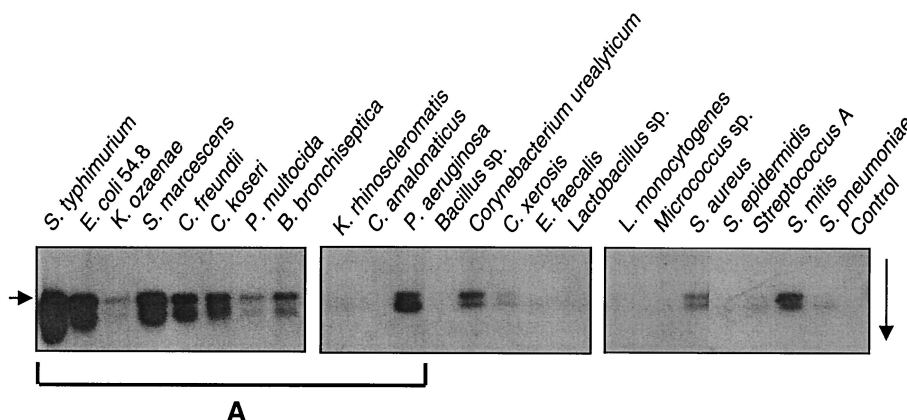


FIG. 3. Hybridization of PCR-amplified bacterial DNA with the *S. enterica* serovar Typhimurium NAT probe PCR products amplified from bacterial genomic DNA were blotted as described in the text. A negative control consisting of an 835-bp amplification product of the human NAT2 coding region was blotted in the control lane. The hybridization temperature and $MgCl_2$ content used for PCR were 50°C and 3 mM (left blot) or 46°C and 4 mM (central and right blots). Similar hybridization results were obtained for both sets of PCR conditions for the *S. enterica* serovar Typhimurium DNA blot. In addition to the expected 790-bp band (left arrow), an unidentified 500-bp band was also detected. A similar doublet was found for *S. enterica* serovar Typhimurium at shorter exposure time. Some of the strains (group A) also gave a positive signal by hybridization of *EcoRI*-digested genomic DNA. The right arrow indicates the direction of electrophoretic migration.

netic parameter values reported in distinct studies by Chung et al. (10, 13, 14, 17).

Bacterial NAT activities appear to have features similar to those of the human NAT1 and NAT2 isoenzymes. They are most similar to NAT1 in terms of reaction spectrum, as shown by their low *N*-acetylation activity with the NAT2-selective substrates SMZ and PA (Table 2) and by their high catalytic efficiency with 5-ASA and 2-AF compared to that with SMZ (Tables 3 to 5). This may be related, in 11 of the 13 bacterial sequences retrieved, to the presence of a conserved Phe residue aligning with Phe¹²⁵ in the human NAT1 sequence (data not shown). This residue may be associated with the low NAT1-type substrate selectivity for SMZ (24). In contrast, most of the bacterial NATs studied had poor or undetectable activity with PABA, which makes them similar to human NAT2 (Table 2). The ranges of apparent K_m values for SMZ, 5-ASA, and 4-ASA also place bacterial NATs closer to human NAT2 than to NAT1. Consistently, the NAT from *M. smegmatis* exhibits a low apparent K_m (25 μ M) for the NAT2 substrate isoniazid (49). We found that bacterial NATs share with human NAT2 a higher catalytic efficiency for 5-ASA than for its isomer, 4-ASA (Tables 3 to 5). The bacterial sequences studied have no conserved residue aligning with Arg¹²⁷ in the human NAT1 sequence (data not shown). This residue may be associated with the high selectivity of human NAT1 for 4-ASA (24). Finally, bacterial NATs appear to have functional specificities that distinguish them from their eukaryotic counterparts, making them useful as a new model for study of the structure-function relationships of arylamine *N*-acetyltransferases.

Enterobacteriaceae species (Table 1) widely acetylated one or more of the tested arylamines. Most of them cross-reacted with the previously described rabbit antiserum raised against purified recombinant *S. enterica* serovar Typhimurium NAT (49) and gave an immunoblot signal comigrating with the *S. enterica* serovar Typhimurium NAT band (data not shown), consistent with other data (M. Payton, A. Mushtaq, J. Sinclair, J. Sandy,

T.-W. Yu, M. Noble, and E. Sim, submitted for publication). However, unlike a previous study (48), we did not detect NAT activity in *P. vulgaris*, possibly due to differences in the strains used. The *E. coli* 54.8 and K-12 strains consistently differed in NAT activity in this study. This may be due to the high polymorphism within the *E. coli* species reflected by differences in genome length (5) or in metabolic phenotypes (36) between strains. *P. alcalifaciens* and *A. xylosoxidans* also had no detectable activity for the six arylamine substrates tested in our study. The low nutritive content of our bacterial growth medium is unlikely to play a significant role in this lack of enzyme activity because these species are members of the normal intestine microflora and the contents of the colonic lumen are considered nutritively poor. Although these three inactive species gave no signal in genomic DNA hybridization experiments, they gave a clear NAT-specific immunoreactivity signal (data not shown). This suggests that these organisms may contain NATs with a different and unknown substrate selectivity. The *Pasteurellaceae* species *P. multocida* did not acetylate 2-AF in our test conditions, but the positive results of DNA hybridization experiments (Fig. 3) suggest that this species contains a genomic sequence with some similarities to the *S. enterica* serovar Typhimurium NAT gene.

The four *Vibrionaceae* species studied were only weakly immunoreactive with the *S. enterica* serovar Typhimurium-specific antiserum (data not shown) and had marginal NAT activities. *H. pylori* acetylated 2-AF much less efficiently than in previous studies (10, 18) (Table 2). We were also unable to retrieve a putative NAT sequence from the *H. pylori* genome database even though two unrelated strains have been now completely sequenced (1, 60). Only a partial putative sequence was obtained from the *V. cholerae* genome (data not shown). Consistently, no genomic DNA hybridization signal was obtained for *V. cholerae*, *A. hydrophila*, and *P. shigelloides* (data not shown). This suggests that the NATs of the *Vibrionaceae* differ greatly in structure and reaction spectrum from those found in other proteobacteria. In contrast, within obligate

anaerobes, we found significant NAT activity for PABA and 5-ASA in the *Bacteroides* sp. (Table 2) and a small activity with 2-AF and 5-ASA in *C. difficile* (data not shown), consistent with previous data for these genera (48). However, the absence of a detectable immunoblot signal for the *Bacteroides* sp. (data not shown) may be related to the absence of an epitope in common with *S. enterica* serovar Typhimurium NAT.

Among the *Proteobacteria* and *Bacteroides* species tested here, *P. aeruginosa* was the most effective *N*-acetylase in terms of both reaction spectrum and catalytic efficiency, since it exhibited the highest activity for 2-AF, 5-ASA, 4-ASA, and PABA (Tables 2, 3, 4, and 5). *P. aeruginosa* gave NAT-specific DNA hybridization (Fig. 3) and cross-immunoreactivity (data not shown) signals. A complete putative NAT sequence was also obtained from this species (data not shown). Moreover, the results of our DNA hybridization experiments (Fig. 3) and the identification of several putative NAT sequences (data not shown) suggest that NATs are widely present in gram-positive species with low and high G+C contents.

Microbial interactions and drug administration are determinants for the composition of the intestinal microflora. Therefore, the identification of bacterial NATs may serve as a means of understanding the enzyme activity in different clinical situations. The aminosalicylate 5-ASA is one of the most effective treatments for inflammatory bowel diseases in humans (50). It is mainly metabolized into *N*-acetyl-5-ASA, which is thought to be therapeutically inactive (35). We detected *N*-acetylation activity with 5-ASA in human feces, with a 1- to 10-fold inter-individual variability in enzyme velocities (see Results). This activity mostly reflects that of obligate anaerobes, including gram-positives, as they constitute the great majority of the colonic microflora (4, 56). These results are consistent with the ability of the *Bacteroides* sp. (Table 2) and of the entire obligate anaerobic colonic microflora in culture (63) to *N*-acetylate 5-ASA. The absence of a detectable NAT activity for 2-AF in feces samples is also consistent with the lack of activity found with the *Bacteroides* sp. (Table 2). The endogenous NAT1 activity of intestinal mucosal cells (29) is unlikely to contribute to the observed *N*-acetylation of 5-ASA in feces, given that (i) maximal *N*-acetyl-5-ASA synthesis was reported for high dilutions of fecal material in humans (63) and (ii) the endogenous pancreatic enzymes present in the intestinal content are greatly inactivated by the microflora in rats (51). We detected NAT activity with 5-ASA in species of *Proteobacteria* and *Bacteroides* from the intestinal microflora, including members known to be overrepresented in inflammatory bowel diseases. These microbes include the aerobes *E. coli*, *P. aeruginosa*, and *Klebsiella* (31) and the obligate anaerobes *Bacteroides* (3, 46) and *C. difficile* (52). Of the various arylamines tested here, 5-ASA was the most efficiently acetylated by bacterial NATs (Tables 2, 3, 4, and 5). Bacterial *N*-acetylation may therefore be responsible, in addition to the intestinal metabolism (68), for 5-ASA inactivation. However, *N*-acetyl-5-ASA has itself been found to inhibit the growth of obligate anaerobes such as *C. difficile* (52). We also found that NATs from *P. aeruginosa*, *E. coli*, and various *Citrobacter* species had stronger activity for 5-ASA than for its isomer, 4-ASA (Tables 3, 4, and 5). Similar therapeutic benefits have been reported for both isomers in specific cases of ulcerative colitis, and patient tolerance of 4-ASA is higher due to the adverse effects of 5-ASA (42). The use of

4-ASA in inflammatory bowel disease patients presenting intolerance to 5-ASA may therefore be recommended, given its low level of inactivation by intestinal microflora. *N*-Acetyl-5-ASA may be unstable in the intestine and/or hydrolyzed by nonspecific amidases to regenerate 5-ASA. However, administered acetyl-5-ASA was reported not to be deacetylated, and acetyl-5-ASA is considered more stable than 5-ASA (35). Thus, we suggest that the bacterial acetylation of 5-ASA could modulate the concentration equilibrium between the two forms, promoting the acetylated form in the gut. Other works have highlighted the importance of the metabolism of intestinal bacteria in drug side effects (44). Consistently, NAT activity in *Mycobacterium* species was suggested to modulate the therapeutic response to isoniazid treatment of tuberculosis. It should be important to investigate such prokaryotic NATs for polymorphism and potential implications for drug resistance (54).

In contrast to previous reports (8, 18), only a small proportion of the bacterial species studied here acetylated PABA (Table 2). In addition, it was previously observed that bacterial NATs have lower activities with PABA than with 2-AF (33, 48), although with similar apparent K_m (8, 10, 18). In our study, reactions with PABA had much lower catalytic efficiencies than those with 2-AF or with the structurally related 5-ASA, mainly due to the higher apparent K_m values measured for PABA (Table 3). The results of reversible inhibition experiments suggested that PABA acts as a competitive inhibitor for the *N*-acetylation of 2-AF in *S. enterica* serovar Typhimurium 60.62 (data not shown). These data are consistent with the essential role of PABA as a precursor for folate synthesis in bacteria and suggest a double level of preservation of the PABA pool in the bacterial cell with regard to *N*-acetylation (34, 49).

Bacterial NATs have been shown to have the potential to generate strongly alkylating cationic compounds involved in carcinogenesis, through *N*- and/or *O*-acetylation of arylamine or hydroxyarylamine metabolites (27). Microflora and endogenous NATs may thus affect susceptibility to human bowel cancers, in addition to other bacterial carcinogen-producing pathways such as nitroreduction and azoreduction (56). The data presented here may ultimately make it possible to design new tester strains with higher efficiencies in mutagenicity tests. To this end, we suggest that the *P. aeruginosa* NAT should be further investigated at the molecular level. Indeed, this study shows that this enzyme is potentially useful in terms of both the substrate diversity and the activity levels required in such tests. However, further kinetic studies on purified enzyme are necessary, since differences in enzyme activities can be due to differences in catalytic properties but also to differences in expression levels.

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REFERENCES

- Alm, R. A., L. S. Ling, D. T. Moir, B. L. King, E. D. Brown, P. C. Doig, D. R. Smith, B. Noonan, B. C. Guild, B. L. deJonge, G. Carmel, P. J. Tummino, A. Caruso, M. Uria-Nickelsen, D. M. Mills, C. Ives, R. Gibson, D. Merberg, S. D. Mills, Q. Jiang, D. E. Taylor, G. F. Vovis, and T. J. Trust. 1999. Genomic-sequence comparison of two unrelated isolates of the human gastric pathogen *Helicobacter pylori*. *Nature* **397**:176–180. (Erratum, **397**:719.)
- Ames, B., and L. S. Gold. 1997. Environmental pollution, pesticides, and the prevention of cancer: misconceptions FASEB J. **11**:1041–1052.
- Bamba, T., H. Matsuda, M. Endo, and Y. Fujiyama. 1995. The pathogenic role of *Bacteroides vulgatus* in patients with ulcerative colitis. *J. Gastroenterol.* **30**:45–47.
- Berg, R. D. 1996. The indigenous gastrointestinal microflora. *Trends Microbiol.* **4**:430–435.
- Berghthorsson, U., and H. Ochman. 1998. Distribution of chromosome length variation in natural isolates of *Escherichia coli*. *Mol. Biol. Evol.* **15**:6–16.
- Blum, M., D. M. Grant, O. M. McBride, M. Heim, and U. A. Meyer. 1990. Human arylamine *N*-acetyltransferase genes: isolation, chromosomal localization and functional expression DNA. *Cell. Biol.* **9**:193–203.
- Bradford, M. M. 1976. A rapid and sensitive method for quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* **72**:248–254.
- Chang, F. C., and J. G. Chung. 1998. Evidence for arylamine *N*-acetyltransferase activity in the *Escherichia coli*. *Curr. Microbiol.* **36**:125–130.
- Chang, F. C., J. G. Chung, W. C. Chang, L. T. Wu, G. W. Chen, and S. H. Chang. 1997. Arylamine *N*-acetyltransferase activity in *Staphylococcus aureus*. *J. Microbiol. Immunol. Infect.* **30**:170–181.
- Chang, S. H., J. G. Chung, L. J. Huang, S. C. Chen, and S. C. Kuo. 1998. Ibuprofen affects arylamine *N*-acetyltransferase activity in *Helicobacter pylori* from peptic ulcer patients. *J. Appl. Toxicol.* **18**:179–185.
- Chen, G. W., C. F. Hung, S. H. Chang, J. G. Lin, and J. G. Chung. 1999. Purification and characterization of an arylamine *N*-acetyltransferase from *Lactobacillus acidophilus*. *Microbios* **98**:159–174.
- Chung, J. G., H. Kuo, T. Lin, C. Ho, J. Lee, J. Lai, G. Levy, and W. Weber. 1996. Evidence for arylamine *N*-acetyltransferase in the nematode *Anisakis simplex*. *Cancer Lett.* **106**:1–8.
- Chung, J. G. 1999. The effects of vitamin E on arylamine *N*-acetyltransferase activity in strains of *Helicobacter pylori* from peptic ulcer patients. *Food Chem. Toxicol.* **37**:655–661.
- Chung, J. G. 1998. Inhibitory actions of ellagic acid on growth and arylamine *N*-acetyltransferase activity in strains of *Helicobacter pylori* from peptic ulcer patients. *Microbios* **93**:115–127.
- Chung, J. G. 1998. Purification and characterization of an arylamine *N*-acetyltransferase from the bacteria *Aeromonas hydrophila*. *Curr. Microbiol.* **37**:70–73.
- Chung, J. G., H. M. Kuo, L. T. Wu, J. M. Lai, J. H. Lee, and C. F. Hung. 1997. Evidence for arylamine *N*-acetyltransferase in *Hymenolepis nana*. *J. Microbiol. Immunol. Infect.* **30**:1–17.
- Chung, J. G., M. F. Tsou, H. H. Wang, H. H. Lo, S. E. Hsieh, Y. S. Yen, L. T. Wu, S. H. Chang, C. C. Ho, and C. F. Hung. 1998. Rhein affects arylamine *N*-acetyltransferase activity in *Helicobacter pylori* from peptic ulcer patients. *J. Appl. Toxicol.* **18**:117–123.
- Chung, J. G., H. H. Wang, M. F. Tsou, S. E. Hsieh, H. H. Lo, Y. S. Yen, S. S. Chang, L. T. Wu, J. H. Lee, and C. F. Hung. 1997. Evidence for arylamine *N*-acetyltransferase activity in the bacterium *Helicobacter pylori*. *Toxicol. Lett.* **91**:63–71.
- Creighton, T. E. 1993. Amino acid residues, p. 6–20. In *Proteins: structures and molecular properties*, 2nd ed. W. H. Freeman and Co., New York, N.Y.
- Deloménie, C., G. H. Goodfellow, R. Krishnamoorthy, D. M. Grant, and J.-M. Dupret. 1997. Study of the role of the highly conserved residues Arg⁹ and Arg⁶⁴ in the catalytic function of human *N*-acetyltransferases NAT1 and NAT2 by site-directed mutagenesis. *Biochem. J.* **323**:207–215.
- Doll, M. A., and D. W. Hein. 1995. Cloning, sequencing and expression of NAT1 and NAT2 encoding genes from rapid and slow acetylators inbred rats. *Pharmacogenetics* **5**:247–251.
- Dupret, J.-M., and D. M. Grant. 1992. Site-directed mutagenesis of recombinant human arylamine *N*-acetyltransferase expressed in *Escherichia coli*. *J. Biol. Chem.* **267**:7381–7385.
- Ferguson, R., M. Doll, T. Rustan, and D. Hein. 1996. Cloning, expression and functional characterization of rapid and slow acetylator polymorphic *N*-acetyltransferase encoding genes of the syrian hamster. *Pharmacogenetics* **6**:55–66.
- Goodfellow, G. H., J.-M. Dupret, and D. M. Grant. 2000. Identification of amino acids imparting acceptor substrate selectivity in human arylamine acetyltransferases NAT1 and NAT2. *Biochem. J.* **348**:159–166.
- Grant, D. M., M. Blum, M. Beer, and U. A. Meyer. 1991. Monomorphic and polymorphic human arylamine *N*-acetyltransferases: a comparison of liver isozymes and expressed products of two cloned genes. *Mol. Pharmacol.* **39**:184–191.
- Grant, D. M., P. D. Josephy, H. L. Lord, and L. D. Morrison. 1992. *Salmonella typhimurium* strains expressing human arylamine *N*-acetyltransferases: metabolism and mutagenic activation of aromatic amines. *Cancer Res.* **52**:3961–3964.
- Hein, D. W., M. A. Doll, T. D. Rustan, K. Gray, Y. Feng, R. J. Ferguson, and D. M. Grant. 1993. Metabolic activation and deactivation of arylamine carcinogens by recombinant human NAT1 and polymorphic NAT2 acetyltransferases. *Carcinogenesis* **14**:1633–1638.
- Hein, D. W., D. M. Grant, and E. Sim. 2000. Update on consensus arylamine *N*-acetyltransferase gene nomenclature. *Pharmacogenetics* **10**:291–292.
- Hickman, D., J. Pope, S. D. Patil, G. Fakis, V. Smelt, L. A. Stanley, M. Payton, J. D. Unadkat, and E. Sim. 1998. Expression of arylamine *N*-acetyltransferase in human intestine. *Gut* **42**:402–409.
- Ho, C., T. Lin, Y. Lai, J. Chung, G. Levy, and W. Weber. 1996. Kinetics of acetyl coenzyme A-arylamine *N*-acetyltransferase from rapid and slow acetylator frog tissues. *Drug Metab. Dispos.* **24**:137–141.
- Horing, E., D. Gopfert, G. Schroter, and U. von Gaisberg. 1991. Frequency and spectrum of microorganisms isolated from biopsy specimens in chronic colitis. *Endoscopy* **23**:325–327.
- Hsieh, S. E., H. H. Lo, Y. S. Chen, and J. G. Chung. 1997. The effect of vitamin C on *N*-acetyltransferase activity in *Klebsiella pneumoniae*. *Food Chem. Toxicol.* **35**:1151–1157.
- Hsieh, S. E., H. H. Lo, and J. G. Chung. 1998. The characteristics of arylamine *N*-acetyltransferase in *Pseudomonas aeruginosa*. *Curr. Microbiol.* **36**:353–360.
- Ilett, K. F., F. F. Kadlubar, and R. F. Minchin. 1999. 1998 international meeting on the arylamine *N*-acetyltransferases: synopsis of the workshop on nomenclature, biochemistry, molecular biology, interspecies comparisons, and role in human disease risk, *Drug Metab. Dispos.* **27**:957–959.
- Ireland, A., J. D. Priddle, and D. P. Jewell. 1990. Comparison of 5-aminosalicylic acid and *N*-acetylaminosalicylic acid uptake by the isolated human colonic epithelial cell. *Gut* **33**:1343–1347.
- Ito, H., N. Kido, Y. Arakawa, M. Ohta, T. Sugiyama, and N. Kato. 1991. Possible mechanisms underlying the slow lactose fermentation phenotype in *Shigella* spp. *Appl. Environ. Microbiol.* **57**:2912–2917.
- Kelly, S. L., and E. Sim. 1994. Arylamine *N*-acetyltransferase in BALB/c mice: identification of a novel mouse isoenzyme by cloning and expression in vitro. *Biochem. J.* **302**:347–353.
- Kitz, R., and I. B. Wilson. 1962. Esters of methanesulfonic acid as irreversible inhibitors of acetylcholinesterase. *J. Biol. Chem.* **237**:3245–3249.
- Lo, H. H., and J. G. Chung. 1999. The effects of plant phenolics, caffeic acid, chlorogenic acid and ferulic acid on arylamine *N*-acetyltransferase activities in human gastrointestinal microflora. *Anticancer Res.* **19**:133–139.
- Maron, D. M., and B. N. Ames. 1983. Revised methods for the Salmonella mutagenicity test. *Mutat. Res.* **113**:173–215.
- Marteau, P., and C. Cellier. 1997. Effets indésirables de l'acide 5-aminosalicylique. *Gastroenterol. Clin. Biol.* **21**:377–386.
- Marteau, P., and M. Halphen. 1995. Comparative randomized open study of the efficacy and tolerance of enemas with 2 gr of 4-aminosalicylic acid (4-ASA) and 1 gr of 5-aminosalicylic acid (5-ASA) in distal forms of hemorrhagic rectocolitis. *Gastroenterol. Clin. Biol.* **19**:31–35.
- McCoy, E. C., H. S. Rosenkranz, and R. Mermelstein. 1981. Evidence for the existence of a family of bacterial nitroreductases capable of activating nitrated polycyclics to mutagens. *Environ. Mutagen.* **3**:421–427.
- Nakayama, H., T. Kinouchi, K. Kataoka, S. Akimoto, Y. Matsuda, and Y. Ohnishi. 1997. Intestinal anaerobic bacteria hydrolyse sorivudine, producing the high blood concentration of 5-(*E*)-(2-bromovinyl)uracil that increases the level and toxicity of 5-fluorouracil. *Pharmacogenetics* **7**:35–43.
- Neidhardt, F. C. 1987. Chemical composition of *Escherichia coli*, p. 3–6. In F. C. Neidhardt et al. (ed.), *Escherichia coli and Salmonella typhimurium: cellular and molecular biology*. American Society for Microbiology, Washington, D.C.
- Neut, C., J. F. Colombel, F. Guillemot, A. Cortot, P. Gower, P. Quandalle, M. Ribet, C. Romond, and J. C. Paris. 1989. Impaired bacterial flora in human excluded colon. *Gut* **30**:1094–1098.
- Ohsako, S., M. Ohtomi, Y. Sakamoto, K. Uyemura, and T. Deguchi. 1988. Arylamine *N*-acetyltransferase from chicken liver: cloning of cDNA and expression in Chinese hamster ovary cells. *J. Biol. Chem.* **263**:7534–7538.
- Okumura, F., O. Ueda, S. Kitamura, and K. Tatsumi. 1995. *N*-Acetylation and *N*-formylation of carcinogenic arylamines and related compounds in dogs. *Carcinogenesis* **16**:71–76.
- Payton, M., R. Auty, R. Delgoda, M. Everett, and E. Sim. 1999. Cloning and characterization of arylamine *N*-acetyltransferase genes from *Mycobacterium smegmatis* and *Mycobacterium tuberculosis*: increased expression results in isoniazid resistance. *J. Bacteriol.* **181**:1343–1347.
- Prantera, C., and M. L. Scribano. 1999. Current treatment for prevention of relapse and recurrence in Crohn's disease. *Ital. J. Gastroenterol. Hepatol.* **31**:515–518.
- Reddy, B. S., J. R. Pleasants, and B. S. Wostmann. 1969. Pancreatic enzymes in germfree and conventional rats fed chemically defined, water-soluble diet free from natural substrates. *J. Nutr.* **97**:327–334.
- Sandberg-Gertzen, H., J. Kjellander, B. Sundberg-Gilla, and G. Jarnerot.

1985. *In vitro* effects of sulphasalazine, azodisal sodium, and their metabolites on *Clostridium difficile* and some other faecal bacteria. *Scand. J. Gastroenterol.* **20**:607–612.
53. Sasaki, Y., S. Ohsako, and T. Deguchi. 1991. Molecular and genetic analyses of arylamine *N*-acetyltransferase polymorphism of rabbit liver. *J. Biol. Chem.* **266**:13243–13250.
54. Sim, E., M. Payton, M. Noble, and R. Minchin. 2000. An update on genetic structural and functional studies of arylamine *N*-acetyltransferases in eucaryotes and procaryotes. *Hum. Mol. Genet.* **9**:2435–2441.
55. Simmonds, N. J., A. D. Millar, D. R. Blake, and D. S. Rampton. 1999. Antioxidant effects of aminosaliclates and potential new drugs for inflammatory bowel disease: assessment in cell-free systems and inflamed human colorectal biopsies. *Aliment. Pharmacol. Ther.* **13**:363–372.
56. Simons, G. L., and S. L. Gorbach. 1981. Intestinal flora in health and disease, p. 1361–1380. *In* L. R. Johnson (ed.), *Physiology of the gastrointestinal tract*. Raven Press, New York, N.Y.
57. Sinclair, J. C., R. Delgoda, M. E. M. Noble, S. Jarmia, N. K. Goh, and E. Sim. 1998. Purification, characterization, and crystallization of an *N*-hydroxyarylamine *O*-acetyltransferase from *Salmonella typhimurium*. *Protein Expr. Purif.* **12**:371–380.
58. Sinclair, J. C., J. Sandy, R. Delgoda, E. Sim, and M. E. M. Noble. 2000. Structure of arylamine *N*-acetyltransferase reveals a catalytic triad. *Nat. Struct. Biol.* **7**:560–564.
59. Thomson, A. B. R., and C. N. Williams. 1999. Trends in inflammatory bowel disease therapy. *Can. J. Gastroenterol.* **13**:775–776.
60. Tomb, J. F., O. White, A. R. Kerlavage, R. A. Clayton, G. G. Sutton, R. D. Fleischmann, K. A. Ketchum, H. P. Klenk, S. Gill, B. A. Dougherty, K. Nelson, J. Quackenbush, L. Zhou, E. F. Kirkness, S. Peterson, B. Loftus, D. Richardson, R. Dodson, H. G. Khalak, A. Glodek, K. McKenney, L. M. Fitzgerald, N. Lee, M. D. Adams, J. C. Venter, et al. 1997. The complete genome sequence of the gastric pathogen *Helicobacter pylori*. *Nature* **388**:539–547. (Erratum, **389**:412.)
61. Tsou, M. F., J. G. Chung, L. T. Wu, K. S. Cheng, and C. F. Hung. 1998. Characterization of arylamine *N*-acetyltransferase in *Enterobacter aerogenes*. *Microbios* **94**:133–143.
62. Tsou, M. F., C. F. Hung, H. F. Lu, L. T. Wu, S. H. Chang, H. L. Chang, G. W. Chen, and J. G. Chung. 2000. Effects of caffeic acid, chlorogenic acid and ferulic acid on growth and arylamine *N*-acetyltransferase activity in *Shigella sonnei* (group D). *Microbios* **101**:37–46.
63. van Hogezaand, R. A., H. M. Kennis, A. van Schaik, J. P. Koopman, P. A. van Hees, and J. H. Tongeren. 1992. Bacterial acetylation of 5-aminosalicylic acid in faecal suspensions cultured under aerobic and anaerobic conditions. *Eur. J. Clin. Pharmacol.* **43**:189–192.
64. Watanabe, M., M. J. Ishidate, and T. Nohmi. 1990. Sensitive method for the detection of mutagenic nitroarenes and aromatic amines: new derivatives of *Salmonella typhimurium* tester strains possessing elevated *O*-acetyltransferase levels. *Mutat. Res.* **234**:337–348.
65. Watanabe, M., T. Sofuni, and T. Nohmi. 1992. Involvement of Cys⁶⁹ residue in the catalytic mechanism of *N*-hydroxyarylamine *O*-acetyltransferase of *Salmonella typhimurium*: sequence similarity at the amino acid level suggests a common catalytic mechanism of acetyltransferase for *S. typhimurium* and higher organisms. *J. Biol. Chem.* **267**:8429–8436.
66. Whitaker, D. P., and M. W. Goosey. 1993. Purification and properties of the enzyme arylamine *N*-acetyltransferase from the housefly *Musca domestica*. *Biochem. J.* **295**:149–154.
67. Zeitz, M. 1999. Consequences of galenic considerations and clinical results for therapy of ulcerative colitis. *Med. Klin.* **94**(Suppl. 1):41–43. (In German.)
68. Zhou, S. Y., D. Fleisher, L. H. Pao, C. Li, B. Winward, and E. M. Zimmermann. 1999. Intestinal metabolism and transport of 5-aminosalicylate. *Drug Metab. Dispos.* **27**:479–485.