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Starch Gel Electrophoresis of Fructose-6-Phosphate Phosphoketolase in the Genus *Bifidobacterium*

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Three groups of nomenspecies of the genus *Bifidobacterium* were distinguished by the different mobility of their fructose-6-phosphate phosphoketolase in starch-gel electrophoresis; there is apparently a close relatedness between electrophoretic type of phosphoketolase and habitat.

Although the taxonomic position of the genus *Bifidobacterium* is still controversial (2, 5, 6), the view is widely accepted that the bifid bacteria can be distinguished from other bacterial groups like lactobacilli, actinomycetes, and anaerobic corynebacteria by the peculiar pathway employed for carbohydrate dissimilation, i.e., the "fructose-6-phosphate shunt" (1, 8, 12), leading to the formation of lactic and acetic acids in the ratio 1.0:1.5 as chief end products.

The subdivision of the genus *Bifidobacterium* into many nomenspecies (Table 1) has been recently suggested by some investigators (4, 7, 9, 10). It must be admitted that in many cases the differences between the species are small: *B. infantis* and *B. parvulorum* have identical morphology and differ only in glucoside fermentation; the same is true for *B. liberorum* and *B. lactentis*. The ability to develop at 46.5 C (*see* Table 1) is of little importance: all bifids can grow well at 43 to 44 C. Other physiological characteristics like behavior toward oxygen, nitrate reduction, catalase, H_2S, and acetoin formation are uniform in all bifids. The presence or absence of aldolase can be of value in discriminating the nomenspecies isolated from intestines of man and bees (aldolase negative) from those isolated from feces of other animals or from rumen of cattle (aldolase positive) (10). Morphology is of little help. Only *B. asteroides* from honey bee intestine has a peculiar form of cells and aggregates; some morphological patterns can be perceived, but they are common to groups of nomenspecies. The deoxyribonucleic acid (DNA) base composition is uniform, about 60% guanine plus cytosine (13); only *B. globosum* is an exception to the rule (65% guanosine plus cytosine); no data are available concerning the species proposed by Mitsuoka (4). One could gain the impression that the separation of specific taxa in the genus *Bifidobacterium* is premature, so few are the known phenotypic distinctive characters. Two considerations militate against this conclusion. (i) Bifid bacteria are found not only in feces of man but in many other habitats like rumen of cattle, feces of various animals, intestine of honey bees, in anaerobic laboratory digesters (Toerien, *personal communication*), and in surface waters (Scardovi et al., *unpublished data*). (ii) Preliminary investigations on DNA homologies among the nomenspecies isolated from rumen of cattle, intestine of bees, and some of the species from human or other animal feces revealed the absence of reciprocal relatedness (11). Specific taxa in the genus *Bifidobacterium* can therefore be recognized on a genetic basis. In the search for an additional tool for the speciation of the genus *Bifidobacterium*, we studied the electrophoretic properties of fructose-6-phosphate phosphoketolase, a key enzyme in the energy-yielding metabolism of this bacterial group.

The strains employed (Table 2) are under study in our laboratory and have been checked repeatedly for purity and for constancy of physiological and morphological characters.

Cells grown on the medium of the composition elsewhere reported (8) were disrupted mechanically with glass beads in a Nossal apparatus. Clarified extracts in tris(hydroxymethyl)aminomethane(Tris)-ethylenediaminetetraacetic acid (EDTA)-borate buffer at *p*H 8.0 (Tris, 6.0 g; boric acid, 4.0 g; Na_2-EDTA·$2H_2O$, 0.6 g/liter), with a protein content of 6 to 12 mg/ml, were run with the starch-gel horizontal electrophoresis system of Smithies (14), with hydrolyzed starch gel (Connaught) prepared with the Tris-EDTA-

TABLE 1. *Key of the nomenspecies of the genus Bifidobacterium*

Fermentative characters[a]				Nomenspecies	Main source
Arabinose:0 Lactose:+	Xylose:0 Ribose:0 Mannose:0	Growth at 46.5 C:0	Maltose:0 Raffinose:0	*B. bifidum* (Tissier)	Feces of man
			Maltose:+ Raffinose:+	*B. globosum* (Scardovi)	Rumen of cattle
		Growth at 46.5 C:+	Maltose:+ Raffinose:+	*B. thermophilum* (Mitsuoka)	Feces of pigs and chickens
	Ribose:+ Mannose:+	Mannitol:0 Sorbitol:0	Amygdalin:0 Esculin:0	*B. infantis* (Reuter)	Feces of man
			Amygdalin:+ Esculin:+	*B. parvulorum* (Reuter)	Feces of man
		Mannitol:+ Sorbitol:+		*B. breve* (Reuter)	Feces of man
Lactose:0	Cellobiose:0 Gluconate:0			*B. ruminale* (Scardovi)	Rumen of cattle
	Cellobiose:+ Gluconate:+			*B. indicum* (Scardovi)	Intestine of bee
Arabinose:0	Xylose:+ Salicin:+ Mannitol:0			*B. liberorum* (Reuter)	Feces of man
	Salicin:0 Mannitol:+			*B. lactentis* (Reuter)	Feces of man
Arabinose:+ Lactose:+	Xylose:+ Esculin:+ Amygdalin:+			*B. adolescentis* (Reuter)	Feces of man
	Esculin:0(+) Amygdalin:0(+)	Glycogen:0 Starch:0	Melezitose:+	*B. longum* var. *longum* (Reuter, Mitsuoka)	Feces of man
			Melezitose:0	*B. longum* var. *animalis*[b] (Reuter, Mitsuoka)	Feces of various animals
				B. suis (Matteuzzi)[b]	Feces of pigs
		Glucogen:+ Starch:+		*B. pseudolongum* (Mitsuoka)	Feces of various animals
Lactose:0				*B. asteroides* (Scardovi)[c]	Intestine of bee
				B. coryneforme (Scardovi)	Intestine of bee

[a] 0, Not fermented; +, fermented; (+), slowly fermented.
[b] These two species have different morphology and are not genetically related (Matteuzzi, D., et al., Z. Allg. Mikrobiol., *in press*).
[c] *B. asteroides* has characteristic morphology of cells and aggregates and little DNA homology to *B. coryneforme*.

borate buffer and with the buffer 10 times concentrated in the electrode vessels. Three samples at a time were placed in the gel on strips of Whatman 3MM filter paper, and analyses were run at 4 to 5 C with voltage gradient of 6 v/cm for about 18 hr. The inner gel layer was cut into 2-mm thick segments, of which the enzymatic activity was measured by the analytical procedure for acetyl phosphate (3) after incubation for 30 min in 1 ml of reactive mixture containing (in micromoles): Tris buffer (*p*H 6.5), 100; fructose-6-P, 12; sodium fluoride, 20; phosphate, 10; and cysteine-hydrochloride, 3. The actual distance of migration varied from run to run, so the enzyme of strain RU230 (*B. globosum*) was used as reference in all experiments and was assigned an

TABLE 2. *Electrophoretic mobilities of fructose-6-phosphate phosphoketolase in the nomenspecies of the genus Bifidobacterium*

Species	Strain	Source	Distance of migration[a]	References
B. asteroides	C51	*Apis mellifica*	16.12 ± 0.34	Our collection
B. indicum	C410	*Apis indica*	16.46 ± 0.22	Our collection
B. coryneforme	C215	*Apis mellifica*	16.50 ± 0.25	Our collection
B. globosum	RU230	Rumen of cattle	10.00	Our collection
B. ruminale	RU326	Rumen of cattle	10.66 ± 0.44	Our collection
Mannitol fermenting	RU424	Rumen of cattle	15.39 ± 0.50	Our collection
B. suis	SU900	Feces of pig	10.01 ± 0.34	Our collection
B. thermophilum	14-44	Feces of pig	10.03 ± 0.50	T. Mitsuoka
B. pseudolongum	Mo-2-10	Feces of mouse	9.84 ± 0.55	T. Mitsuoka
B. pseudolongum	PNC-2-9G	Feces of pig	10.50 ± 0.22	T. Mitsuoka
B. longum var. *animalis*	C10-45	Feces of calf	10.21 ± 0.48	T. Mitsuoka
B. infantis	B720	Feces of man	14.90 ± 0.30	Our collection
B. breve	B901	Feces of man	15.55 ± 0.40	Our collection
B. breve	B632	Feces of man	15.42 ± 0.28	Our collection
B. longum	B929	Feces of man	14.89 ± 0.31	Our collection
B. longum	B844	Feces of man	15.00 ± 0.30	Our collection
B. bifidum	B790	Feces of man	15.36 ± 0.50	Our collection
B. bifidum	E-319-f	Feces of man	15.20 ± 0.30	G. Reuter
B. adolescentis	B872	Feces of man	15.13 ± 0.30	Our collection

[a] Distances of migration are calculated relative to an arbitrary value of 10 assigned to the enzyme of *B. globosum* strain RU230.

arbitrary value of 10. The segment showing the highest activity was taken as center of the band; all extracts were analyzed in at least five separate runs each.

It is immediately apparent from the figures reported in Table 2 that the phosphoketolases of the strains examined do not all have the same electrophoretic mobility. The enzyme of the strains of the nomenspecies isolated from feces of various animals and rumen of cattle behave like that of the reference strain RU230 of *B. globosum*, whereas the phosphoketolase of the species isolated from human feces moves faster toward the anode. Although the differences in the distances of migration among the species within these two groups appear not to be significant, we searched further for possible small differences in migration rate by running mixtures of extracts. Only a single band was obtained in all cases.

Among the strains investigated, strain RU424 merits comment. This and other strains of our collection are mannitol fermenters, occasionally isolated from rumen of cattle on trypticase-phytone-mannitol agar plates; it was identified by its fermentation pattern and DNA homology (F. Crociani, V. Scardovi, and L. D. Trovatelli, Ann. Microbiol., *in press*) as *B. adolescentis*, a species found mostly in feces of man. The electrophoretic behavior of its phosphoketolase is in accordance with this label. Strain C-10-45 of Mitsuoka has a fermentation pattern like that of *B. longum*, a species found in feces of man, but its attribution to a new animal variety of this species, made by Mitsuoka (4), is apparently not valid because it has no DNA homology with true *B. longum;* it is instead genetically related to *B. pseudolongum*, a species inhabiting the feces of animals. The behavior of its phosphoketolase fits the DNA homology pattern.

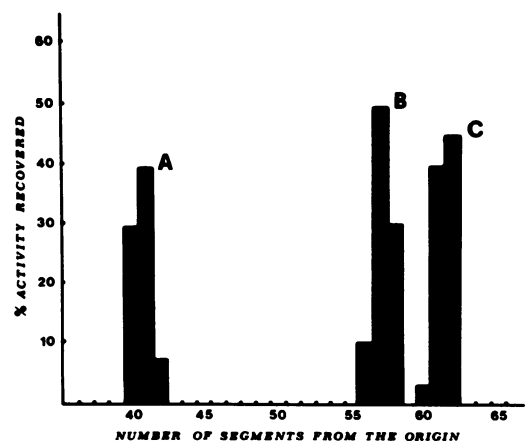

FIG. 1. *Electrophoretic separation of fructose-6-phosphate phosphoketolases in pooled extracts of Bifidobacterium globosum strain RU230 (band A), B. bifidum strain E-319-f (band B), and B. asteroides strain C51 (band C).*

The phosphoketolase of the species isolated from the intestine of bees apparently follows the pattern of the species of the human intestine, but some significant differences in the distances of migration were recorded (*see* Table 2). The extracts of the strains from bees were therefore run in mixtures with each of the extracts of the strains from man. The gel layers were cut into 1-mm fragments to improve resolution. Two separate bands of activity were obtained in all experiments, thus indicating convincingly that the species *B. asteroides*, *B. indicum*, and *B. coryneforme* possess another electrophoretic type of fructose-6-phosphate phosphoketolase. An example of such mixed runs is given in Fig. 1.

The electrophoretic mobility of fructose-6-phosphate phosphoketolase, although not a useful character for the differentiation of species in the genus *Bifidobacterium*, is related to their ecology.

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