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Glycosidase Profiles of Members of the Family *Enterobacteriaceae*

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A total of 712 strains representing 47 taxa of the family *Enterobacteriaceae* were tested for the ability to hydrolyze 14 4-methylumbelliferyl (4-MU)-linked substrates within 3 h of incubation. In addition to the well-known differentiation potential of the hydrolysis of 4-MU–β-D-galactopyranoside, 4-MU–β-D-glucuronide, and 4-MU–β-D-xylopyranoside, the hydrolysis of some other fluorogenic substrates (e.g., 4-MU–β-D-fucopyranoside, 4-MU–*N*-acetyl-β-D-galactosaminide, and 4-MU–α-D-galactopyranoside) can also be used for species differentiation within the family *Enterobacteriaceae*.

Qualitative enzyme tests using chromogenic or fluorogenic substrates are used for differentiation of a wide range of different bacteria. Within the family *Enterobacteriaceae*, β-galactosidase, β-glucuronidase, and β-xylosidase have been included in several diagnostic schemes as very useful for identification purposes (1, 5, 13, 18). Further studies including commercial kits (API ZYM [Analytab Products, Plainview, N.Y.]) (10) or the application of quantitative enzyme tests (8) showed further the value of those tests for separation purposes. Although the application of traditional tests in *Enterobacteriaceae* diagnostics as described by Farmer et al. (7) cannot be replaced by qualitative enzyme tests, the latter may be useful as additional characteristics for differentiation. The purpose of this study was to evaluate the diagnostic value of 14 fluorogenic glycosidase substrates for differentiation of 47 taxa within the family *Enterobacteriaceae*.

A total of 712 strains of the family *Enterobacteriaceae* were tested. Of these, 323 were obtained from the National Collection of Type Cultures, Colindale, London, England, and the rest were obtained from different clinical specimens identified by conventional methods (7). All strains were further identified by using the TTE-AS system (ICN-Flow Laboratories, Meckenheim, Federal Republic of Germany) (11). Before testing, all isolates were grown on MacConkey Agar (Merck, Darmstadt, Federal Republic of Germany) for 24 h at 37°C. To assess purity, cultures were checked throughout the study by colony morphology.

A total of 14 4-methylumbelliferyl (4-MU)-linked substrates, obtained from Serva, Heidelberg, Federal Republic of Germany, or Sigma, Deisenhofen, Federal Republic of Germany, were tested (Table 1). Most of the substrates were predissolved in a few drops of dimethyl sulfoxide. Except for 4-MU–fucopyranosides und 4-MU–β-D-xylopyranoside, all substrates were dissolved at a final concentration of 2×10^{-4} M in 0.1 M Tris-HCl buffer. 4-MU–fucopyranosides and 4-MU-β-D-xylopyranoside were dissolved in 0.1 M phosphate buffer, because fucosidases and xylosidases are inhibited by Tris-HCl buffer (12). The pH was 8.0 throughout. All test solutions were sterilized by filtration and dispensed in 100-μl amounts into microplates (ICN Flow). To each well was added 100 μl of a heavy bacterial suspension in 0.9% NaCl (McFarland turbidity standard tube no. 8). After inoculation, the microplate was sealed with a sterile plastic sealer. Following incubation for 3 h at 37°C, test plates were read with a Fluoroscan II fluorimeter (ICN Flow Laboratories) connected to a personal computer (Deskpro286; Compaq, Munich, Federal Republic of Germany). Prior to measurement of test results, the fluorimeter was blanked with 10^{-4} M quinoline dissolved in 0.1 N H_2SO_4, resulting in a zero-fluorescence value of −1,000. The fluorescence of a negative control containing no fluorogenic substrate was always recorded.

Results were measured at wavelengths of 355 nm for excitation and 460 nm for emission. After calculation of the difference ($S = T - C$) between the relative fluorescence intensities of the test (T) and the control (C) wells, a test was coded positive if S exceeded 50.

Reproducibility of the reactions was determined by testing 44 isolates (one of each taxon) three times on separate days, and an overall probability of erroneous results (p_i) of 5.3% was calculated with formula 4 of Sneath and Johnson (17), which is within acceptable limits.

All test strains were negative for hydrolysis of 4-MU–β-L-fucopyranoside and 4-MU–α-D-mannopyranoside and positive for hydrolysis of 4-MU–β-D-mannopyranoside. Test results for the remaining 11 substrates are shown as percentages of positive tests for each taxon in Table 1. The results for β-galactosidase (*o*-nitrophenyl-β-D-galactopyranoside test) were essentially in line with those published by Farmer et al. (7), although the percentages of positive results were slightly different because of the different numbers of strains. β-Glucuronidase activity was found in *Escherichia coli*, *Shigella* sp., and *Salmonella* sp., as already reported by Kilian and Bülow (12). Within the genus *Escherichia*, this property was found to be restricted to *E. coli*. None of the *E. blattae*, *E. fergusonii*, *E. hermannii*, or *E. vulneris* isolates showed β-glucuronidase activity. Interestingly, we found β-glucuronidase in few strains of *Edwardsiella hoshinae* and *Yersinia kristensenii*, which was not previously reported in the literature. However, the diagnostic value of this test, especially for detection of *E. coli* (6, 9, 16, 18), is supported.

For the β-xylosidase reaction, only restricted data are available. This test was introduced by Brisou et al. (5), who found this enzyme activity in almost all *Klebsiella pneumoniae* and all *K. oxytoca*, *Enterobacter aerogenes*, and *E. cloacae* strains, which was confirmed by our results. Furthermore, the results were in line with those published for *E. gergoviae* (4) and *Yersinia* sp. (2) but only partly in line with the results of Godsey et al. (8), who showed no activity for *K. pneumoniae*, *E. aerogenes*, or *Shigella sonnei*. The

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TABLE 1. Glycosidase profiles of members of the family *Enterobacteriaceae* obtained by using 4-MU-linked substrates after 3 h of incubation

Species	No. of strains	% Positive for test[a]										
		ARA	CEL	αGAL	βGAL[b]	*N*-Ac βGAL	αGLU	βGLU[b]	βGLUO[b]	*N*-Ac βGLU[b]	βFUC	βXYL[b]
Citrobacter freundii	76	95	8	95	93	1	1	84	0	3	93	3
Citrobacter diversus	12	100	100	8	100	8	67	100	0	67	100	0
Citrobacter amalonaticus	15	93	80	7	100	40	13	80	0	80	60	60
Edwardsiella tarda	4	25	0	50	0	75	0	0	0	75	0	0
Edwardsiella hoshinae	6	0	0	83	0	100	0	17	50	100	0	0
Enterobacter aerogenes	5	100	100	100	100	80	60	100	0	100	100	100
Enterobacter cloacae	14	100	57	79	100	71	21	100	0	93	100	100
Enterobacter sakazakii	4	100	100	100	100	100	75	100	0	100	100	75
Enterobacter gergoviae	15	100	80	100	100	87	7	100	0	87	100	0
Enterobacter taylorae	7	100	100	0	100	100	0	100	0	100	100	0
Escherichia coli	43	98	0	98	100	0	0	16	100	5	98	0
Escherichia blattae	3	0	0	0	0	100	0	0	0	100	0	0
Escherichia fergusonii	3	100	100	100	100	0	0	67	0	67	100	0
Escherichia hermannii	3	100	100	67	100	0	0	100	0	0	100	0
Escherichia vulneris	8	100	75	100	100	0	0	100	0	0	100	87
Ewingella americana	6	100	17	0	100	100	0	100	0	100	67	17
Hafnia alvei	49	73	2	0	90	78	0	29	0	100	16	0
Klebsiella oxytoca	8	100	0	87	100	0	50	100	0	0	100	62
Klebsiella ozaenae	6	100	0	83	100	0	0	83	0	50	100	33
Klebsiella pneumoniae	12	100	58	100	100	0	75	100	0	75	100	92
Kluyvera sp.[c]	18	100	83	89	100	11	56	100	0	78	100	72
Morganella morganii	44	20	0	0	0	0	0	0	0	89	0	0
Obesumbacterium proteus	6	0	0	0	0	67	0	100	0	100	0	0
Proteus mirabilis	77	18	0	0	0	0	0	0	0	52	0	0
Proteus vulgaris	20	10	0	0	0	0	85	60	0	85	0	0
Providencia alcalifaciens	5	20	0	0	0	0	0	0	0	60	0	0
Providencia rettgeri	7	29	0	0	0	0	0	71	0	100	0	0
Providencia heimbachae	4	0	0	0	0	0	0	0	0	100	0	0
Rahnella aquatilis	6	100	17	100	100	0	17	100	0	0	100	100
Salmonella arizonae	9	100	0	89	100	0	0	22	56	11	78	0
Salmonella typhi	14	7	0	100	0	0	0	43	0	0	0	0
Salmonella enteritidis	6	33	0	67	0	0	0	83	0	0	0	0
Salmonella sp.[d]	28	18	4	96	4	0	0	18	7	36	0	0
Shigella sonnei	4	100	0	100	100	0	0	75	100	25	100	50
Shigella sp.[d]	15	0	0	87	0	0	0	13	33	7	0	0
Serratia marcescens	24	96	37	0	96	100	0	96	0	100	46	0
Serratia rubidaea	7	100	0	100	100	100	100	100	0	100	100	100
Serratia liquefaciens	12	75	17	58	92	92	42	92	0	92	42	0
Serratia odorifera	2	0	0	100	100	100	0	100	0	100	100	100
Serratia fonticola	4	100	25	75	100	100	25	100	0	100	100	100
Serratia ficaria	4	50	50	25	75	50	50	100	0	100	25	0
Yersinia enterocolitica	36	94	47	0	100	100	3	81	0	100	75	3
Yersinia pseudotuberculosis	13	15	0	0	100	85	0	100	0	92	100	92
Yersinia frederiksenii	17	100	24	0	100	100	0	100	0	100	100	41
Yersinia kristensenii	9	11	22	0	89	100	0	100	44	100	11	0
Yersinia intermedia	13	100	8	54	100	100	0	100	0	100	100	0
Yersinia ruckeri	9	11	0	0	44	100	0	44	0	100	11	0

[a] Substrates hydrolyzed: ARA, 4-MU–α-L-arabinopyranoside; CEL, 4-MU–β-D-cellopyranoside, αGAL, 4-MU– α-D-galactopyranoside; βGAL, 4-MU–β-D-galactopyranoside; *N*-Ac βGAL, 4-MU–*N*-acetyl-β-D-galactopyranoside; βGLU, 4-MU–α-D-glucopyranoside; βGLU, 4-MU–β-D-glucopyranoside; βGLUO, 4-MU–β-D-glucuronide; N-Ac βGLU, 4-MU–*N*-acetyl-β-D-glucopyranoside; βFUC, 4-MU–β-D-fucopyranoside; βXYL, 4–MU-β-D-xylopyranoside. All strains were negative for hydrolysis of 4-MU–β-L-fucopyranoside and 4-MU–α-D-mannopyranoside and positive for hydrolysis of 4-MU–β-D-mannopyranoside.

[b] Obtained from Serva; all other substrates were obtained from Sigma.

[c] Strains of *K. ascorbata* and *K. cryocrescens* were tested.

[d] Identified only to the genus level.

reported β-xylosidase reactions for *Citrobacter freundii*, *Morganella morganii*, *Serratia marcescens*, *Proteus mirabilis*, and *E. coli* (8) were in line with our results; however, it should be pointed out that the test results of Godsey et al. (8) were read after 30 min of incubation. The results of the β-xylosidase reaction tested for more than 800 strains in the TTE-AS system for identification of members of the family *Enterobacteriaceae* (11) were confirmed.

Furthermore, in the study of Godsey et al. (8), the results for hydrolysis of 4-MU–α-D-arabinopyranoside, 4-MU–α-D-galactopyranoside, 4-MU–β-D-glucopyranoside, 4-MU–β-D-galactopyranoside, 4-MU–*N*-acetyl-β-D-glucosaminide, and 4-MU–β-D-glucuronide were recorded for 10 species. These results were largely in line with our results. The few discrepant results may be due to the short time of incubation (30 min) used by Godsey et al. (8).

The results of Muytjens et al. (15) obtained with the α-glucosidase reaction for differentiating *E. sakazakii* from

other *Enterobacter* spp. were not confirmed, because we also found α-glucosidase activities in other *Enterobacter* species (Table 1). However, the glycosidase reactions can vary because the enzymes may be inducible, as reported for the β-glucosidase responsible for esculin hydrolysis (14).

To our knowledge, results for the β-D-fucosidase reaction have not previously been reported in the literature. The enzyme profiles of almost all of the species tested were similar to the results of the β-D-galactosidase reaction (Table 1), which is not surprising, because the fucose molecule (6-deoxygalactose) largely resembles the galactose molecule.

Although our data are largely in line with data in the literature, several differences in our results compared with data in the literature were found because of varying parameters like incubation temperature, inoculum size, and duration of incubation, as already stated by Brenner (3). Despite these minor differences, Table 1 gives a compilation of glycosidase profiles for a large number of strains belonging to 47 different taxa of the family *Enterobacteriaceae*.

Some of these tests can be easily used for differentiation of species in addition to the biochemical reactions given by Farmer et al. (7).

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