Multiple Electrophoretic Forms of Transaldolase and 6-Phosphogluconic Dehydrogenase and Their Relationships to the Taxonomy and Ecology of the Bifidobacteria

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The electrophoretic patterns of the transaldolases and 6-phosphogluconate dehydrogenases (6PGD) of 1,206 strains, each belonging to one of 24 named species of the genus *Bifidobacterium*, were determined by means of starch-gel electrophoresis. All of these strains were previously assigned to species on the basis of their deoxyribonucleic acid homology relationships and were selected so as to include all known phenotypes and habitats. Fourteen electrophoretic forms of transaldolase and 19 of nicotinamide adenine dinucleotide phosphate-dependent 6PGD were identified and numbered. Each strain displayed one band for each enzyme. Glucose-grown cells of the *B. pullorum* strains and of most of the *B. dentium* strains were devoid of detectable levels of 6PGD. The zymograms of more than 60% of the strains studied were species specific. Nearly half of the other strains had significant overlapping of transaldolase and 6PGD patterns, and they were assigned therefore to species on the basis of an additional marker, 3-phosphoglyceraldehyde dehydrogenase production, the results of which are not reported here in detail. Of the species studied, *B. asteroides* exhibited the greatest variability in electrophoretic types of both transaldolase and 6PGD. Correlations between electrophoretic data, deoxyribonucleic acid homology relationships between the species, and ecology are discussed.

Fructose 6-phosphate phosphoketolase (F6PPK) is the key enzyme of carbohydrate dissimilation in the genus *Bifidobacterium*. Although its relative electrophoretic mobility is generally not a useful character for species differentiation, there are some differences in the properties of F6PPK in some *Bifidobacterium* species generally confined to specific habitats. The most anodic form of F6PPK was from *B. asteroides* (from the honey bee intestine); a second, less mobile F6PPK was from *B. globosum*, *B. thermophilum*, and *B. suis* (commonly isolated from the feces of various animals); a third F6PPK isozyme of intermediate electrophoretic mobility was present in *B. breve*, *B. longum*, and *B. adolescentis* (human strains from intestine, dental caries, and vagina) (24). A comparative study of the F6PPKs from the type strains of *B. globosum* and *B. dentium* disclosed that the enzyme from *B. globosum* was more heat stable than that from *B. dentium*, that the optimum activity was at pH 5 to 6 for *B. globosum* and at pH 7 for *B. dentium*, and that the "animal" type of F6PPK (from *B. globosum*) was activated by Mg^{2+} and was less active in the presence of Mn^{2+} , whereas the reverse was true with the "human" type of F6PPK (from *B. dentium*), where only Mn^{2+} fully activated the enzyme. The molecular weights were different

(1.6×10^5 and 2.9×10^5 , respectively), and the enzyme from *B. globosum* also cleaved xylulose 5-phosphate, whereas the *B. dentium* F6PPK was specific for fructose 6-phosphate (33).

A preliminary study on the electrophoretic forms of other enzymes, such as transaldolase, transketolase, 6-phosphogluconate dehydrogenase (6PGD), and aldolase, was made with 49 representative strains of *Bifidobacterium* species; several isozymes were detected, but no clear correlation with the habitats was found (23). In the meanwhile, a vast number of bifidobacteria from diverse habitats (feces of animals under different diets, breast- or bottle-fed infants, sewage, etc.) were studied in our laboratory and were identified mainly by means of deoxyribonucleic acid (DNA)-DNA hybridization. Four additional "homology groups" were recognized and proposed as new species (28). Because of the disposal of a large number of strains of all currently known *Bifidobacterium* species, we thought it advisable to enlarge our electrophoretic studies to cover the largest possible number of *Bifidobacterium* strains as a start in exploring the internal structure of the genus.

The two enzymes dealt with in this study, transaldolase and 6PGD, are undoubtedly of different importance in the carbohydrate metabolism of bifidobacteria. Transaldolase, an essen-

tial enzyme in the "fructose 6-phosphate shunt," is characteristic of bifidobacteria because it acts upon fructose 6-phosphate and erythrose 4-phosphate after the initial formation of the latter from the former by a phosphoketolase; 6PGD is apparently a functionless enzyme, at least in glucose-grown cells, which, as a rule, lack detectable glucose 6-phosphate dehydrogenase (4, 25). Different extents of variability in the two enzymes can reasonably be expected during the course of bifidobacterial evolution (e.g., see Gillespie and Kojima [6]). A preliminary immunological study on the structural relatedness of bifidobacterial transaldolases has recently been performed with the ultimate goal of elucidating the evolutionary relationships of these organisms (34). The work described here is an extension of this study.

MATERIALS AND METHODS

Bacterial strains. The *Bifidobacterium* strains used in this study are listed in Table 1. The two DNA homology groups described by Scardovi and Trovatielli (27) are referred to as "minimum" and "subtile" pending their formal proposal as new species.

Cell-free extracts. Cells of most of the strains were grown on Trypticase-phytone-yeast extract medium (TPY), of which the composition is reported elsewhere (22). The substrate (900 ml in 1-liter conical flasks) was sterilized at 110°C for 30 min, gassed with carbon dioxide passed through a porous glass disk for 1 min (final pH, 6.2 to 6.4), and inoculated with 100 ml of culture. *B. magnum* strains were grown in TPY medium enriched with Tween 80 (1 ml/liter). *B. pul-lorum* strains were grown on MRS medium (3). *B. asteroides* strains were grown on TPY medium (pH 7.0) which had been gassed with CO₂ and which was contained in completely filled cylindrical 1-liter vessels. After 18 to 20 h of incubation at 39°C, the cells were harvested, washed with phosphate buffer (0.05 M, pH 6.5), and disrupted by sonication. The extracts, clarified by centrifugation at 20,000 rpm for 30 min in the cold, had a protein content varying from 5 to 25 mg/ml (12).

Starch-gel electrophoresis. The starch-gel horizontal electrophoretic system of Smithies was used (36). Hydrolyzed starch (Connaught Medical Research Labs., Toronto, Canada) was used at a concentration of 90 g/liter of the appropriate buffer (see below). Plastic frames (12.0 by 37.0 by 0.9 cm or 6.0 by 37.0 by 0.9 cm) were used to hold the slabs. Generally, 5 to 10 μ l of extract (or dilution thereof) on Whatman 3MM paper strips (0.5 by 0.5 cm) was run in the cold for 15 to 20 h with a current of 15 to 20 mA for transaldolase and 40 to 50 mA for 6PGD. Particular sets of extracts were used as references throughout this study (see below). Results were recorded photographically.

The following buffers were used. Transaldolase—bridge buffer (per liter): tris(hydroxymethyl)-amino methane, 16.3 g; citric acid monohydrate, 9.0 g (pH 7.0); gel buffer: bridge buffer diluted 1:15. 6PGD—bridge buffer: 0.4 M monosodium citrate (pH 7.0 with citric acid) (this solution was renewed every two runs);

gel buffer: 0.005 M histidine plus 1.5 g of sodium chloride per liter (pH 7.0) (see ref. 1).

Staining procedures. (i) **Transaldolase.** The developing solution contained (per 100 ml): fructose 6-phosphate (Na salt, 98% purity, Sigma), 400 mg; sodium arsenate, 370 mg; glycine, 240 mg; nicotinamide adenine dinucleotide, 13 mg; D-erythrose 4-phosphate (60 to 75% purity, Sigma), 16 mg; phenazine methosulfate, 2 mg; Nitro Blue Tetrazolium (Sigma), 20 mg; and about 130 U of glyceraldehyde 3-phosphate dehydrogenase; the solution was adjusted to pH 7.0.

(ii) **6PGD.** Tris(hydroxymethyl)aminomethane-hydrochloride buffer (0.5 M) (pH 7.0), 10 ml; 6-phosphogluconic acid (trisodium salt, Sigma), 250 mg; nicotinamide adenine dinucleotide phosphate, 20 mg; Nitro Blue Tetrazolium, 20 mg; phenazine methosulfate, 2 mg; and water, 90 ml.

(iii) **3-Phosphoglyceraldehyde dehydrogenase.** The staining solution contained (per 100 ml): DL-glyceraldehyde-3-phosphate (prepared from diethylacetal, barium salt; Sigma), 200 mg (neutralized solution from Dowex 50); 0.5 M tris(hydroxymethyl)aminomethane-hydrochloride buffer, (pH 8.5), 20 ml; sodium arsenate, 370 mg; phenazine methosulfate, 2 mg; Nitro Blue Tetrazolium, 20 mg; and nicotinamide adenine dinucleotide, 13 mg.

For staining, the starch slabs were flooded with 25 ml of the appropriate staining solution and kept at 37°C until discernible color developed (from 15 min to 1 to 2 h). The slabs were washed, covered with a plastic sheet, and kept at 4 to 5°C. During this storage, the zymograms gained in color and sharpness.

RESULTS

General characters of the zymograms and numbering of the isozymes. The ranges of the electrophoretic mobilities of the enzymes in the species examined are shown in Fig. 1 and 2. The distances travelled by the fastest and by the slowest moving bands were sufficiently different to permit a clear-cut recognition of all the isozymes detected in this study, namely, 14 of transaldolase and 19 of 6PGD (see Fig. 3). The fastest moving (i.e., most anodic) band was designated by the number 1, and the slowest (i.e., least anodic) was designated by the number 10 for transaldolase and 13 for 6PGD. The zymograms presented as Fig. 1 and 2 summarize an advanced, but not the final, stage of the present study; additional isozymes were found in the species more extensively investigated later: *B. asteroides*, *B. indicum*, *B. boum*, and *B. thermophilum*. It was thought desirable to refer to these additional isozymes by lower-case letters suffixed to the numbers already used.

Each strain exhibited only one band for either enzyme; in the few cases where two distinct bands were observed, mixed cultures, ascertained by subcloning and successive electrophoresis, were involved. Some species, for example *B. asteroides*, showed a tendency to give trailed

TABLE 1. Source and electrophoretic forms of the transaldolase and 6PGD isozymes of the strains studied

Strains	Source	Electrophoretic form of	
		Transaldolase	6PGD
<i>Bifidobacterium cuniculi</i> Scardovi et al.			
RA93 (= ATCC 27916), Type strain (28)	Feces of rabbit	1	4
RA94, RA95, RA98, RA99, RA104, RA105	Feces of rabbit	1	4
<i>Bifidobacterium pullorum</i> Trovatelli et al.			
P145 (= ATCC 27685), Type strain (37); P182, P183	Feces of chicken	2	absent
<i>Bifidobacterium globosum</i> Scardovi et al.			
RU230 ^a (= ATCC 25864), Type strain (29)	Rumen of cattle	2	6
RU240 ^a ; RU256 ^a ; RU257 ^a ; RU323 ^a ; RU457 ^a (29)	Rumen of cattle	2	6
RU907, RU911 (38)	Rumen of cattle	2	6
RU809, RU819, RU830, RU833, RU838, RU844 (mannitol+, ref. 2)	Rumen of cattle	2	6
SU841 ^b ; SU844, SU847 ^b ; SU861, SU881 ^b ; SU884, SU890 ^b ; SU914, SU886 ^c (39)	Feces of piglet	2	6
T110	Feces of rat	2	7
T15; T21; T34; T95; T113; T117; T130; T151	Feces of rat	2	6
T55; T58 ^a ; T78	Feces of rat	2	5
T60; T64; T75	Feces of rat	2	4
T142	Feces of rat	2	3a
AG69	Feces of lamb	2	7
AG29, AG40, AG46, AG49, AG53, AG57, AG70, AG73, AG82	Feces of lamb	2	6
P7, P20, P24, P28, P30 ^d , P33, P42, P49, P92	Feces of chicken	2	6
P112, P113	Feces of chicken	2	5
P111, P115	Feces of chicken	2	4
RA27 ^c ; RA35 ^d ; RA50 ^c ; RA109 ^d ; RA167 ^d	Feces of rabbit	2	6
RA143; RA147	Feces of rabbit	2	5
RA144, RA157	Feces of rabbit	2	4
RA120, RA122, RA125, RA128, RA138	Feces of rabbit	2	3a
VT97, VT98, VT366, VT376, VT377, VT378, VT379, VT384, VT387 ^c ; VT390	Feces of calf	2	6
VT446, VT448; VT449; VT450	Feces of calf	2	6
F229, F387, F461, F468 ^a ; F512, F544 ^d ; F574, F591; F593, F607	Sewage	2	6
B607; B608 ^b ; B614	Feces of infant	2	6
Notes: ^a Arabinose and xylose -; ^b inulin+; ^c mannitol and inulin+, but fructose -; ^d fructose -; ^e reported as <i>B. pseudolongum</i> (39)			
<i>Bifidobacterium pseudolongum</i> Mitsuoka			
PNC-2-9G (= ATCC 25526), Type strain (16)	Feces of pig	2	7
29-Sr-T (received from Mitsuoka)	Feces of chicken	2	7
Mo-2-10 (received from Mitsuoka)	Feces of mouse	2	7
"Subtile" DNA homology group (27)			
F395 (= ATCC 27537), Reference strain; F340; F394; F396; F398	Sewage	3	2
<i>Bifidobacterium choerinum</i> Scardovi et al.			
SU806 (= ATCC 27686), Type strain (28); SU827, SU836, SU837, SU838	Feces of piglet	3	4
SU840, SU877, SU882, SU885, SU888, SU891	Feces of piglet	3	4
F390, F441 ^a	Sewage	3	4
Note: ^a Xylose not fermented.			
<i>Bifidobacterium pseudocatenulatum</i> Scardovi et al.			
B1279 (= ATCC 27917), Type strain (28); B1280, B1286, B1330, B1336, B1342	Feces of infant	4	1
B1457, B1460, B1555, B1663, B1666, B1669, B1670, B1735, B1889, B1957, B1960	Feces of infant	4	1
B1961, B1965, B1969, B1970, B1977, B2072, B2073, B2077, B2078, B2282, B2284	Feces of infant	4	1
B2286, B2351, B2359, B2361, B2445, B2446, B2540, B2541, B2544, B2545	Feces of infant	4	1
B1830 ^a , B1832 ^a , B1834 ^a , B1874, B1875, B1879, B1880, B1886, B1888, B2067	Feces of infant	4	3
B2247 ^b , B2248 ^b , B2249 ^b , B2250 ^b , B2251 ^b , B2252 ^b , B2262 ^b , B2263 ^b , B2266 ^b	Feces of infant	4	3
B2339, B2340, B2650, B2652, B2654, B2656	Feces of infant	4	3
VT6, VT9 ^a , VT74 ^c , VT77 ^c , VT164, VT171, VT173, VT183, VT187, VT204, VT207	Feces of calf	4	3
VT254, VT265, VT270, VT278, VT279, VT287, VT337, VT339, VT341, VT348	Feces of calf	4	3
VT351, VT353, VT354, VT360, VT368, VT370, VT373	Feces of calf	4	3
VT393, VT395, VT396, VT398, VT401, VT405, VT409	Feces of calf	5	3
F54, F72, F262, F331, F356, F360, F469 ^d	Sewage	4	1
Additional strains ^e : F18, F19, F71, F101, F165, F166, F171, F177, F209	Sewage	4	1
F240, F335, F344, F400, F469 ^f	Sewage	4	1
Notes: ^a Mannitol +; ^b only 60 % related to <i>B. pseudocatenulatum</i> and <i>B. catenulatum</i> reference strains (Table 2, ref. 28); strains B2262 and B2266 were arabinose -; while B2262 and B2263 and B2266 were xylose -; ^c see Table 3, ref. 28, for their homology data; ^d formerly regarded as a member of <i>B. catenulatum</i> (Table 1, ref. 22); ^e diagnosis based on sugar fermentation (starch+; see footnote to Table 6, ref. 28); ^f other differences in sugar fermented reported elsewhere (28).			
<i>Bifidobacterium dentium</i> Scardovi and Crociani			
B764 (= ATCC 27534), Type strain (22)	Dental caries	4	indet. ^f
<i>Melezitose-positive^a</i> : B698, B709, B767 ^c ; B774, B777, B968, B971, B973			
B974, B977, B980, B982, B984, B986, B987, B992, B994, B1004, B1006, B1007	Dental caries	4	indet. ^f
B1012, B1013, B1014, B1015, B1016, B1017, B1019, B1023, B1025, B1027, B1031	Dental caries	4	indet. ^f
B1032, B1033, B1035, B1036, B1038, B1043, B1046, B1049, B1051, B1053, B1054	Dental caries	4	indet. ^f
B1056, B1057, B1058, B1061, B1064, B1067, B1069, B1080, B1081, B1086, B1090 ^d	Dental caries	4	indet. ^f
B1091, B1093 ^d , B1096 ^d , B1105 ^d , B1106 ^d , B1107, B1109 ^d , B1117, B1119, B1121	Dental caries	4	indet. ^f

TABLE 1—continued

Strains	Source	Electrophoretic form of	
		Transaldolase	6PGD
B1123;B1127;B1128;B1129;B1130;B1131;B1137;B1145;B1151;B1154;B1156	Dental caries	4	indet. ^f
B1022;B1040;B1065;B1071;B1074;B1142;B1148	Dental caries	5	indet. ^f
<i>Melezitose-negative</i> ^a : B696;B697 ^c ;B728;B730;B735 ^c ;B741;B744;B746	Dental caries	4	indet. ^f
B771;B773;B781;B782;B785;B786;B989;B997;B1000;B1135	Dental caries	4	indet. ^f
B1075	Dental caries	5	indet. ^f
B788;B792;B872;B932 ^a	Human vagina	4	indet. ^f
B750;B754;B757;B760 ^a	Feces of adult	4	indet. ^f
B525;B538;B1994;B1998;B2562 ^c ;B2564 ^c ;B2568 ^c ;B2571 ^c ;B2572 ^c ;B2617	Feces of infant ^b	4	2
B2618;B2619;B2620;B2621;B2623;B2624;B2625	Feces of infant ^b	4	2
B5563 ^c ;B2565 ^c ;B2566 ^c ;B2567 ^c ;B2569 ^c ;B2570 ^c	Feces of infant ^b	5	2
B96 and B127 (received from Dr. Beerens, Lille)	Oral cavity	4	2
RA116 and RA117	Feces of rabbit	4	indet. ^f
X573 (= ATCC 15423) <i>Actinomyces eriksonii</i> Georg et al. (5)	Man lung abscess	4	2
X407 (= ATCC 15424) <i>Actinomyces eriksonii</i> Georg et al. (5)	Man pleural fluid	4	2
3859 <i>B. appendicitis</i> (Prévot Collection, Institut Pasteur)	Human appendix	4	indet. ^f
Notes: ^a Only the underlined strains were used for DNA-DNA hybridization (22); ^b all of the strains listed were used for DNA-DNA hybridization (unpublished); ^c sorbitol+; ^d inulin +; ^e isolated from the same fecal specimen; ^f indeterminable with spot staining.			
<i>Bifidobacterium magnum</i> Scardovi and Zani			
RA3 (= ATCC 27540), Type strain (31)	Feces of rabbit	5	7
RA1;RA9;RA10;RA77;RA78;RA89;RA184;RA185;RA189;RA190	Feces of rabbit	5	7
RA203;RA209;RA220;RA221;RA222	Feces of rabbit	5	7
<i>Bifidobacterium catenulatum</i> Scardovi and Crociani			
B669 (= ATCC 27539), Type strain (22)	Feces of adult	5	6
B660;B661;B676;B684 (= ATCC 27675)	Feces of adult	5	6
B1331 ^a ;B1333 ^a ;B1334 ^a ;B1338 ^a ;B1341 ^a ;B1738;B1748 ^a ;B1955;B2218	Feces of infant	5	6
B2242 ^b ;B2415;B2416 ^a ;B2418 ^a ;B2419	Feces of infant	5	6
B2120;B2121;B2126;B2127;B2130;B2316 ^{ad}	Feces of infant	5	8
F64;F94 ^c ;F134;F141;F189 ^b ;F217;F258;F276;F290;F291;F294	Sewage	5	6
F297;F320;F336 ^b ;F364 ^b ;F428	Sewage	5	6
Notes: ^a Fructose -; ^b mannitol+ (F336 also sorbitol -); ^c xylose -; ^d strains B2120 to B2130 from a single specimen, strain B2316 was isolated from the same individual 30 days later.			
<i>Bifidobacterium animalis</i> (Mitsuoka) Scardovi and Trovatelli			
R101-8 ^b (= ATCC 25527), Type strain (27)	Feces of rat	5	9
T6 ^a ;T27 ^a ;T98 ^b ;T160 ^a ;T169	Feces of rat	5	9
P16;P17;P23 ^a (= ATCC 27536);P32 ^c ;P39 ^c ;P45 ^b	Feces of chicken	5	8
RA12;RA13;RA14 ^c ;RA15;RA16 ^c ;RA18;RA20;RA23 ^{bc}	Feces of rabbit	5	8
F337;F349;F437;F439	Sewage	5	8
Notes: ^a Mannose +; ^b cellobiose -; ^c melezitose +.			
<i>Bifidobacterium angulatum</i> Scardovi and Crociani			
B677 (= ATCC 27535), Type strain (22)	Feces of adult	5	5
F158;F196;F406;F424;F426	Sewage	5	5
<i>Bifidobacterium infantis</i> Reuter			
S12 (= ATCC 15697), Type strain (20)	Feces of infant	8	4
B1269 (= ATCC 27920), Reference for DNA homology grouping	Feces of infant	5	4
S76e (= ATCC 15702), Type strain <i>B. liberorum</i> Reuter (20)	Feces of infant	8	5
659 (= ATCC 25962), Type strain <i>B. lactentis</i> Reuter (20)	Feces of infant	8	4
<i>Pentoses-negative</i> strains (like strain S12):			
B625 ^b ;B642 ^b ;B649 ^b ;B651	Feces of infant	6	5
B634 ^a	Feces of infant	6	3
<i>Pentoses-positive</i> strains			
B1351;B1378;B1412;B1436 ^d ;B1490;B1509;B1528;B1599;B1602;B1635 ^c	Feces of infant	5	4
B1638;B1648;B1652;B1654;B1658;B1673;B1707;B1709;B1717;B1719	Feces of infant	5	4
B1721;B1730;B1731;B1732;B1799;B1805;B1822;B1824;B1851;B1853	Feces of infant	5	4
B1854;B1858;B1860;B1866 ^b ;B1882;B1883;B1915;B1918;B1922;B1934	Feces of infant	5	4
B1939;B1948;B1951;B1954;B1956;B2016;B2026;B2032;B2037;B2042	Feces of infant	5	4
B1522;B1541;B1545;B1582 ^e	Feces of infant	5	5
B947;B948	Human vagina	5	4
Notes: ^a Unique strain melezitose and gluconate +; ^b mannitol+ (like strain 659); B649 is also sorbitol+; ^c melezitose, mannitol, sorbitol and starch -; ^d same specimen as B1427, B1429 and 1434 (see under <i>B. longum</i>); ^e same specimen as B1629 and 1634 (see <i>B. longum</i>); ^f same specimen as B1672 (see <i>B. longum</i>); ^g same specimen as B1580 and 1581 (see <i>B. longum</i>); ^h same specimen as B1862, B1864 and B1872 (see <i>B. longum</i>).			

TABLE 1—continued

Strains	Source	Electrophoretic form of	
		Transaldolase	6PGD
<i>Bifidobacterium longum</i> Reuter			
E194b (= ATCC 15707), Type strain (biotype a) (20)	Feces of adult	8	6
S3 (= ATCC 15708), biotype b (20)	Feces of infant	8	5
B612;B613;B647 ^a ;B687 ^b ;B688 ^b (see ref. 30)	Feces of infant	6	5
<i>Melezitose</i> + and <i>ribose</i> + strains: B1315;B1327;B1427;B1429;B1434	Feces of infant	8	5
B1629 ^c ;B1634 ^c ;B1695;B1835;B1972;B1975;B1990;B1993;B1996;B2031	Feces of infant	8	5
B2086 ^c ;B2087 ^c ;B2089 ^c ;B2095 ^c ;B2101;B2105;B2108;B2211;B2212	Feces of infant	8	5
B2325;B2327;B2426	Feces of infant	8	5
B1862 ^d ;B1864 ^d ;B1872 ^d	Feces of infant	8	6
B1427 ^f ;B1429 ^f ;B1434 ^f ;B1536;B1580 ^g ;B1581 ^g ;B1672 ^h	Feces of infant	6	5
<i>Melezitose</i> - and <i>ribose</i> + strains: B1873;B1881;B1978;B1979;B2055	Feces of infant	8	5
B2057;B2349;B2350;B2352;B2356;B2639;B2641	Feces of infant	8	5
B2395;B2399;B2462;B2470;B2471;B2472	Feces of infant	6	5
<i>Melezitose</i> - and <i>ribose</i> - strains: B1478;B1480;B1482;B1483;B2405 ⁱ ;B2407 ⁱ	Feces of infant	8	indet. ^j
B2473 ⁱ ;B2477 ⁱ ;B2479 ⁱ ;B2484 ⁱ ;B2599;B2601;B2633;B2636	Feces of infant	8	indet. ^j
<i>Melezitose</i> - and <i>gluconate</i> + strains: B2158;B2160;B2174;B2192	Feces of infant	5	5
Notes: ^a Pentoses -; ^b melezitose -; ^c see <i>B. infantis</i> B1635; ^d see <i>B. infantis</i> B1866; ^e see text for DNA homology; ^f see <i>B. infantis</i> B1436; ^g see <i>B. infantis</i> B1582; ^h see <i>B. infantis</i> B1683; ⁱ isolates from the same individual (higher numbers isolated 30 days later); ^j indeterminable with spot staining.			
<i>Bifidobacterium infantis</i> - <i>B. longum</i> 'intermediates'			
<i>Melezitose</i> - and <i>inulin</i> - , <i>ribose</i> + strains: VT10;VT11;VT29;VT114;VT116	Feces of calf	6	5
VT120;VT126;VT128;VT134;VT144;VT148;VT210;VT219;VT220;VT230	Feces of calf	6	5
VT234;VT235;VT240;VT244;VT246;VT247;VT248;VT251;VT296;VT297	Feces of calf	6	5
VT303;VT400;VT413;VT419 ^a ;VT420 ^a ;VT422 ^a ;VT429;VT430;VT440	Feces of calf	6	5
<i>Melezitose</i> - , <i>inulin</i> - and <i>ribose</i> - strains: VT42;VT155;VT163 ^b ;VT301	Feces of calf	6	5
VT302;VT311;VT318;VT319;VT329;VT330 ^c ;VT332 ^c	Feces of calf	6	5
Notes: ^a Starch and dextrin+; ^b fructose -; ^c mannitol and inulin +			
<i>Bifidobacterium breve</i> Reuter			
Arabinose and xylose not fermented			
<i>Mannitol</i> + , <i>sorbitol</i> + , <i>melezitose</i> - strains ^a (<i>B. breve</i> biotype a (20)):			
S1 (= ATCC 15700), Type strain, biotype a (20)	Feces of infant	6	7
S50 (= ATCC 15698), Type strain of <i>B. parvulorum</i> , biotype a (20)	Feces of infant	6	7
B632; B637;B644;B648 (see ref. 30)	Feces of infant	6	7
B799;B898;B912;B941;B943 (see ref. 30)	Human vagina	6	7
B1501;B2020;B2021;B2025;B2036;B2142;B2150 ^c ;B2157 ^c ;B2166;B2194	Feces of infant	6	7
B2195;B2210;B2228 ^d ;B2272;B2274;B2276;B2296;B2299;B2319 ^e ;B2330	Feces of infant	6	7
B2368;B2379;B2448;B2495;B2500 ^f ;B2511;B2520;B2556;B2583;B2657	Feces of infant	6	7
B2668;B2675;B2687	Feces of infant	6	7
VT267;VT268;VT271	Feces of calf	6	7
B609 ^g ;B622 ^g (ref. 30);B2023;B2131;B2136;B2341;B2507;B2607	Feces of infant	6	6a
B637 (ref. 29);B1619;B1837;B1847;B2180;B2186;B2203;B2236	Feces of infant	6	6
B2243;B2320 ^g ;B2590;B2591;B2592;B2598	Feces of infant	6	6
B862 (see ref. 30)	Human vagina	6	6
<i>Mannitol</i> + , <i>sorbitol</i> + , <i>melezitose</i> + strains (<i>B. breve</i> biotype b, (20)):			
B1264;B1272;B1276;B1303;B1925 ^h ;B1962;B2060;B2109;B2114	Feces of infant	6	7
B633 (ref. 31); B1621;B1630;B2173;B2176	Feces of infant	6	6a
B1562 ^h ;B1563 ^h ;B1566 ^h ;B1567 ^h ;B1569 ^h ;B1836	Feces of infant	6	6
<i>Mannitol</i> - , and <i>sorbitol</i> - strains (<i>B. parvulorum</i> pattern, 20) :			
<i>melezitose</i> - (biotype a, 20): B2456;B2457;B2530;B2533;B2548;B2553	Feces of infant	6	7
<i>melezitose</i> + (biotype b, 20): B2429;B2432	Feces of infant	6	7
B1568	Feces of infant	6	6
Arabinose and xylose fermented ^d			
B1606	Feces of infant	6	7
B628 ^k (see ref. 32)	Feces of infant	6	6a
B2163 ^k ;B2164 ^k ;B2235 ^k	Feces of infant	6	6
B624 ^k ;B627 ^k (see ref. 30)	Feces of infant	6	5
Notes: ^a Twenty-two strains of this group are cellobiose - , and twenty-one are mannose - ; ^b same specimen as B2023; ^c same specimen as B2163 and B2164; ^d same specimen as B2235; ^e same specimen as B2320; ^f lactose - ; ^g sorbitol - ; ^h same specimen as B1568; ⁱ ribose - ; ^j these strains are mannitol and sorbitol - but melezitose - ; ^k gluconate+.			
<i>Bifidobacterium coryneforme</i> Scardovi and Trovattelli			
C215 (= ATCC 25911), Type strain (26)	Intestine of bee	6	6
C148;C155;C216;C218;C242;C339	Intestine of bee	6	6
<i>Bifidobacterium boum</i> Scardovi et al.			
RU917 (= ATCC 27917), Type strain (28)	Rumen of cattle	6	8
RU246;RU289 ^b ;RU331;RU358;RU395 ^b ;RU473;RU617;RU627;RU929	Rumen of cattle	6	8
RU276 ^a ;RU346 ^a ;RU518;RU608;RU665;RU683	Rumen of cattle	6	9
RU312;RU318;RU400 ^a	Rumen of cattle	6	9a
SU865 ^a ;SU910 ^a ;SU912 ^{ab}	Feces of piglet	6	8
Notes: ^a Lactose+; ^b inulin -			

TABLE 1—continued

Strains	Source	Electrophoretic form of	
		Transaldolase	6PGD
<i>Bifidobacterium suis</i> Matteuzzi et al.			
SU859 (= ATCC 27533), Type strain (14)	Feces of piglet	6	8
SU864 ^a , SU868 (= ATCC 27531); SU900; SU903; SU905; SU906; SU908; SU909	Feces of piglet	6	8
SU924; SU932; SU940	Feces of piglet	6	8
SU899; SU901; SU916; SU921; SU941; SU829 ^a ; SU846 ^a ; SU850 ^a ; SU851 ^a ; SU923 ^a	Feces of piglet	6	5
Notes: ^a Urease-negative strains (13)			
<i>Bifidobacterium bifidum</i> (Tissier) Orla-Jensen			
E319f G. Reuter var. a (20); S28a (= ATCC 15696) C. Reuter var. b	Feces of infant	7	7
ATCC 11863 (212A) <i>Lactobacillus bifidus</i> subsp. <i>pennsylvanicus</i> György (7)	Feces of infant	7	7
B1737 ^a ; B1760 ^b ; B1764; B1928; B1958 ^b ; B1968 ^b ; B2004 ^b ; B2009 ^b ; B2091	Feces of infant	7	7
B2093; B2193; B2475; B2531 ^b ; B2554 ^b ; B2614; B2658 ^c ; B2660 ^c ; B2662 ^c	Feces of infant	7	7
B2670 ^d ; B2679 ^d ; B2696	Feces of infant	7	7
B1446 ^c ; B1447 ^c ; B1448 ^c ; B1455 ^c	Feces of infant	7	8
VT21; VT165; VT181; VT188; VT195/3; VT197/2; VT197/3; VT199	Feces of calf	7	7
VT261; VT286; VT320	Feces of calf	7	7
Notes: ^a Ribose-; ^b melibiose+; ^c maltose and sucrose+, fructose- (strain B2662 is ribose-); ^d maltose+; ^e isolated from the same fecal specimen of breast-fed infant.			
<i>Bifidobacterium thermophilum</i> Mitsuoka (Syn.: <i>B. ruminale</i>)			
P2-91 (= ATCC 25525), Type strain (16)	Feces of piglet	8	9
RU326 (= ATCC 25866), Type strain of <i>B. ruminale</i> (29)	Rumen of cattle	8	9
<i>Melezitose- and lactose-</i> strains (Mitsuoka's biotype a (16))			
RU294; RU349; RU366; RU368; RU402; RU433; RU439; RU647; RU673	Rumen of cattle	8	9
RU699; RU780; RU782; RU788; RU790; RU792	Rumen of cattle	8	9
RU458	Rumen of cattle	8	9a
RU363 ^a ; RU408; RU423; RU426; RU466; RU470; RU564; RU629; RU725	Rumen of cattle	8	8
RU891; RU894; RU897	Rumen of cattle	8	8
F489; F494	Sewage	8	8
RU288; RU301; RU303; RU365; RU398; RU409; RU417; RU471; RU516; RU571	Rumen of cattle	8	7
<i>Melezitose- and lactose+</i> strains (Mitsuoka's biotype c (16))			
F50 ^b ; F150 ^b ; F334; F368; F438; F451; F452; F456; F491; F493 ^c ; F499; F523; F524	Sewage	8	9
SU854; SU862; SU863 (see ref. 39)	Feces of piglet	8	9
SU895 ^d ; SU938 (see ref. 39)	Feces of piglet	8	8
F146; F460 ^d ; F539	Sewage	8	8
VT81; VT83; VT88; VT90; VT95 ^c	Feces of calf	7	7
<i>Melezitose+ and lactose+</i> strains (Mitsuoka's biotype d (16))			
F333; F454 ^d ; F466	Sewage	8	9
14-44 P16-6; NISSIN (Mitsuoka's biotype b, c and d resp.)	Feces of pig	8	8
Notes: ^a Same specimen as RU365; ^b arabinose, xylose and gluconate+ (F50 is also starch-); ^c starch-; ^d arabinose and xylose+; ^e arabinose+.			
<i>Bifidobacterium adolescentis</i> Reuter			
E194a (= ATCC 15703), Type strain, biotype a (20)	Feces of adult	8	5
<i>Mannitol+ and sorbitol+</i> (Reuter's biotype a (20))			
F3 ^a ; F5 ^a ; F9 ^a ; F10; F17 ^a ; F21; F23; F147; F253; F268	Sewage	8	5
<i>Mannitol- and sorbitol-</i> (Reuter's biotype b (20))			
F161; F250; F302	Sewage	8	5
RU424; RU863; RU864; RU870 Group II (ref. 2) see also (30)	Rumen of cattle	8	5
RU704 ^{ad} ; RU724 ^{ad} ; RU822 ^{ad} ; RU716 ^{ad} Group III (ref. 2)	Rumen of cattle	8	5
<i>Mannitol- and sorbitol+</i> (Reuter's biotype c (20))			
F2; F35 ^a ; F41; F80; F109 ^c ; F110; F119; F123; F159; F160; F167; F200; F275 ^a	Sewage	8	5
F283; F305; F318; F328; F351; F352; F353; F365; F366; F377; F410	Sewage	8	5
<i>Mannitol- and sorbitol-</i> (Reuter's biotype d (20))			
F152 ^{ac} ; F272 ^{ac} ; F455 ^{ac} ; F471 ^{ac}	Sewage	8	5
E305 (= ATCC 15704), E298b (= ATCC 15705), E319a (= ATCC 15706)	Feces of adult	8	5
(Reuter's biotypes b, c and d respectively (20))			
Notes: ^a Gluconate-; ^b strains F80, F123, F283, F365 and F366 are inulin-; ^c cellobiose- (F152, F455 are also salicin-); ^d arabinose and xylose-			
<i>Bifidobacterium indicum</i> Scardovi and Trovatelli			
C410 (= ATCC 25912), Type strain (26); C403; C409; C411	Intestine of: <i>Apis cerana</i> (Malaysia) ^k	9	9
C418 (= ATCC 25913)	<i>Apis cerana</i> (Japan) ^l	9	9
C526; C557	<i>Apis cerana</i> (Philippines) ^m	9	9b
C528; C606 ^a	"	9	8
C523; C524; C533; C536; C537; C538; C539; C540; C541; C547; C550; C552; C553	"	9	6a
C555; C560; C568; C569; C570; C571; C572; C574; C576; C577; C578; C579; C580	"	9	6a
C586; C587; C588; C589; C591; C593; C594; C595; C596; C597; C598; C599; C600	"	9	6a
C601; C603 ^a	"	9	6a
C435; C436; C437; C440; C442; C443 ^b ; C445; C447; C448	(Philippines) ⁿ	7	8
C444 ^b	"	9	6a

TABLE 1—continued

Strains	Source	Electrophoretic form of	
		Transaldolase	6PGD
C486 ⁱ	<i>Apis dorsata</i> (Philippines) ^m	9	6
C459 ^d	"	9	6a
C478	"	8	9a
C501	"	8	8
C449,C450,C453 ^c ,C463 ^c ,C465 ^f ,C469 ^g ,C481,C483 ^b ,C484 ^h ,C500,C501 ⁱ	"	8	6
C509,C511,C512,C513	"	8	6
C454 ^c ,C495,C496,C497,C498,C499,C507,C508	"	7	9a
C423	"	7	9
C421,C424,C425,C426,C428,C451,C456 ^d ,C462 ^e ,C474,C476,C482,C487 ⁱ	"	7	8
C488 ⁱ ,C490 ⁱ ,C491,C492,C493,C503 ⁱ ,C517	"	7	8
C427,C502,C514,C515,C516,C518,C519	"	7	7
C467 ^g ,C468 ^g ,C504 ^j ,C506 ^j	"	7	6
C458 ^d	"	7	6
C466 ^f	"	6	8
C485 ^h	"	6	6
Notes: ^a to ^j Strains whose designations bear the same superscript letter were isolated from the same individual; ^a bees received from Chemara Research Station, Layang, Malaysia (march 1967); ^b bees received from Anan, Tokushima Prefecture, Japan (nov. 1967); ^m bees received from College of Agriculture, Univ. of Philippines, Laguna (May 1968); ⁿ same origin as that immediately above, but received in March 1968.			
<i>Bifidobacterium asteroides</i> Scardovi and Trovattelli			
C51 (= ATCC 25910), Type strain (26)	<i>Apis mellifera</i> (Europe) ^a	8	10a
C229,C235	"	9a	10a
C391	"	9	13
C393,C394,C395,C396,C398	"	9	12
C244	"	9	10b
C139	"	9	10a
C674 ^c ,C675 ^c	(Philippines) ^b	9	10a
C118	(Europe) ^a	8b	13
C123	"	8b	10b
C214,C277,C281,C294	"	8b	10a
C624	(Philippines) ^b	8b	10a
C203,C377 ^d	(Europe) ^a	8a	10b
C283	"	8a	10a
C639,C668	(Philippines) ^b	8a	10a
C363 ^d	(Europe) ^a	8a	9a
C226	"	8	12
C3 (= ATCC 25909),C33,C40	"	8	11
C71,C73,C74,C184,C197,C250,C301,C338,C350,C375 ^d ,C387	"	8	10b
C544,C562 ^c ,C563 ^c ,C565 ^c ,C566 ^c ,C567 ^c ,C584 ^f ,C592	<i>Apis cerana</i> ^b	8	10b
C82,C95,C130,C135,C201	<i>Apis mellifera</i> (Europe) ^a	8	10a
C608,C614,C631,C644,C680 ^g ,C686 ^g	"	8	10a
C432,C582 ⁱ ,C585 ^f	<i>Apis cerana</i> ^b	8	10a
C6,C14,C67,C69	<i>Apis mellifera</i> (Europe) ^a	8	10
C119,C168,C389	"	8	9b
C681 ^g	(Philippines) ^b	8	9b
C89,C333,C352,C357	(Europe) ^a	8	9a
C207	"	8	9
C702	(Philippines) ^b	7a	10b
C676 ^c ,C697,C700	"	7a	10a
C151,C374 ^d	(Europe) ^a	7	10a
C246,C248,C296	"	6	10b
Notes: ^a Countries of origin are reported in ref. 26; ^b bees received from College of Agriculture, Univ. of Philippines, Laguna (June-Nov. 1968); ^c to ^g same superscript letter indicates isolation from the same intestine.			
"Minimum" DNA homology group (27)			
F392 (= ATCC 27538), Reference strain (27); F399	Sewage	10	6

or smeared bands, or one or two more faint anodic bands subordinate to the major one, especially when concentrated extracts were used; appropriate dilution of the extracts obviated this effect. The occurrence of such sub-

bands, observed only with 6PGD staining, was by no means repeatable from run to run; hence, at this point it can only be assumed that there is only one band per enzyme per strain.

Whereas transaldolase staining was invariably

FIG. 1. Coelectrophoretic run of pooled cell-free extracts from 10 reference *Bifidobacterium* strains after staining for transaldolase.

positive even with very dilute extracts (generally 1:10 for this staining), the staining for 6PGD failed with strains of *B. pullorum*, some strains of *B. longum*, and most *B. dentium* strains. The absence, or better the indeterminability, of this enzyme in *B. pullorum* strains has already been shown (37), but the low content of this enzyme in *B. dentium* strains was rather surprising because of the ability of this species to develop with gluconate as a fermentable substrate (22). Trials were made to determine the sensitivity of the staining procedure for 6PGD under our conditions of electrophoresis; 0.05 mU of enzyme appeared to be the lowest quantity for a positive staining after 3 to 4 h of storage in the reaction mixture at 37°C. This means that extracts that were not stainable for 6PGD, which have a protein content of 10 to 20 mg/ml, do not possess a dehydrogenase specific activity higher than 1×10^{-4} to 2×10^{-4} U (used in 20- μ l samples per slot). The *B. dentium* strains, which were positive for 6PGD staining (see below), exhibited specific activities in the range from 0.005 to 0.01 U, i.e., from 50- to 100-fold the minimal stainable level of the enzyme. 6PGD was invariably oxidized nicotinamide adenine dinucleotide phosphate dependent.

Distribution of isozymes. The isozymes of transaldolase and 6PGD found among the strains studied are presented in Tables 1 and 2. In Table 1 the results are reported in detail, and additional information is given concerning the

sources and some distinguishing fermentative characters of the strains studied. In Table 2 are reported: (i) frequencies of the zymographic patterns, (ii) the bands which may be of significance as species markers, (iii) matchings between isozymes in different species, and (iv) indications as of distinguishing between the species by means of 3-phosphoglycerate dehydrogenase staining. The results of this study are graphically presented in Fig. 3; in these diagrams the species-specific zymograms are reported together with gross indications of the frequencies of the enzymic bands found in each species.

The high degree of isozyme variability displayed by *B. asteroides* and *B. indicum* is illustrated in the isozyme distribution histograms given in Fig. 4.

DISCUSSION

The eventual taxonomic and ecological significance of electrophoretic data of the kind reported in this study obviously depends on the number of strains of a given species studied and on the variety of habitats from which the strains were isolated.

B. globosum Scardovi et al. (29) is considered by Rogosa (21) to be identical with *B. pseudolongum* Mitsuoka (16). However, we still maintain that these species are unique due to differences in the guanine plus cytosine contents of their DNA (some 3%) and to their DNA homol-

FIG. 2. Coelectrophoretic run of pooled cell-free extracts from 11 *Bifidobacterium* reference strains after staining for 6PGD.

FIG. 3. Diagrammatic representations of the electrophoretic forms of transaldolase and 6PGD found in the genus *Bifidobacterium*. The numbers on the sides of the diagrams refer to the isozymes of transaldolase (squares) and 6PGD (circles). Symbols: ■ and ●, isozymes found in 75 to 100% of strains; ◐ and ◑, isozymes found in 40 to 60% of strains; □ and ○, isozymes found in less than 30% of strains. The numbers at the bottom of each column refer to the number of strains studied.

TABLE 2. *Transaldolase and 6PGD isozyme frequencies in the Bifidobacterium strains studied*

Species	Number of strains	Electrophoretic form of		Zymogram identical to that of	Distinguishable by means of G3PD ^d
		Transaldolase	6PGD		
<i>B. cuniculi</i>	7 ^a	<u>1</u> (7) ^b	<u>4</u> (7)	None	
<i>B. globosum</i>	103	<u>2</u> (103)	<u>6</u> (81)	None	
			7 (2)	Three strains of <i>B. pseudolongum</i>	No
			5 (7)	None	
			4 (7)	None	
			3a (6)	None	
<i>B. pseudolongum</i>	3	<u>2</u> (3)	<u>7</u> (3)	Two strains of <i>B. globosum</i>	No
<i>B. pullorum</i>	3	<u>2</u> (3)	indet. ^c	None	
"Subtile" DNA homology group	5	<u>3</u> (5)	<u>2</u> (5)	None	
<i>B. choerinum</i>	15	<u>3</u> (15)	<u>4</u> (15)	None	
<i>B. pseudocatenulatum</i>	120	<u>4</u> (113)	<u>1</u> (60)	None	
			<u>3</u> (53)	None	
			5 (7)	None	
<i>B. dentium</i>	140	<u>4</u> (126)	<u>2</u> or indet.	None	
			5 (14)	None	
<i>B. magnum</i>	16	<u>5</u> (16)	<u>7</u> (16)	None	
<i>B. catenulatum</i>	41	<u>5</u> (41)	<u>6</u> (35)	None	
			8 (6)	Eighteen strains of <i>B. animalis</i>	Yes
<i>B. animalis</i>	24	<u>5</u> (24)	<u>8</u> (18)	Six strains of <i>B. catenulatum</i>	Yes
			9 (6)	None	
<i>B. angulatum</i>	6	<u>5</u> (6)	<u>5</u> (6)	Four strains of <i>B. longum</i> and four strains of <i>B. infantis</i>	No
<i>B. infantis</i>	65	<u>5</u> (57)	<u>4</u> (53)	None	
			5 (4)	Four strains of <i>B. angulatum</i> and four strains of <i>B. longum</i>	No
		6 (5)	5 (4)	Eighteen strains of <i>B. longum</i> , all of the <i>B. longum</i> - <i>B. infantis</i> intermediate strains, and ten strains of <i>B. breve</i>	No
			3 (1)	None	
		8 (3)	4 (2)	None	
			5 (1)	None	
<i>B. longum</i>	80	<u>8</u> (58)	<u>6</u> (4)	None	
			5 (40)	All strains of <i>B. adolescentis</i>	No
			indet. (14)	None	
		6 (18)	5 (18)	Ten strains of <i>B. suis</i> , all of the strains <i>B. longum</i> - <i>B. infantis</i> intermediate strains, four strains of <i>B. infantis</i> , and two of <i>B. breve</i>	No
			5 (4)	All of the <i>B. angulatum</i> strains, and four strains of <i>B. infantis</i> .	No
<i>B. longum</i> - <i>B. infantis</i> "intermediates"	46	<u>6</u> (46)	<u>5</u> (46)	Ten strains of <i>B. suis</i> , eighteen strains of <i>B. longum</i> , four strains of <i>B. infantis</i> and two of <i>B. breve</i>	No
<i>B. breve</i>	106	<u>6</u> (106)	<u>7</u> (65)	None	
			6a (14)	None	
			6 (25)	All of the <i>B. Coryneforme</i> strains and one of <i>B. bifidum</i>	?
			5 (2)	Ten strains of <i>B. suis</i> , eighteen strains of <i>B. longum</i> , four <i>B. longum</i> - <i>B. infantis</i> intermediate strains	No
<i>B. coryneforme</i>	8	<u>6</u> (8)	<u>6</u> (8)	Twenty-five strains of <i>B. breve</i> and one of <i>B. indicum</i>	Yes
<i>B. boum</i>	22	<u>6</u> (22)	9a (3)	None	
			9 (6)	None	
			<u>8</u> (13)	Twelve strains of <i>B. suis</i> and one of <i>B. indicum</i>	Yes
<i>B. suis</i>	22	<u>6</u> (22)	<u>8</u> (12)	Thirteen strains of <i>B. boum</i> and one of <i>B. indicum</i>	Yes
			<u>5</u> (10)	Eighteen strains of <i>B. longum</i> , four strains of <i>B. infantis</i> , two of <i>B. breve</i> and all of the <i>B. longum</i> - <i>B. infantis</i> intermediate strains	No
<i>B. bifidum</i>	39	<u>7</u> (39)	8 (4)	Thirty strains of <i>B. indicum</i> (from <i>A. dorsata</i>)	Yes
			<u>7</u> (35)	Five strains of <i>B. thermophilum</i>	Yes
<i>B. thermophilum</i>	74	<u>8</u> (69)	9a (1)	Four strains of <i>B. asteroides</i>	No
			<u>9</u> (36)	One strain <i>B. asteroides</i>	?
			<u>8</u> (22)	One strain <i>B. indicum</i>	Yes
			7 (10)	None	
			7 (5)	Thirty-five strains of <i>B. bifidum</i> and seven strains of <i>B. indicum</i>	Yes
<i>B. adolescentis</i>	54	<u>8</u> (54)	<u>5</u> (54)	Forty strains (= 50%) of <i>B. longum</i>	No

TABLE 2—continued

Species	Number of strains	Electrophoretic form of Transaldolase 6PGD		Zymogram identical to that of	Distinguishable by means of G3PD			
<i>B. asteroides</i>	85	9a (2)	10a (2)	None				
			9 (10)	13 (1)	None			
		8b (7)	12 (5)	None				
			10b (1)	None				
			10a (3)	None				
			13 (1)	None				
			10b (1)	None				
		8a (6)	10a (5)	None				
			10b (2)	None				
			10a (3)	None				
			9a (1)	None				
			<u>8</u> (51)	12 (1)	None			
		<i>B. indicum</i>	122	<u>9</u> (53)	11 (3)	None		
					10b (19)	None		
					10a (15)	None		
					10 (4)	None		
					9b (4)	None		
					9a (4)	One strain <i>B. thermophilum</i> and one strain of <i>B. indicum</i>	No	
					9 (1)	Thirty-six strains of <i>B. thermophilum</i>	?	
					10b (1)	None		
					10a (3)	None		
					10a (2)	None		
<i>B. indicum</i>	122	<u>9</u> (53)	10b (3)	None				
			9b (2)	None				
			9 (5)	None				
			8 (2)	None				
			6 (1)	None				
			6a (43)	None				
			9a (1)	Four strains of <i>B. asteroides</i> and one strain of <i>B. thermophilum</i>	No			
			8 (1)	Twenty-two strains of <i>B. thermophilum</i>	Yes			
			6 (15)	None				
			9a (8)	None				
			8 (30)	Four strains of <i>B. bifidum</i>	Yes			
			7 (7)	Thirty-five strains of <i>B. bifidum</i> and five strains of <i>B. thermophilum</i>	Yes			
			<i>B. indicum</i>	122	<u>9</u> (53)	6 (5)	None	
						8 (1)	Twelve strains of <i>B. suis</i> and thirteen strains of <i>B. boum</i>	?
						6 (1)	Twenty-five strains of <i>B. brece</i> and all of the strains of <i>B. corneforme</i>	?
<i>"Minimum"</i> DNA homology group 2								
<u>10</u> (2)	<u>6</u> (2)	None				?		

^a The underlined numbers refer to the most commonly found isozymes in each species (species-specific markers).

^b Numbers in parentheses are numbers of strains displaying the same isozyme.

^c indet., Indeterminable with spot staining (see under Results).

^d G3PD, 3-Phosphoglycerate dehydrogenase.

ogy level (near 70%). The similarity in their zymogram patterns seems to confirm Rogosa's opinion, but the strains of *B. pseudolongum* included in this study are too few to give significant results. All of the strains of *B. globosum* share the transaldolase isozyme 2 but display at least five 6PGD isozymes, the most frequent of which, number 6, is shared by all of the strains isolated from rumen and sewage and from the feces of piglets, calves, lambs, and human infants. The other isozymes are found, although less frequently than isozyme 6, in the strains

isolated from rat, chicken, and rabbit feces. It should be mentioned that *B. globosum* is the only species found in such a large variety of animal intestinal habitats. The variability of 6PGD seems to be in some way habitat dependent. In the original description of *B. globosum*, this organism is reported as never fermenting xylose, mannitol, or gluconate and only rarely fermenting arabinose (29). The pentoses, mannitol, and gluconate were fermented by the strains of *B. globosum* isolated by Crociani et al. (2). Other biovars were found by Trovatelli and

FIG. 4. Frequency histograms of the transaldolase and 6PGD electrophoretic forms of strains of *B. asteroides* and *B. indicum*. Numbers on the abscissa correspond to those reported in Fig. 3.

Matteuzzi (38) and by Zani et al. (39). Most of the *B. globosum* strains studied here fermented arabinose and xylose, as did strains of *B. pseudolongum*. No particular isozyme pattern was found to correlate with these biovars. It should be mentioned that *B. globosum* biovars that ferment arabinose and xylose and *B. pseudolongum* cannot be distinguished from *B. angulatum* on the basis of sugars fermented; their distinction should be based on the guanine plus cytosine content of their DNA, DNA homology, and morphology (22). The differences in their electropherograms (see Table 2) could be of diagnostic value.

Transaldolase isozyme 4 was found in 94% of the strains of *B. pseudocatenulatum* and in 90% of the strains of *B. dentium*, whereas the less mobile band 5, found in a minority of the strains of both species, was found in all of the strains of *B. magnum*, *B. catenulatum*, *B. animalis*, and *B. angulatum* and in most of the strains of *B. infantis* (see Table 2). Among these species, *B. pseudocatenulatum* and *B. catenulatum* are the most closely related genetically: their DNA homology is in the range of 60 to 80% (28). Differences in the structure of their cells wall peptidoglycan, in the guanine plus cytosine content

of their DNA, and in their fermentation of starch were distinctive traits which correlated with the difference in their DNA base sequences. The zymograms of these two species are quite different, especially with respect to the mobilities of their 6PGDs (Table 2); this adds support to their taxonomic distinction. It should be mentioned that among the 64 strains of *B. pseudocatenulatum* studied, the following 11 were included: six strains (B2247 to B2252, inclusive) from the feces of a breast-fed infant, and two (VT74 and VT77) from the feces of suckling calf. These strains, phenotypically members of *B. pseudocatenulatum* and with zymograms the same as that of *B. pseudocatenulatum* (pattern 4-3), behaved as "intermediates" between *B. pseudocatenulatum* and *B. catenulatum* with regard to DNA-DNA hybridization (ref. 28, Table 6). Furthermore, the strains of *B. pseudocatenulatum* isolated from suckling calves have 6PGD isozyme 3, whereas most of those from the feces of infants have the more anodic isozyme 1 (see Table 1).

Although identical electrophoretic mobilities of enzymes do not necessarily mean identical primary structures, the strong similarity of the electrophoretic patterns of *B. pseudocatenulatum* and *B. dentium* (see Table 2) is somewhat surprising. The two species are less than 20% related (DNA homology), and they diverge in a number of characters like guanine plus cytosine content of DNA, cell wall peptidoglycan structure, sugars fermented, and habitat (22, 28). *B. dentium*, the unique bifidobacterium found constantly in human caries (unpublished results), has also been found in the oral cavity and appendix and in abscesses of humans (see below); its presence in the feces of human infants and of rabbits is very rare (unpublished data). On the other hand, *B. pseudocatenulatum* is common in the feces of human infants, in suckling calves, and in sewage (28). The presence of 6PGD isozyme 2 seems to be distinctive for *B. dentium* cells grown on gluconate; cells grown on glucose, with the exception of those of strains isolated from the feces of human infants and from abscesses in humans (*Actinomyces eriksonii*), do not possess stainable levels of this enzyme (see Materials and Methods). The two strains of *A. eriksonii* (ATCC 15423 and ATCC 15424), isolated by Georg et al. (5) and classified as *Bifidobacterium eriksonii* by Holdeman and Moore (8), produced the same zymogram pattern as *B. dentium* (pattern 4-2; see Table 1). This is in accord with the high DNA homology (80 to 100%; Scardovi, unpublished data) we have found between these two strains and the type strain of *B. dentium* (B764 = ATCC 27534) used as reference. Recently Mitsuoka et al. (17) pro-

posed that the name *B. eriksonii* is a synonym of *B. adolescentis* on the basis of their phenotypic identity. Actually, *B. dentium* is indistinguishable from *B. adolescentis* on phenotypic grounds; at present, only DNA homology and zymograms are helpful in distinguishing between these organisms (see Table 2).

Preliminary data on the DNA homology relationships between *B. infantis* Reuter (syn.: *B. liberorum* and *B. lactentis*) and *B. longum* Reuter, obtained with Reuter's strains and a few additional isolates from the feces of breast-fed infants, indicated considerable interspecies relatedness (32). A large number of strains isolated in our laboratory from the feces of either breast- or bottle-fed infants was subsequently studied by means of DNA-DNA hybridization, and the interspecific levels of DNA homology between the two species were found to vary in the range of 65 to 80%. However, as many as 180 strains isolated successively from the feces of suckling calves could not be identified with either species by means of this technique: they were over 80% related to the reference strains of both species. Conversely, selected strains of *B. infantis* and *B. longum* displayed nearly identical high degrees of relatedness (75 to 80%) to selected reference strains from calves (unpublished data). The strains from calves are referred to here as intermediates between *B. infantis* and *B. longum*; their existence could place some doubt on the advisability of recognizing *B. infantis* and *B. longum* as separate species and would seem to support the idea that these two species actually form a "continuum" where the central position could well be occupied by the strains from calf feces. In any event, sugar fermentation patterns are largely shared by the *B. infantis* and *B. longum* homology groups. Arabinose and xylose, which according to Reuter (20) are not fermented by *B. infantis*, are fermented by most of our strains of *B. infantis*, which are all melezitose negative, as was reported by Reuter for his strains. Melezitose-positive and -negative strains were found in the *B. longum* group as well as ribose-positive and -negative strains. All of the calf strains fermented arabinose and xylose; melezitose and ribose were variably fermented (Table 1; also, unpublished data). No wonder, therefore, that the transaldolase and 6PGD zymograms overlap extensively in these bifidobacteria (see Tables 1 and 2 and Fig. 3B). Although 92% of the *B. infantis* strains displayed transaldolase 5 and 6PGD band 4, a pattern not shared by any other bifidobacteria (the other species that have transaldolase 5 have less anodic 6PGD bands), only 72% of the *B. longum* strains have the less anodic transaldolase isozyme 8. The transaldolase isozyme 6 of intermediate mobility

is common to all of the strains from calves and to 8 and 20% of the *B. infantis* and *B. longum* strains, respectively.

All *B. breve* strains, which are commonly found in the feces of human infants, in human vagina, and in the feces of suckling calves, have a form of transaldolase (isozyme 6; see Tables 1 and 2 and Fig. 3B) that is apparently identical with that of the *B. infantis*-*B. longum* intermediates from calves. This is understandable: recent investigations (unpublished data) have shown that *B. breve* is the species most closely related to either *B. infantis* or *B. longum* (50 to 75% DNA homology). Rather unexpectedly, *B. coryneforme*, *B. boum*, and *B. suis* possess the same transaldolase (isozyme 6), although these species differ from *B. breve* in a number of phenotypic traits and in habitat (see Table 1) and are genetically unrelated to *B. breve* (14, 28, 30). This similarity is merely superficial, however; the transaldolases of *B. breve*, *B. infantis*, and *B. longum* are immunologically different from those found in the other species mentioned (34). The transaldolases of the *B. breve* strains that fermented arabinose and xylose and those of the strains from the human vagina and the feces of suckling calves were not electrophoretically distinct from those of the strains isolated from the feces of human infants (see Table 1).

A new species, *B. boum*, has recently been described (28). This species was found in the rumen contents of cattle, which is also the habitat of *B. thermophilum* Mitsuoka (syn [21]: *B. ruminale* Scardovi et al.). The two species are separated primarily on DNA homology (60 to 70% relatedness) and on differences in the structures of their cell wall peptidoglycans (28). The difference in the electrophoretic mobilities of their specific transaldolases adds support to the taxonomic separation of these species; the electrophoretic forms of their 6PGDs are identical (Tables 1 and 2). Of the *B. thermophilum* strains, five were isolated from the feces (two specimens) of suckling calves. These strains can be distinguished from those isolated from the rumen of cattle, from sewage and from the feces of pigs because they have transaldolase isozyme 7 (Table 1), an isozyme with a mobility intermediate between that (isozyme 8) of the vast majority of *B. thermophilum* strains and that (isozyme 6) of the genetically related *B. boum*. These findings are similar to those obtained with the *B. infantis*-*B. longum* intermediate strains isolated from the same habitat: these strains possessed a transaldolase band with a mobility intermediate between those of *B. infantis* and *B. longum*. In the first case, however, the strains (VT81, VT83, VT88, VT90, and VT95) were undoubtedly members of *B. thermophilum* on

the basis of DNA-DNA hybridization results (unpublished data), whereas in the latter case the strains were genetically intermediate between *B. infantis* and *B. longum* (see above). These findings have possible ecological significance because they seem to indicate that the bifidobacteria found in the feces of suckling animals are in some way different from those found in the rumen contents of the same animal, even though these organisms belong to the same genus-species.

B. bifidum is characterized by the zymogram pattern 7-7; 6PGD band 8 was found in four strains only (Tables 1 and 2). The 7-7 pattern is shared by the few strains of *B. thermophilum* isolated from the feces of calves (see above), whereas the pattern 7-8 is displayed by a large number of *B. indicum* strains isolated from *Apis dorsata* (Table 2). In any event, *B. bifidum* strains can be distinguished because they possess a more anodic 3-phosphoglyceraldehyde dehydrogenase. *B. bifidum* strains isolated from the feces of calves and of breast-fed infants have the same transaldolase and 6PGD electrophoretic pattern (Table 1). These two habitats, therefore, harbor not only the same *Bifidobacterium* species—viz. *B. bifidum*, *B. pseudocatenulatum*, *B. infantis*, *B. longum*, and *B. breve*—but also identical enzymatic biovars of these species.

The 54 strains of *B. adolescentis* investigated in this study displayed zymogram pattern 8-5 (Table 2) even though they were of different origins, viz. human feces, sewage, and rumen of cattle (Table 1), and possessed different fermentation patterns (Reuter's biovars [20] and pentose-negative strains from rumen [2]). Because we had only four strains from the feces of adult humans (Reuter's strains, see Table 1), a habitat which has been reported to be the one preferred by this species (16, 20), our data might not be representative of this species. The *B. adolescentis* strains studied could not be distinguished on the basis of zymogram pattern from nearly half of the *B. longum* strains isolated from the feces of infants and included in this study. Furthermore, these two groups of strains possess 3-phosphoglyceraldehyde dehydrogenases with identical electrophoretic mobilities (unpublished data). Preliminary immunological data on the transaldolases of the bifidobacteria (34) seem to confirm this apparent relatedness: the transaldolase of *B. adolescentis* is immunologically more related to that of *B. longum* than to those of *B. catenulatum*, *B. dentium*, *B. angulatum*, and *B. pseudocatenulatum*. However, *B. adolescentis* is apparently more related phenotypically and genetically (DNA homology) to these species than to *B. longum* (unpublished data).

It is evident that the DNA relatedness between *B. adolescentis* and *B. longum*, determined so far only with a limited number of isolates (32), needs to be more precisely defined, especially since the taxonomic status of *B. longum* seems to be unclear (see above).

B. asteroides and *B. indicum* have been isolated from the intestines of *Apis mellifera* L. (mostly of the race *ligustica*, but also *mellifera* and *caucasica*), *Apis cerana* Fabr. (*A. indica*), and *Apis dorsata* Fabr. The specimens of *A. mellifera* were obtained from various localities in Italy as well as from Greece, France, Germany, Norway, the Soviet Union, the Philippines, and other countries (details in ref. 26). The origins of *A. cerana* and *A. dorsata* are given in Table 1. *B. asteroides* and *B. indicum* are highly variable with respect to the transaldolases and 6PGDs they produce (see Tables 1 and 2 and Fig. 4). As many as eight transaldolases and nine 6PGDs were detected in *B. asteroides* (85 strains), and four and seven, respectively, in *B. indicum* (124 strains). Here again, however, each strain exhibited only one band for each allozyme. Transaldolase zymograms are of little value in differentiating the two species because of the large number of band matchings; on the other hand, limited overlapping occurs with the 6PGD isozymes (see Fig. 4 and 5). *B. asteroides* is unique among the bifidobacteria in that it possesses the less mobile variants of 6PGD (Fig. 3B). The differences in the morphology, nutrition, and structure of cell wall peptidoglycan (11) of *B. asteroides* and *B. indicum* are such as to warrant their separation into different taxa (26). However, their reciprocal DNA homology (some 30%; see ref. 32) is indicative of a certain relatedness because little or no homology was demonstrated with all other bifidobacteria used as references. Admittedly, only a few interspecific homology experiments were performed (32); additional experiments now under way will probably reveal higher levels of interspecific DNA relatedness.

The frequency distribution of transaldolase isozymes (Fig. 5) and the data summarized in Table 2 reveal some interesting facts. The distribution of *B. indicum* transaldolases is asymmetrical, with two isozymes (viz. 7 and 9) displaying nearly the same high frequency. Isozyme 7 is found almost exclusively in the strains isolated from *Apis dorsata*, whereas the less anodic isozyme 9 is found in the strains from *Apis cerana* (*A. indica*) (see Table 1). Extensive investigations are needed to determine whether the bifidobacteria from *A. cerana* can be distinguished from those from *A. dorsata*, two species of asiatic bees of which specimens were received from Malaysia, Japan, and the Philippines, on

the basis of DNA-DNA hybridization. The few data reported elsewhere (32), obtained with the DNA of strain C410 (=ATCC 25912) isolated from *A. cerana*, are suggestive that this can be achieved. The frequency distribution of isozymes is by far more symmetrical in *B. asteroides*, and there is no indication, according to experiments now under way, that groups of isolates of this species can be separated by means of DNA-DNA hybridization. As many as 23 different combinations of electrophoretic types of transaldolase and 6PGD are listed for *B. asteroides* in Table 2. If one considers that not less than six electrophoretic forms of 3-phosphoglyceraldehyde dehydrogenase have been preliminarily recognized among these strains (unpublished data), one could probably characterize each *B. asteroides* isolate by a strain-specific pattern of these three enzymes. It is not known, obviously, whether this high degree of variability is intrinsic to the genetic structure of the organism itself or is related to the rapidly changing environment the organism meets as the physiology of the host changes during its short life (see ref. 9, 15, 18, 19).

The bifidobacterial transaldolase of least electrophoretic mobility was found in the two strains isolated from sewage and assigned to the "minimum" DNA homology group (27). However, their 6PGD isozyme is apparently more anodic than expected (Fig. 3B). This observation, which at present is only anecdotal, stems from a cursory comparison of the relative positions of the isozymes graphically presented in Fig. 3; the decrease in the electrophoretic mobility of the transaldolases from *B. cuniculi* to *B. asteroides* is seemingly accompanied by a decrease in the mobility of their 6PGD isozymes. Electrophoretic studies on other enzymes are needed for elucidating this point, which might have some evolutionary significance.

REPRINT REQUESTS

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