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BRIEF NOTE

Differentiation of *Shigella* species from *Escherichia coli* by glycerol dehydrogenase activity

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It has been determined that *Escherichia coli* and the four *Shigella* species (except *S. boydii* serotype 13) form a single genomospecies (Brenner, 1984). *E. coli* and the so-called *Shigella* species are biochemically and serologically closely related (Ewing, 1986). The "O" antigens of many serotypes of *Shigella* are either identical to or closely related to those of *E. coli* (Ewing, 1986). The biochemical identification of *Shigella* sometimes presents difficulties in differentiating it from the biochemically atypical *E. coli* strains. The genus *Shigella* is composed of non-motile bacteria. With the exception of certain biotypes of *S. flexneri* 6, *Shigella* do not form gas from fermentable carbohydrates and, compared to *Escherichia, Shigella* are less active in their utilization of carbohydrates. Christensen's citrate, sodium acetate, sodium mucate and decarboxylation of lysine are of considerable value in the differentiation of members of the genus *Shigella* from *Escherichia,* particularly from anaerogenic nonmotile biotypes of *E. coli* (Ewing, 1986). *Shigella* do not grow fermentatively on Christensen's citrate; sodium mucate is utilized by some cultures of *S. sonnei* but not by other *Shigella.* Sodium acetate is utilized as a sole source of carbon by some biotypes of *S. flexneri* 4a but not by other *Shigella.* Lysine is not decarboxylated by *Shigella.* The characteristic "invasiveness" of *Shigella* strains in the guinea pig keratoconjunctivitis test (Sereny test) (Sereny, 1957) is also shown by enteroinvasive *E. coli.*

The diversity of the glycerol pathway has been documented in enterobacterial species (Bouvet *et al.*, 1994, 1995). Species which can grow fermentatively on glycerol possess typical enzymes of the anaerobic glycerol pathway (glycerol dehydrogenase type I (glyDH-I), dihydroxyacetone kinase, glycerol dehydratase and 1,3-propanediol dehydrogenase). *E. coli* cannot grow fermentatively on glycerol because of the absence of enzymes of the anaerobic glycerol pathway. An NAD^+-linked glycerol dehydrogenase, glyDH-II, induced by glycerol and hydroxyacetone (thus distinct from glyDH-I) responsible for salvaging the glycerol moiety of degradation products of phospholipids and triglycerides, has been described in *E. coli* (Jin *et al.*, 1983; Lin, 1976; St. Martin *et al.*, 1977; Tang *et al.*, 1979, 1982). The structural gene of the *E. coli* glyDH-II has been mapped and cloned (Truniger and Boos, 1994). The identity of glyDH-II of *E. coli* with D-1-amino-2-propanol oxidoreductase has been reported and its participation in vitamin B_{12} metabolism has been suggested (Kelley and Dekker, 1985). The levels of the glyDH-II activity are markedly induced by oxidized substrates (*i.e.* aminoacetone, hydroxyacetone, dihydroxyacetone). GlyDHs from a few bacterial species including *Hafnia alvei* and *"Salmonella enterica"* were found to be distinct from *E. coli* glyDH. GlyDH-III from *H. alvei* is induced by glycerol and not by hydroxyacetone whereas glyDH-IV of *"S. enterica"* is induced by hydrox-

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yacetone only (Bouvet *et al.*, 1994). Recently, the distribution of glyDHs type I, II, III and IV and 1,3-propanediol dehydrogenase among the *Enterobacteriaceae* was studied (Bouvet *et al.*, 1995). GlyDHs other than type I which are not associated with glycerol fermentation are widely distributed among the *Enterobacteriaceae.*

This study evaluated glyDHs as complementary tests for differentiating *E. coli* from *Shigella* species.

A total of 1,000 recent isolates belonging to *E. coli* (626 strains) and *Shigella* species (374 strains) from human clinical specimens (stool, urine, blood) or veterinary specimens (*E. coli* only) received at the French Salmonella and Shigella Reference Center (CNS) were included. *E. coli* strains belonging to 164 different O serogroups had been received from the International *Escherichia* and *Klebsiella* Center. Biochemical identification and serotype determination were carried out by conventional methods (Ewing, 1986) and "Biotype-100" carbon source strips (BioMérieux, La Balme-les-Grottes, France) and software "Recognizer" (Institut Pasteur Taxolab, Paris, France). The study included 67 biochemically atypical *E. coli* strains. *E. coli* diarrhoeogenic pathotypes: enterotoxinogenic (ETEC), enteroinvasive (EIEC), enteropathogenic (EPEC) and enterohaemorrhagic (EHEC) types have been previously identified using DNA probes specific for pathogenicity genes (Bohnert *et al.*, 1988). Two new serotypes 14 and 15 (Groo *et al.*, 1989; Ansaruzzaman *et al.*, 1995) of *Shigella dysenteriae* were included. All these strains were subcultured twice in Luria broth medium before performing the tests.

For the detection of glycerol dehydrogenase, bacteria were grown overnight at 30°C on tryptocasein soy agar plates (TCS, Diagnostics Pasteur, Marnes-la-Coquette, France) supplemented with 1% (vol/vol) glycerol or 70 mM hydroxyacetone (HA) both sterilized by filtration. Cultures grown on TCS-glycerol were incubated in an anaerobic jar with catalyst, H_2+CO_2 generators (GasPak, BBL Becton Dickinson Microbiology Systems, Cockeysville, MD) and methylene blue indicator strip. Cultures grown on TCS-hydroxyacetone were incubated aerobically.

The bacterial growth from TCS-glycerol or TCS-hydroxyacetone agar plates was collected and suspended in reaction buffer to an absorbance (at 600 nm) of about 0.6. The reaction buffer contained 0.1 M K_2CO_3, 30 mM $(NH_4)_2SO_4$ and was adjusted to pH 9.0. It was stored in a well-stoppered bottle at 4°C up to one month. Bacterial suspensions from TCS-glycerol or TCS-hydroxyacetone agar plates were immediately dispensed (150 µl) into 96-well microtitre plates (Dynatech AG, Denkerdorf, Germany). Then, 30 µl portions of a reagent (NAD, 210 mg; glycerol, 600 µl; nitro-blue tetrazolium, 42 mg; phenazine methosulphate, 2 mg; distilled water to 10 ml) (kept frozen at −20°C in the dark) were added to the wells and the plates were gently shaken and incubated in the dark at room temperature. The plates were examined for the development of a purple colour within 15 to 30 min. The colour in the uninoculated control medium remained unchanged (yellow). The control strains which we used were *Serratia odorifera* Grimont 10-73^T (negative), and *E. coli* CNS 75-88 (positive). To validate the test, five colonies of ten isolates were studied and no discrepancy was observed. Growth temperature (30 or 37°C) had no effect on the activity of this enzyme. Hydroxyacetone was from Aldrich (Strasbourg, France; cat. no. 13,818-5). All other biochemicals were obtained from Sigma Chemicals Co. (St. Louis, MO) and were reagent grade.

Table I indicates that 610/626 (97%) of *E. coli* strains tested produced a glyDH type II induced by glycerol and hydroxyacetone, and no *Shigella* strain demonstrated such activity except *S. flexneri* serotype 6 biotype Manchester. The remaining 16 *E. coli* strains lacking the glyDH-II were identified as follows: 8 biochemically typical *E. coli*, 7 biochemically atypical

glyDH = glycerol dehydrogenase.
HA = hydroxyacetone.
TCS = tryptocasein soy (medium).

Table I. Distribution of glyDH induced by glycerol and/or hydroxyacetone, among *E. coli* and *Shigella* species.

	GlyDH induced by: glycerol		hydroxyacetone	
E. coli	+	(610/626) (*)	+	(617/626)
S. boydii				
serotypes 1, 5, 8, 9, 10, 12, 16, 17, 18	–	(0/30)	–	(0/30)
serotypes 7, 11, 13, 14, 15	–	(0/22)	+	(22/22)
serotypes 2, 3	–	(0/20)	d	(4/20)
serotype 4	–	(0/16)	d	(13/16)
S. dysenteriae				
serotype 1	–	(0/15)	+	(15/15)
serotypes 2, 3, 4, 5, 7, 9, 10, 11, 12, 14, 15	–	(0/27)	–	(0/27)
serotypes 6, 8	–	(0/4)	d	(2/4)
S. flexneri				
serotypes 1, 2, 3, 4, 5, X, Y	–	(0/116)	–	(0/116)
serotype 6 biotype Manchester	+	(4/4)	+	(4/4)
serotype 6 biotype Manchester "variant"	–	(1/8) (**)	+	(8/8)
serotype 6 biotype Boyd 88	–	(0/12)	+	(12/12)
serotype 6 biotype Herfordshire	–	(0/10)	+	(10/10)
S. sonnei biotypes a, d, e, f, g	–	(0/90)	–	(0/90)

(*) +=97 to 100% positive; –=0 to 1% positive; d=2 to 96% positive. The numbers in parentheses indicate the number of positive strains/number of strains tested. *E. coli*-negative strains were: CNS (French Salmonella Reference Center) 929876, CNS 85-209, CNS 87-249, CNS 87-299, CNS 930215, CNS 933112, CNS 937249, CNS 930888, CNS 930241, CNS 934567, CNS 933630, CNS 938458, CNS 931964, CNS 9311876, CNS 934103 and 4370-53.

(**) *S. flexneri* serotype 6 biotype Manchester "variant" positive strain was 165-88.

E. coli (non-motile and/or not aerogenic, no decarboxylation of lysine) and one enteroinvasive *E. coli*.

A glyDH activity type IV, induced only by HA, was detected in a few serotypes of *Shigella*: *S. dysenteriae* serotype 1, *S. flexneri* serotype 6 biotypes Boyd 88, Manchester "variant" and Herfordshire, and *S. boydii* serotypes 7, 11, 13, 14 and 15. In contrast, all biotypes of *S. sonnei*, seven serotypes of *S. flexneri*, eleven serotypes of *S. dysenteriae* and nine serotypes of *S. boydii* gave negative results. Different results occurred in a few serotypes: *S. dysenteriae* serotypes 6 and 8 and *S. boydii* serotype 4. Only the type strain and the strain used as reference in serological studies of *S. boydii* serotypes 2 and 3 gave a positive test.

E. coli and *Shigella* species are closely related genetically, biochemically and serologically, and intermediate strains may be difficult to identify. The glyDH test is a simple and rapid tool which can be an aid in differentiating *Shigella* from biochemically atypical *E. coli* strains in addition to common tests.

Key-words: Glycerol dehydrogenase, *Shigella*, *Escherichia coli*; Differentiation.

Différenciation de *Shigella* de *Escherichia coli* par l'activité glycérol déshydrogénase

Un test enzymatique simple, rapide et peu coûteux a été utilisé pour détecter la présence d'une glycérol-déshydrogénase de type II (induite par le glycérol et l'hydroxyacétone) ou de type IV (induite seulement par l'hydroxyacétone) chez 1.000 souches appartenant à *Escherichia coli* et *Shigella*. Une glycérol-déshydrogénase de type II a été trouvée chez 97 % des souches de *E. coli* testées. Cette activité n'a été détectée chez aucune espèce de *Shigella* à l'exception de *S. flexneri* sérotype 6 biotype Manchester. Une glycérol-déshydrogénase de

type IV a été trouvée chez *S. dysenteriae* sérotype 1, *S. flexneri* sérotype 6 biotypes Boyd 88, Manchester «variant» et Herfordshire, et *S. boydii* sérotypes 7, 11, 13, 14 et 15. Les autres sérotypes de *Shigella* ne présentent aucune de ces deux activités enzymatiques.

Mots-clés: Glycérol-déshydrogénase, *Shigella, Escherichia coli;* Différenciation.

References

Ansaruzzaman, M., Kibriya, A.K.M.G., Rahman, A., Neogi, P.K.B., Faruque, A.S.G., Rowe, B. & Albert, M.J. (1995), Detection of provisional serovars of *Shigella dysenteriae* and designation as *S. dysenteriae* serotypes 14 and 15. *J. Clin. Microbiol.*, 33, 1423-1425.

Bohnert, M.G., d'Hauteville, H.M. & Sansonetti, P.J. (1988), Detection of enteric pathotypes of *Escherichia coli* by hybridization using six DNA probes. *Ann. Inst. Pasteur/Microbiol.*, 139, 189-202.

Bouvet, O.M.M., Lenormand, P., Carlier, J.P. & Grimont, P.A.D. (1994), Phenotypic diversity of anaerobic glycerol dissimilation shown by seven enterobacterial species. *Res. Microbiol.*, 145, 129-139.

Bouvet, O.M.M., Lenormand, P., Ageron, E. & Grimont, P.A.D. (1995), Taxonomic diversity of the anaerobic glycerol dissimilation in the *Enterobacteriaceae. Res. Microbiol.*, 146, 279-290.

Brenner, D.J. (1984), Family I. Enterobacteriaceae, pp. 408-420, *in* "Bergeys's manual of systematic bacteriology", Krieg, N.R. and Holt, J.G. (ed.) vol. 1. Will' ms and Wilkins, Baltimore.

Ewing, W.H. (1986), Edwards and Ewing's identification of *Enterobacteriaceae*, 4th ed. Elsevier Science Publis., Amsterdam.

Groo, R.J., Thomas, L.V., Cheasty, T., Rowe, B. & Lindberg, A.A. (1989), Four new provisional serovars of *Shigella. J. Clin. Microbiol.*, 27, 829-831.

Jin, R.Z., Tang, J.C.-T. and Lin, E.C.C. (1983), Experimental evolution of a novel pathway for glycerol dissimilation in *Escherichia coli. J. Mol. Evol.*, 19, 429-436.

Kelley, J.J. & Dekker, E.E. (1985), Identity of *Escherichia coli* D-1-amino-2-propanol:NAD^+ oxidoreductase with *E. coli* glycerol dehydrogenase but not with *Neisseria gonorrhoeae* 1,2-propanediol:NAD^+ oxidoreductase. *J. Bacteriol.*, 162, 170-175.

Lin, E.E. (1976), Glycerol dissimilation and its regulation in bacteria. *Annu. Rev. Microbiol.*, 30, 535-578.

Sereny, B. (1957), Experimental keratoconjunctivitis shigellosa. *Acta. Microbiol. Acad. Sci. Hung.*, 4, 367-376.

St. Martin, E.J., Freedberg, W.B. & Lin, E.C.C. (1977), Kinase replacement by a dehydrogenase for *Escherichia coli* glycerol utilization. *J. Bacteriol.*, 131, 1026-1028.

Tang, J.C.-T., Ruch, F.E., Jr & Lin, E.C.C. (1979), Purification and properties of nicotinamide adenine dinucleotide-linked dehydrogenase that serves an *Escherichia coli* mutant for glycerol catabolism. *J. Bacteriol.*, 140, 182-187.

Tang, J.C.-T., St. Martin, E.J. & Lin, E.C.C. (1982), Derepression of an NAD-linked dehydrogenase that serves an *Escherichia coli* mutant for growth on glycerol. *J. Bacteriol.*, 152, 1001-1007.

Truniger, V. & Boos, W. (1994), Mapping and cloning of *gldA*, the structural gene of the *Escherichia coli* glycerol dehydrogenase. *J. Bacteriol.*, 176, 1796-1800.