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Electrophoretic Patterns of Proteins in the Genus *Bifidobacterium* and Proposal of Four New Species

B. BIAVATI,[1] V. SCARDOVI,[1] AND W. E. C. MOORE[2]

Istituto di Microbiologia Agraria, Università di Bologna, Bologna, Italy,[1] and Department of Anaerobic Microbiology, Virginia Polytechnic Institute and State University, Blacksburg, Virginia 24061[2]

The polyacrylamide gel electrophoretic patterns of soluble cellular proteins from 1,094 strains of bifidobacteria were compared with available deoxyribonucleic acid (DNA)-DNA homology data and with the phenotypic and biochemical reactions of these strains. There was excellent correlation between the 25 distinct protein patterns and 24 DNA-DNA homology groups in the genus. Differentiation among species on the basis of common phenotypic properties often was unreliable. Our results demonstrate that the species previously known as "*Bifidobacterium eriksonii*" is a synonym of *Bifidobacterium dentium* Scardovi and Crociani; that "*Actinomyces parabifidus*" is a synonym of *Bifidobacterium infantis* Reuter; that *Bifidobacterium globosum* (ex Scardovi, Trovatelli, Crociani, and Sgorbati 1969) sp. nov., nom. rev. (type strain, ATCC 25865) and *Bifidobacterium pseudolongum* Mitsuoka are closely related but distinct entities; and that *Bifidobacterium minimum* sp. nov. (type strain, ATCC 27538) and *Bifidobacterium subtile* sp. nov. (type strain, ATCC 27537) are valid species. Our analyses also indicate that *Bifidobacterium coryneforme* (ex Scardovi and Trovatelli 1969) sp. nov., nom. rev. (type strain, ATCC 25911) is a valid species. A group of strains intermediate between *B. infantis* and *B. longum*, "*Bifidobacterium infantis-longum*," occurs in calf feces. Identical or nearly identical protein patterns were produced by strains that had 80% or greater DNA homology.

Among the 20 species of the genus *Bifidobacterium* cited on the Approved Lists of Bacterial Names (33), several genetically distinct species from humans and other animals cannot be differentiated reliably by commonly used enzymatic or fermentation reactions (5) or by zymograms of transaldolases and 6-phosphogluconate dehydrogenases (23). Several authors have reported the use of polyacrylamide gel electrophoresis (PAGE) of soluble cellular proteins to distinguish species or serovars (serotypes) of bacteria (1, 4, 8, 9, 11, 32, 35) or to determine the similarity of unidentified isolates (15). The present study was designed to determine the reliability and sensitivity of a PAGE procedure (15) to distinguish genetically related and unrelated species of bifidobacteria. For this purpose we compared the electrophoretograms of *Bifidobacterium* type strains and many other strains that were previously identified according to their deoxyribonucleic acid (DNA)-DNA homology characteristics or phenotypic characteristics or both.

MATERIALS AND METHODS

Bacterial strains. The strains examined are listed in Table 1.

Methods. The biochemical properties of the strains were determined by methods previously described by Holdeman et al. (5). The DNA-DNA homology values used were from previously published data (10, 23, 24, 26, 27, 29–31, 37, 38) and were obtained by the methods described by Scardovi et al. (24, 29).

For electrophoretic analyses, cells were inoculated into 5 ml of supplemented brain heart infusion broth (5) containing 0.1% calcium carbonate and 0.025% (final concentration) Tween 80 and then incubated at 37°C for 24 h. Growth was harvested by centrifugation at 8,000 × *g* for 10 min. Electrophoretic analysis of cellular soluble proteins was performed by using the procedures of Moore et al. (15). The similarity of patterns on different gels was determined by placing cut photographs adjacent to each other. Strains with similar protein patterns were reanalyzed on single gels for direct visual comparisons.

RESULTS AND DISCUSSION

The DNA-DNA homology relationships among species of the genus *Bifidobacterium* have been studied extensively. Many of the species show close genetic relationships, and several species have similar phenotypic characteristics. Therefore, the species in this genus provide an ideal test of the sensitivity and reliability of the application of PAGE to differentiate bacterial species.

The available type strains and other strains of bifidobacteria that have known DNA homologies with the type strains were included in the

TABLE 1. Species, number of strains and sources of 1094 bifidobacteria examined by electrophoresis

B. adolescentis:
- 292 Adult human intestine and feces
- 19 Sewage

B. angulatum:
- 5 Sewage
- 1 Adult human intestine

B. animalis:
- 4 Chicken feces
- 4 Rat feces
- 1 Rabbit feces
- 1 Sewage

B. asteroides:
- 8 Bee intestine

B. bifidum:
- 21 Adult human intestine and feces
- 14 Human infant feces
- 5 Human vagina
- 2 Calf feces
- 2 Human clinical:
  - 1 Culdocentesis fluid
  - 1 Pancreatic fluid

B. boum:
- 6 Bovine rumen
- 2 Piglet feces

B. breve:
- 12 Human adult intestine and feces
- 8 Human infant feces
- 5 Human clinical:
  - 3 Peritoneal fluid
  - 1 Rectal abscess
  - 1 Colostomy
- 3 Human vagina
- 3 Unlisted
- 1 Calf feces

B. catenulatum:
- 175 Adult human intestine and feces
- 15 Sewage
- 1 Human infant feces
- 1 Human vagina
- 1 Unlisted

B. choerinum:
- 8 Piglet intestine
- 2 Sewage

"B. coryneforme":
- 6 Bee intestine

B. cuniculi:
- 5 Rabbit feces

B. dentium:
- 30 Human clinical:
  - 2 Dental abscess
  - 3 Sputum
  - 4 Oral cavity
  - 5 Abdominal wound
  - 1 Ascitic fluid
  - 5 Lung abscess
  - 2 Chest fluid
  - 1 Chest incision
  - 2 Jaw abscess
  - 2 Neck abscess
  - 2 Leg wound
  - 1 Wound

B. dentium (cont'd)
- 24 Human dental plaque
- 14 Adult human intestine and feces
- 5 Unlisted
- 2 Human vagina
- 2 Human infant feces
- 2 Rabbit feces

"B. globosum":
- 2 Lamb feces
- 2 Bovine rumen
- 2 Calf feces
- 2 Rabbit feces
- 1 Rat feces
- 1 Human infant feces
- 1 Sewage

B. indicum:
- 9 Bee intestine

B. infantis:
- 16 Human infant feces
- 3 Unlisted
- 1 Human cervix
- 1 Dog feces
- 1 Chicken feces

"B. infantis-longum":
- 5 Calf feces

B. longum:
- 264 Adult human intestine and feces
- 7 Human clinical:
  - 2 Blood
  - 1 Umbilicus
  - 1 Abdominal cavity
  - 1 Perirectal abscess
  - 1 Renal
  - 1 Placenta
- 4 Human infant feces

B. magnum:
- 10 Rabbit feces

"B. minimum":
- 2 Sewage

B. pseudocatenulatum:
- 3 Human infant feces
- 3 Calf feces
- 1 Sewage

B. pseudolongum:
- 3 Swine feces
- 1 Sewage

B. pullorum:
- 3 Chicken feces

"B. subtile":
- 5 Sewage

B. suis:
- 7 Piglet intestine and feces

B. thermophilum:
- 13 Piglet stomach
- 2 Piglet feces
- 4 Bovine rumen
- 1 Calf feces
- 2 Sewage

TABLE 2. Published DNA homology relationships of *Bifidobacterium* species

Species (literature citation)	% of homology within (range)	Comments
B. adolescentis (21)	70–102 (24)	Below 50% homology with all the other species[a]
B. angulatum (24)	76–95 (24)	Below 50% homology with all the other species[a]
B. animalis (12, 26)	72–99 (26)	Below 50% homology with all the other species[a]
B. asteroides (25)	Not published	50% homology with *B. choerinum* (27)
B. bifidum (17, 36)	100 (29)	Below 50% homology with all the other species[a]
B. boum (27)	69–96 (27)	Closely related to *B. thermophilum* at 60–70% (27)
B. breve (21)	75–100 (29)	Closely related to *B. infantis* and *B. longum* at 50–75% (23)
B. catenulatum (24)	78–101 (27)	Closely related to *B. pseudocatenulatum* at 60–80% (27)
B. choerinum (27)	75–120 (27)	50% homology with *"B. globosum"*, *B. pseudolongum*, and *B. asteroides* (27)
"B. coryneforme" (25)	Not published	Closely related to *B. indicum* at 60% (31)
B. cuniculi (27)	94–102 (27)	Closely related to *B. globosum* at 50–67% (27)
B. dentium (24)	69–110 (24)	Below 50% homology with all the other species[a]
"B. globosum" (28)	78–106 (29) 72–104 (38)	Closely related to *B. pseudolongum* at 69–73% (29)
B. indicum (25)	Not published	Closely related to *B. coryneforme* at 60% (31)
B. infantis (21)	Not published	Closely related to *B. longum* at 65–80% (23)
"B. infantis-longum"	Not published	Over 80% related to the reference strains of *B. infantis* and *B. longum* (23)
B. longum (21)	Not published	Closely related to *B. infantis* at 65–80% (23)
B. magnum (30)	75–106 (30)	Below 50% homology with all the other species[a]
B.pseudocatenulatum (27)	78–115 (27)	Closely related to *B. catenulatum* at 60–80% (27)
B. pseudolongum (12)	Not published	Closely related to *B. globosum* at 69–73% (29)
B. pullorum (37)	94–107 (37)	Below 50% homology with all the other species[a]
B. suis (10)	100 (10)	Below 50% homology with all the other species[a]
B. thermophilum (12)	79–117 (27)	Closely related to *B. boum* at 60–70% (27)
"B. minimum" (26)	103 (26)	Below 50% homology with all the other species[a]
"B. subtile" (26)	70–94 (26)	Below 50% homology with all the other species[a]

[a]Differentiation of this species from all other species is indicated in several references which provide one or more comparisons (10, 24, 26, 27, 29, 30, 31, 37).

PAGE study. To facilitate comparisons of the DNA homology and PAGE results, a summary (primarily from previously published work) of the DNA-DNA relationships of these strains and species is presented in Table 2.

Electrophoretic patterns of soluble cellular proteins. The distinctive protein patterns of the type strains (and strains that are known to have high DNA homology with the type strains) correlated with the DNA homology relationships among the bifidobacteria and were found to be repeatable (see Fig. 1, lane 8; Fig. 2, lane 13; and Fig. 12, lane 2). This PAGE procedure (15) was highly sensitive and could distinguish between species that have less than 80% interspecies DNA-DNA homology. Within species, strains that have 80% or greater homology had identical, or nearly identical, protein patterns.

The PAGE patterns of 1,094 *Bifidobacterium* strains were determined and compared with the patterns of the type strains of the *Bifidobacterium* species and with the patterns of strains that

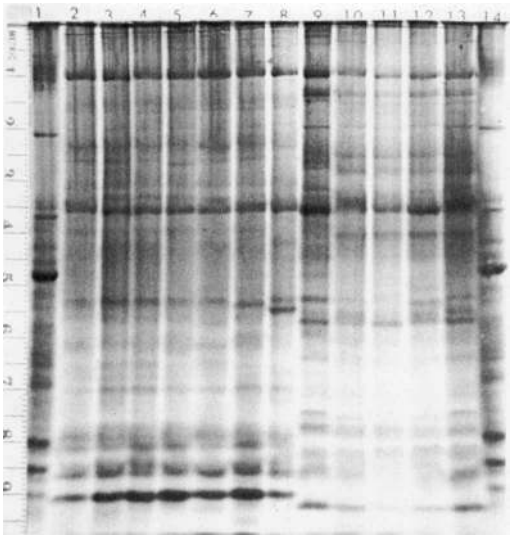

FIG. 1. *B. adolescentis*, lanes 2 to 8: 2 = VPI A3-27B, feces, adult rural South African black; 3 = VPI B6-42, feces, adult Caucasian, U.S.; 4 = VPI C8-1, feces, adult Japanese-Hawaiian; 5 = VPI J1-2, feces, adult rural Japanese; 6 = VPI M3-140, adult human stomach contents, U.S.; 7 = VPI 7501 (Scardovi F351), waste water, Italy; 8 = ATCC 15703 (type strain), intestine of adult human, Germany.

B. angulatum, lanes 9 to 13: 9 = ATCC 27535 (type strain), human feces, Italy; 10 = ATCC 27669, 11 = ATCC 27670, 12 = ATCC 27671, 13 = VPI 7569 (Scardovi F426), all from waste water, Italy.

are known to have high DNA homology with the type strains. The PAGE patterns of representative strains of *Bifidobacterium* species are shown in Fig. 1 to 14. For each species, the strains chosen as representatives included the type strain and the strains from the most widely divergent sources that were available in order to illustrate the maximum variation among strains within each species. When possible, species that are closely related by DNA-DNA homology, species with similar phenotypic properties, and species from similar habitats (in that order) were placed on the same gel to test the sensitivity of the electrophoretic analysis.

On all gels, lanes 1 and 14 show the reference patterns of *Streptococcus faecalis* strain VPI U4-20, which was used as a control for detecting gel-to-gel variation. The two major causes of variation in gel density were the moisture content of the gel powder (which affected the actual amount of acrylamide weighed) and the oxygen content of the gel solution. Complete removal of the oxygen (e.g., with iron) from the acrylamide solution caused immediate solidification when persulfate was added, and the gels could not be poured. Mechanical removal of oxygen under reduced pressure left variable amounts of oxygen in solution. Other causes of variation are under investigation.

From the *S. faecalis* control strain, the dark band that appears between 43 and 49 mm is a useful reference point for comparing different gels. The band at 1 cm is typical of most species of the genus *Bifidobacterium*.

Patterns from *Bifidobacterium adolescentis* and *Bifidobacterium angulatum* strains are shown in Fig. 1. Cultures of the type strain of *B. adolescentis* (Fig. 1, lane 8) have a band at 54 mm that is reproducible (see Fig. 2, lane 13, and Fig. 12, lane 2), as are most of the other minor variations in banding patterns among strains

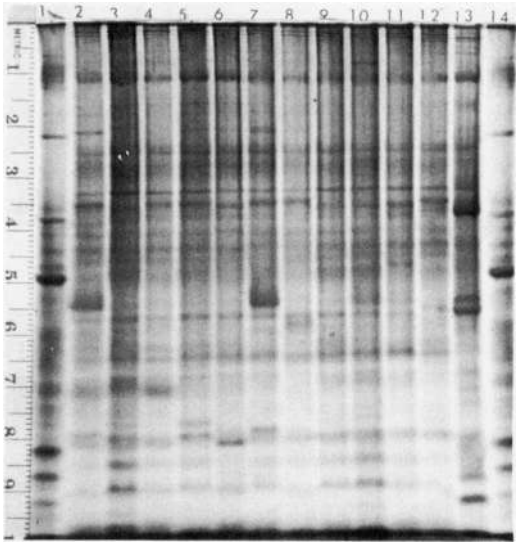

FIG. 2. *B. dentium*, lanes 2 to 8: 2 = ATCC 27678, adult human feces, Italy; 3 = ATCC 27679, human vagina, Italy; 4 = ATCC 27680, human dental caries, Italy; 5 = VPI 0661 (CDC 1209B-1), human autopsy lung tissue, U.S.; 6 = VPI 13250 (Scardovi B2617), human infant feces, Italy; 7 = VPI 13251 (Scardovi Ra116), rabbit feces; 8 = ATCC 27534 (type strain), human dental caries, Italy.

B. dentium ("*B. eriksonii*"), lanes 9 to 12: 9 = ATCC 15424 (labeled *B. adolescentis*, CDC X407 ["*A. eriksonii*"], pleural fluid, adult male), presumably U.S.; 10 = VPI 1932 (CDC X685, originally labeled "*A. eriksonii*"); 11 = ATCC 15423 (labeled *B. adolescentis*, CDC X573, [originally the type strain of "*A. eriksonii*"]), lung abscess, adult male; 12 = VPI 1935 (CDC X425 "*A. eriksonii*"), human abscess.

B. adolescentis, lane 13 = ATCC 15703 (type strain).

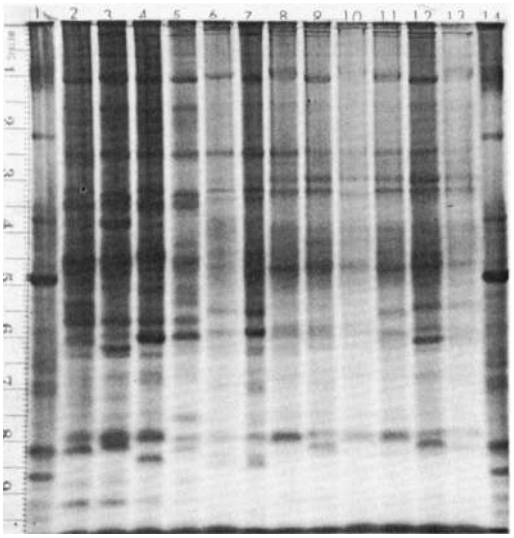

FIG. 3. *B. infantis*, lanes 2 to 5: 2 = ATCC 15697 (type strain), human infant feces, Germany; 3 = ATCC 25962, human infant feces, Germany; 4 = ATCC 15702, human infant feces, Germany; 5 = ATCC 17930 (labeled *B. bifidum* CDC W723, [*Actinomyces parabifidus*] Strain Timberland [19]; L. Pine 308 [3, 20]).

"B. infantis-longum" (an intermediate 'biotype' between *B. infantis* and *B. longum* by DNA homology, fermentation reactions, and electrophoretic pattern), lanes 6 and 7: 6 = VPI 13294 (Scardovi VT420), calf feces; 7 = VPI 13295 (Scardovi VT302), calf feces.

B. longum, lanes 8 to 12: 8 = ATCC 15707 (type strain), adult human intestine, Germany; 9 = VPI R1-33, adult human feces, U.S.; 10 = VPI J8-25, adult human feces, rural Japan; 11 = VPI X2-30, adult human feces, U.S.; 12 = VPI M3-122, adult human stomach contents; 13 = VPI 7269, human infant feces, U.S.

within the species. This species is commonly isolated from the intestines and feces of adult humans, but it was not found in any fecal samples from more than 100 infants in Italy.

In addition to the "genus band" at 1 cm, strains of *B. adolescentis* and *B. angulatum* share a band at 36 mm and show strain-to-strain variation with bands at 53 to 57 mm. Close inspection reveals several bands that are uniformly distinct for the two species.

The PAGE patterns of *Bifidobacterium dentium* and *B. adolescentis* strains are shown in Fig. 2. *B. dentium* Scardovi and Crociani 1974, *B. adolescentis* Reuter 1963, and *"Actinomyces eriksonii"* Georg et al. 1965 have very similar fermentation patterns (see Table 5). *B. dentium* and *B. adolescentis* are distinct species according to the results of DNA homology studies (24) and have distinct PAGE patterns. The PAGE patterns of *B. dentium* and *"Bifidobacterium eriksonii"* (*"A. eriksonii"*) are very similar. The taxonomic status of *"A. eriksonii"* has changed several times since the species was first described. Because of the similarity between *"A. eriksonii"* and bifidobacteria, the species was designated *"B. eriksonii"* by Holdeman and Moore (7). In 1974, Mitsuoka et al. (13) studied the cultural and phenotypic reactions of *B. adolescentis* and *"B. eriksonii"* and considered the two species to be identical. *"A. eriksonii"* is listed as a species incertae sedis in *Bergey's Manual of Determinative Bacteriology*, 8th ed. (34), and the name was not included on the Approved Lists of Bacterial Names (33), apparently because it was assumed to be a later synonym of *B. adolescentis*. Scardovi et al. (23)

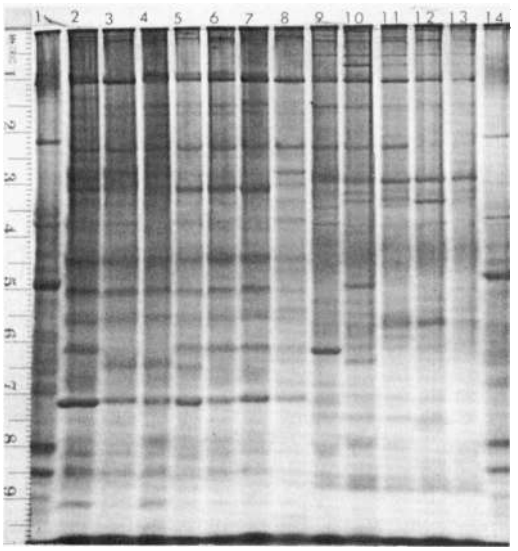

FIG. 4. *B. bifidum* (type species of the genus), lanes 2 to 8: 2 = VPI M4-361, adult human ascending colon; 3 = VPI S5E-45, adult human feces (after all-meat diet for 3 days), U.S.; 4 = ATCC 15696, human infant intestine, Germany; 5 = VPI 5645 (Scardovi B790), human vagina, Italy; 6 = VPI 13223 (Scardovi VT181), calf feces, Italy; 7 = VPI 12064, human pancreatogram fluid, U.S.; 8 = ATCC 29521 (type strain), Tissier's strain from the Prevot collection, human infant feces, France.

B. breve, lanes 9 to 13: 9 = ATCC 15700 (type strain), human infant feces, Germany; 10 = VPI C11-10, adult Japanese-Hawaiian feces, Hawaii; 11 = ATCC 15698, human infant intestine, Germany; 12 = VPI 5642 (Scardovi B720), human vagina, Italy; 13 = VPI 13234 (Scardovi VT268), calf feces, Italy.

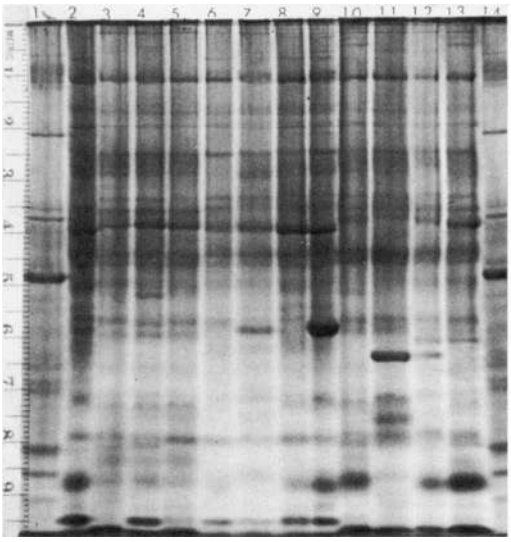

FIG. 5. *B. catenulatum*, lanes 2 to 9: 2 = VPI M3-204, duodenum contents, adult human black, U.S.; 3 = VPI J4-38, adult human feces, rural Japan; 4 = VPI 7399A (Scardovi B618), human infant feces, Italy; 5 = VPI 6276, human vagina, U.S.; 6 = ATCC 27675, adult human feces, Germany; 7 = VPI 7495 (Scardovi F64), sewage, Italy; 8 = VPI C52-40B, adult Japanese-Hawaiian feces, Hawaii; 9 = ATCC 27539 (type strain), adult human feces, Italy.

B. pseudocatenulatum, lanes 10 to 13: 10 = ATCC 27919 (type strain), human infant feces, Italy; 11 = VPI 13270 (Scardovi B1460), human infant feces, Italy; 12 = VPI 13275 (Scardovi VT74), calf feces, Italy; 13 = VPI 13276 (Scardovi F54), sewage, Italy.

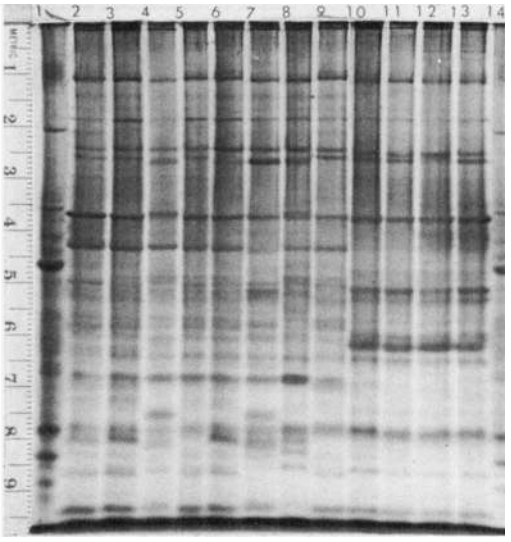

FIG. 6. *"B. globosum"*, lanes 2 to 9: 2 = ATCC 25865 (type strain), bovine rumen, Italy; 3 = VPI 13379 (Scardovi Ag40), lamb feces, Italy; 4 = VPI 13384 (Scardovi F229), sewage, Italy; 5 = VPI 13386 (Scardovi B607), human infant feces, Italy; 6 = VPI 13389 (Scardovi VT376), calf feces, Italy; 7 = VPI 13390 (Scardovi T15), rat feces, Italy; 8 = VPI 13392 (Scardovi Ra120), rabbit feces, Italy; 9 = ATCC 25864, bovine rumen, Italy.

B. pseudolongum, lanes 10 to 13: 10 = ATCC 25526 (type strain), swine feces, Japan; 11 = VPI 13382 (Scardovi Su841), piglet feces, Italy; 12 = VPI 13383 (Scardovi Su914), piglet feces, Italy; 13 = VPI 13385 (Scardovi F468), sewage, Italy.

reported 80 to 100% DNA homology among ATCC 27534 (the type strain of *B. dentium*), ATCC 15423 ("*A. eriksonii*"), and ATCC 15424, the type strain of "*A. eriksonii*." The nearly identical electrophoretic patterns of the strains of *B. dentium* and "*B. eriksonii*" further confirm the reliability of the PAGE results and the synonymy of "*B. eriksonii*" with *B. dentium* rather than with *B. adolescentis*. The name *B. dentium* is retained because neither "*A. eriksonii*" nor "*B. eriksonii*" appeared on the Approved Lists and therefore the earlier names have no taxonomic standing and cannot be considered earlier synonyms of *B. dentium*.

The electrophoretic patterns of soluble proteins of *Bifidobacterium infantis*, *Bifidobacterium longum*, and an intermediate group labeled "*Bifidobacterium infantis-longum*" are shown in Fig. 3. Included with *B. infantis* is ATCC 17930 (lane 5), which was once labeled "*Actinomyces parabifidus*." Although "*A. parabifidus*" was reported to be synonymous with *Bifidobacterium bifidum* (18, 22), ATCC 17930 has neither the biochemical reactions nor the electrophoretic pattern of *B. bifidum*. However, this strain does have the phenotypic characteristics and protein pattern of *B. infantis*, and we have determined that it has 82% DNA-DNA homology with ATCC 27920, the reference strain of *B. infantis*. It also has 76% DNA-DNA homology with the type strain of *B. longum*. A close relationship to *B. longum* is characteristic of all of the strains of *B. infantis* studied.

With respect to its fermentation reactions (see Table 5), *B. infantis* is similar to *B. adolescentis* and *Bifidobacterium catenulatum*, but no strain of *B. infantis* (as determined by electrophoretic protein patterns or by DNA-DNA homology) has been found in feces of adult humans. *B. infantis* has been found in human vaginas and, as previously reported, in feces from infants. Strains previously reported as *B. infantis* from

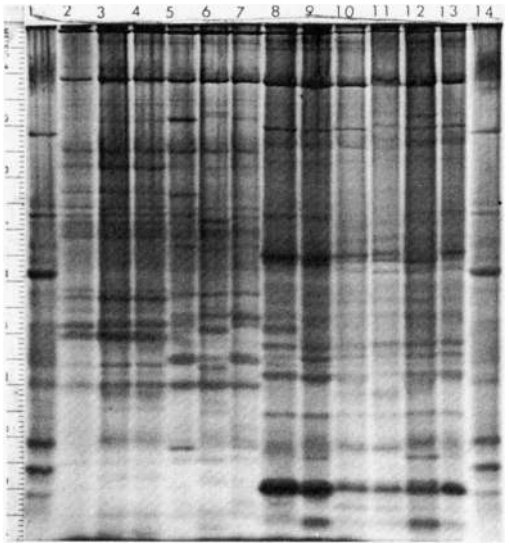

FIG. 7. *B. animalis*, lanes 2 to 7: 2 = ATCC 27673, sewage, Italy; 3 = ATCC 27674, rabbit feces, Italy; 4 = ATCC 27536, chicken feces, Italy; 5 = ATCC 27672, rat feces, Italy; 6 = VPI 13212 (Scardovi T27), rat feces, Italy; 7 = ATCC 25527 (type strain), rat feces, Japan. *B. magnum*, from rabbit feces, Italy, lanes 8 to 13: 8 = ATCC 27540 (type strain), 9 = ATCC 27681, 10 = ATCC 27682, 11 = VPI 13265 (Scardovi Ra89), 12 = VPI 13266 (Scardovi Ra184), 13 = VPI 13268 (Scardovi Ra209).

feces of adult humans (6, 14, 16) have been reidentified in this study either as *B. adolescentis* or as *B. catenulatum*.

The close genetic relationships among *B. infantis*, *B. longum*, and the "*B. infantis-longum*" group (Table 2) and their similar fermentation patterns (see Table 5) are reflected in the similar electrophoretic patterns of their soluble cellular proteins. The similarities suggest that these groups may be subspecies of *B. longum*. However, bands at 92 mm (Fig. 3, lanes 2 through 5) and 30 mm (lanes 8 through 13) appear to be distinctive and to correlate with the strains of *B. infantis* and *B. longum*, respectively, that have greater intragroup DNA homology than intergroup DNA homology.

The electrophoretic patterns of *B. bifidum* and *Bifidobacterium breve* are shown in Fig. 4. *B. bifidum* is among the most easily recognized species in the genus because of its characteristic fermentation pattern (see Table 5). The heavy band at 62 mm produced by the type strain of *B. breve* (Fig. 4, lane 9) varies with the age of the culture and is sometimes present in other strains of the species.

The electrophoretic patterns of *B. catenulatum* and *Bifidobacterium pseudocatenulatum* are shown in Fig. 5. These two species are closely related and share 60 to 80% DNA-DNA homology. They reportedly differ in the guanine-plus-cytosine contents of their DNAs (*B. catenulatum*, 54.7 mol%; *B. pseudocatenulatum*, 57.5 mol% [24, 27]), cell wall peptidoglycan (O. Kandler, personal communication), and isozyme patterns (23). Although a band at 95 mm appears to correlate with *B. catenulatum* strains, differentiation on the basis of electrophoretic protein patterns is questionable. The DNA homology and protein similarities suggest that *B. catenulatum* and *B. pseudocatenulatum* may be subspecies of *B. catenulatum*.

The patterns of "*Bifidobacterium globosum*" Scardovi et al. 1969 (not on the Approved Lists [33]) and *Bifidobacterium pseudolongum* are shown in Fig. 6. These two species share about 70% DNA-DNA homology (see Table 2) and have the same fermentation patterns; their names are considered by Rogosa (22) to be synonyms. However, the reported guanine-plus-

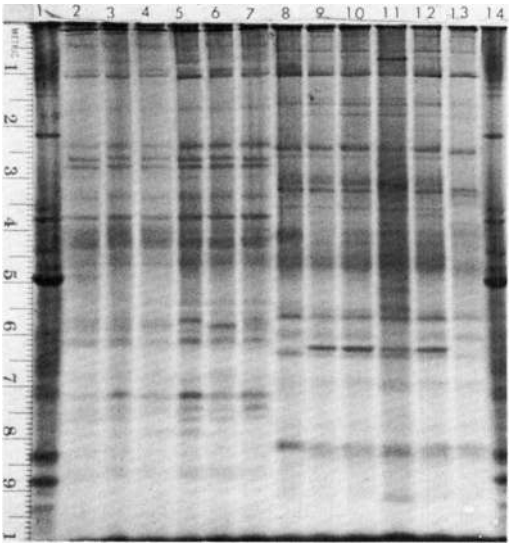

FIG. 8. *B. choerinum*, lanes 2 to 7: 2 = VPI 13235 (Scardovi Su827), piglet feces, Italy; 3 = VPI 13237 (Scardovi Su837), piglet feces, Italy; 4 = VPI 13239 (Scardovi Su882), piglet feces, Italy; 5 = VPI 13242 (Scardovi F390), sewage, Italy; 6 = VPI 13243 (Scardovi F441), sewage, Italy; 7 = ATCC 27686 (type strain), piglet feces, Italy. *B. suis*, from piglet feces, Italy, lanes 8 to 13: 8 = ATCC 27533, 9 = ATCC 27531, 10 = ATCC 27532, 11 = VPI 13281 (Scardovi Su889), 12 = VPI 13282 (Scardovi Su940), 13 = VPI 13284 (Scardovi Su850).

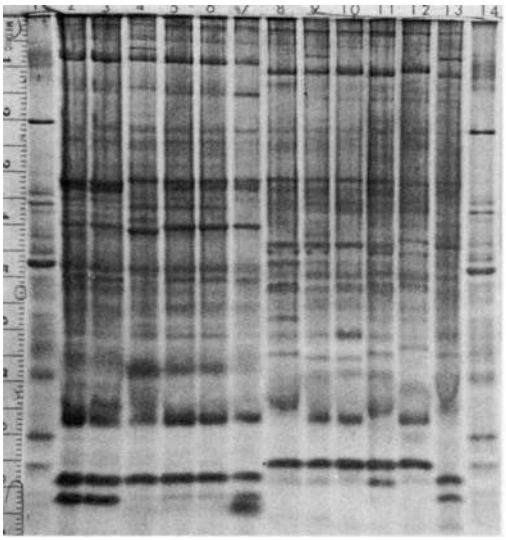

FIG. 9. *B. boum*, lanes 2 to 7: 2 = VPI 13224 (Scardovi Ru246), bovine rumen, Italy; 3 = VPI 13225 (Scardovi Ru276), bovine rumen, Italy; 4 = VPI 13226 (Scardovi Ru400), bovine rumen, Italy; 5 = VPI 13231 (Scardovi Su910), piglet feces, Italy; 6 = VPI 13232 (Scardovi Su912), piglet feces, Italy; 7 = ATCC 27917 (type strain), bovine rumen, Italy. *B. thermophilum*, lanes 8 to 13: 8 = ATCC 25525 (type strain), swine feces, Japan; 9 = ATCC 25866, bovine rumen, Italy; 10 = ATCC 25867, bovine rumen, Italy; 11 = VPI P10A-4, piglet feces, U.S.; 12 = VPI 13288 (Scardovi F539), sewage, Italy; 13 = VPI 13291 (Scardovi VT95), calf feces, Italy.

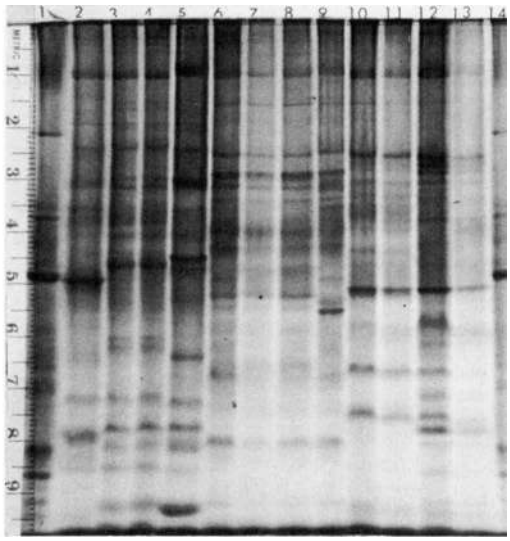

FIG. 10. *B. asteroides*, from intestines of honey bees (*Apis mellifera*), lanes 2 to 5: 2 = VPI 13216 (Scardovi C229), Norway; 3 = VPI 13217 (Scardovi C391), Czechoslovakia; 4 = VPI 13219 (Scardovi 395), Czechoslovakia; 5 = ATCC 25910 (type strain), Italy.

B. coryneforme sp. nov., nom. rev. from intestines of *Apis mellifera*, lanes 6 to 9: 6 = ATCC 25911 (type strain), Norway; 7 = VPI 13303 (Scardovi C216), Norway; 8 = VPI 13304 (Scardovi C218), Norway; 9 = VPI 13305 (Scardovi C242), England.

B. indicum, from intestines of bees, lanes 10 to 13: 10 = ATCC 25912 (type strain) from *Apis cerana*, Malaysia; 11 = VPI 13254 (Scardovi C403), *Apis cerana*, Malaysia; 12 = VPI 13256 (Scardovi C421) from *Apis dorsata*, Philippines; 13 = VPI 13257 (Scardovi C435), *Apis cerana*, Philippines.

cytosine contents of their DNAs differ by 3 mol% (29), and the two nomenspecies were considered to be distinct entities by Scardovi et al. (29). The electrophoretic patterns indicate that these two groups can be recognized as distinct, but closely related, species. Therefore, we propose revival of the name *B. globosum* (see below).

The PAGE patterns of strains of *Bifidobacterium animalis* and *Bifidobacterium magnum* are shown in Fig. 7. The *B. animalis* strains from rats (Fig. 7, lanes 5 through 7) appear to be highly specific and different from the strains from sewage, rabbits, and chickens. In addition to DNA-DNA homology and electrophoretic protein pattern differences, *B. magnum* is also distinguished by its unusually large cellular dimensions (30).

Electrophoretograms of two species commonly isolated from swine are shown in Fig. 8. Although *Bifidobacterium choerinum* and *Bifidobacterium suis* are both common in swine feces, these two species show little or no DNA-DNA homology and have distinct fermentation patterns (see Table 5).

The electrophoretic patterns of *Bifidobacterium boum* and *Bifidobacterium thermophilum* are shown in Fig. 9. These two species share 60 to 70% DNA-DNA homology and are difficult to distinguish on phenotypic bases. However, the protein patterns appear to be distinctive. The genus band at 1 cm was displaced and appeared at 8 mm in all cultures of *B. boum* tested. Strain 13291 (Fig. 9, lane 13) is classified as *B. thermophilum* on the basis of DNA homology. However, this strain has an isozyme with a mobility that is intermediate between that of *B. thermophilum* and that of *B. boum* (23). The intermediate electrophoretic pattern appears to correlate with the isozyme determinations.

The electrophoretic patterns of *Bifidobacteri-*

TABLE 3. Percent DNA homology among strains of *B. asteroides*, *B. indicum*, and *"B. coryneforme"*

DNA from	Reference DNA from	
	B. asteroides	*B. indicum*
B. asteroides	100%	30%
B. indicum	33%	100%
"B. coryneforme"	23%	60%

um asteroides, *"Bifidobacterium coryneforme,"* and *Bifidobacterium indicum*, all isolated from honey bees, are shown in Fig. 10. *"B. coryneforme"* (Fig. 10, lanes 6 through 9) was inadvertently omitted from the Approved Lists (33) and therefore currently has no taxonomic standing. This species is distinct from *B. asteroides* and *B. indicum*, as reported by Scardovi et al. (31) and as determined by DNA homology (Table 3), and it appears to differ in fermentation reactions as well as cellular proteins. Therefore, we propose reinstatement of the name *B. coryneforme* (see below).

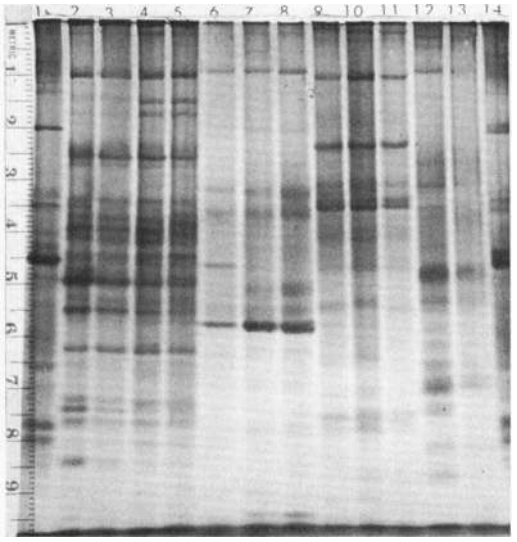

FIG. 11. *B. cuniculi*, from rabbit feces, Italy, lanes 2 to 5: 2 = VPI 13244 (Scardovi Ra94), 3 = VPI 13245 (Scardovi Ra95), 4 = VPI 13246 (Scardovi Ra98), 5 = ATCC 27916 (type strain).

B. pullorum, from chicken feces, Italy, lanes 6 to 8: 6 = ATCC 27685 (type strain), 7 = VPI 13279 (Scardovi P182), 8 = VPI 13280 (Scardovi P183).

B. subtile sp. nov., from sewage, Italy, lanes 9 to 11: 9 = ATCC 27683, 10 = ATCC 27684, 11 = ATCC 27537 (type strain).

B. minimum sp. nov., from sewage, Italy, lanes 12 and 13: 12 = VPI 13297 (Scardovi F399), 13 = ATCC 27538 (type strain).

The electrophoretic patterns of *Bifidobacterium cuniculi* and *Bifidobacterium pullorum* and of two new species are shown in Fig. 11. A direct comparison of the patterns of the type strains of the various *Bifidobacterium* species is presented in Fig. 12 and 13.

In the interpretation of the electrophoretic protein patterns, as in all other microbiological work, one is constantly concerned with the possibility that the results may have been affected by contaminants. In some instances, the protein patterns themselves provide evidence of contamination by displaying patterns that are unusual for the species or genus, as illustrated in Fig. 14.

Phenotypic characteristics. A total of 732 isolates of bifidobacteria from adult human feces (6, 14, 16), from all anatomic areas of adult human and piglet intestinal tracts, and from pig feces were studied. These isolates had been identified previously on the basis of their fermentation patterns. Because the PAGE patterns

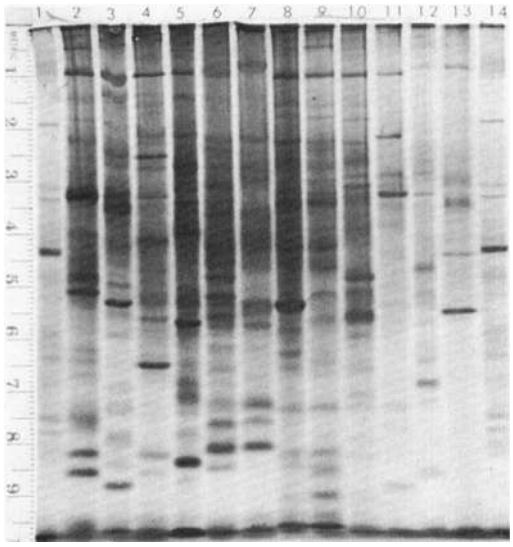

FIG. 12. Electrophoretic patterns of the type strains of 12 species of *Bifidobacterium*. Lane 2 = *B. adolescentis* ATCC 15703, 3 = *B. angulatum* ATCC 27535, 4 = *B. bifidum* ATCC 29521, 5 = *B. breve* ATCC 15700, 6 = *B. infantis* ATCC 15697, 7 = *B. longum* ATCC 15707, 8 = *B. catenulatum* ATCC 27539, 9 = *B. pseudocatenulatum* ATCC 27919, 10 = *B. dentium* ATCC 27534, 11 = *B. subtile* ATCC 27537, 12 = *B. minimum* ATCC 27538, 13 = *B. pullorum* ATCC 27685.

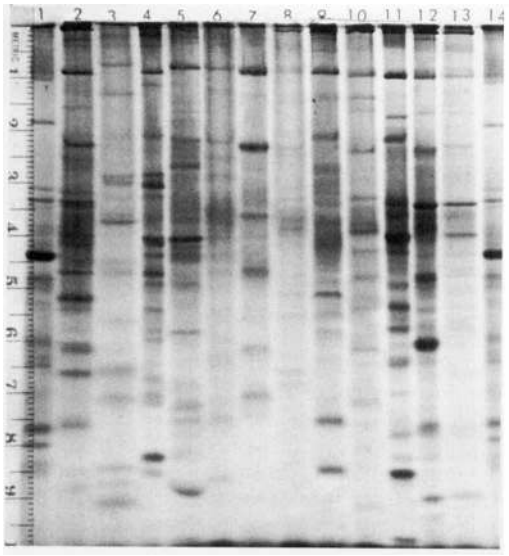

FIG. 13. Type strains of additional species, from animals. Lane 2 = *B. animalis* ATCC 25527, 3 = *B. boum* ATCC 27917, 4 = *B. thermophilum* ATCC 25525, 5 = *B. asteroides* ATCC 25910, 6 = *B. coryneforme* ATCC 25911, 7 = *B. indicum* ATCC 25912, 8 = *B. choerinum* ATCC 27686, 9 = *B. suis* ATCC 27533, 10 = *B. cuniculi* ATCC 27916, 11 = *B. magnum* ATCC 27540, 12 = *B. pseudolongum* ATCC 25526, 13 = *B. globosum* ATCC 25865.

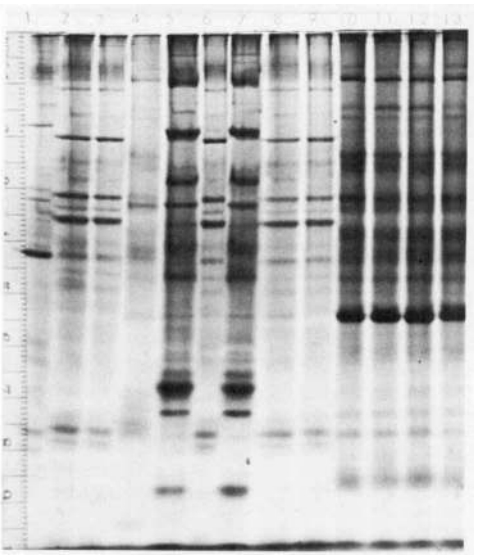

FIG. 14. Results from mixed cultures. Lane 2 = pattern produced by a culture labeled *B. pseudocatenulatum* (the original mixed culture was repeated on this gel for illustrative purposes), 3 = pure culture of diplococci isolated from original mixed culture, 4 = pure culture of *B. pseudocatenulatum* derived from the original mixed culture.

Lane 5 = culture labeled *B. pseudocatenulatum*, 6 = single colony isolate of diplococci derived from the original culture, 7 = single colony isolate of streptococci derived from the original culture. No bifidobacteria were recovered from this culture.

Lane 8 = original culture labeled *B. pseudocatenulatum* (by DNA homology), 9 = pure culture of diplococci derived from the original culture, 10 through 13 = single colony isolates of bifidobacteria derived from the original culture. The electrophoretic patterns of these isolates (VPI 13278) are most similar to those of *B. catenulatum*.

of type strains and of strains that have high DNA-DNA homology with the type strains were nearly identical, distinct from those of other species, and repeatable, it became obvious that PAGE was more reliable for differentiating *Bifidobacterium* species than fermentation reactions were. Therefore, the intestinal strains were reidentified according to their protein electrophoretic patterns. The original identifications, which were based on fermentation reactions, and the identifications based on the PAGE patterns are shown in Table 4.

The fermentation reactions of the type strains of the species studied and of the strains identified by PAGE are listed in Table 5. These results were obtained over several years. Improvements in formulations of media during that time might account for some of the phenotypic variations, but variation was observed among different strains in several species even when they were reexamined with our currently used media.

Table 5 shows that several *Bifidobacterium* species cannot be differentiated reliably on the basis of fermentation reactions. Even the most discriminating tests fail to separate major species of human origin, as shown in Table 6. The reliability of this key sometimes can be improved if the source of an unidentified isolate is known (Table 1); nevertheless, only DNA-DNA homology data or PAGE protein patterns appear to provide highly definitive results.

New species. The protein patterns, fermentation reactions, and previously published DNA-DNA homology data support reinstatement of "*B. coryneforme*" Scardovi and Trovatelli and "*B. globosum*" Scardovi, Trovatelli, Crociani, and Sgorbati and elevation of the "*Bifidobacterium minimum*" and "*Bifidobacterium subtile*" homology groups of Scardovi and Trovatelli to the species level. Accordingly, we propose the four new species of *Bifidobacterium* listed below.

Like other members of the genus *Bifidobac-*

TABLE 4. Identification of bifidobacteria by fermentation reactions and by protein electrophoretic patterns

Identification by fermentation	Identification by electrophoresis[a]
From humans	
310[b] *B. adolescentis*	156 *B. adolescentis* 114 *B. catenulatum* 35 *B. longum* 5 *B. dentium*
202 *B. longum*	201 *B. longum* 1 *B. catenulatum*
90 *B. infantis*	52 *B. adolescentis* 35 *B. catenulatum* 3 *B. breve*
70 *B. breve*	43 *B. adolescentis* 19 *B. catenulatum* 7 *B. breve* 1 *B. longum*
21 *B. bifidum*	21 *B. bifidum*
2 *B. pseudolongum*	2 *B. longum*
From pigs	
12 *Bifidobacterium* species P1	12 *B. thermophilum*

[a]Of the 732 strains examined, there were 251 *B. adolescentis*, 239 *B. longum*, 169 *B. catenulatum*, 21 *B. bifidum*, 10 *B. breve*, and 5 *B. dentium*, from adult humans and 12 *B. thermophilum* from pigs. There were 25 additional isolates from humans that, according to both fermentation reactions and electrophoretic patterns, belonged to three undescribed species.

[b]Number of strains.

terium, these four species are gram-positive, nonsporeforming, nonmotile, non-proteolytic, fermentative, and anaerobic to aerotolerant rods that produce acetic, formic, and lactic acids. They do not produce catalase or gas. The optimum temperature for growth is between 30 and 45°C.

The known sources of these species are listed in Table 1. The DNA homology relationships of these species to other species in the genus are given in Table 2. The biochemical reactions of the type strain and other strains of each of these species are listed in Table 5.

***B. coryneforme* (ex Scardovi and Trovatelli 1969) sp. nov., nom. rev.** (cor.v.ne.for'me. Gr. n. *coryne* a club; L. n. *forma* shape, form; M.L. adj. *coryneformis* club shaped). Cells of the type strain in prereduced anaerobically sterilized peptone-yeast extract-glucose broth (5) are 0.7- to 1.2-μm by 0.9- to 3.0-μm, coccoid, oval, or club-shaped rods that occur singly, in pairs, and in clumps.

Colonies on blood agar plates incubated anaerobically for 48 h at 37°C are punctiform to 1 mm in diameter, circular, entire, convex, translucent to semiopaque, grayish white, shiny, and smooth.

Growth in broth with a fermentable carbohydrate produces dense turbidity with a smooth or "cottony" sediment and a final pH of 4.3 to 5.3. Slight turbidity and a small amount of sediment are produced in peptone-yeast extract broth that does not contain a fermentable carbohydrate. Other characteristics of the type strain and other strains of this species are given in Tables 1 through 3 and 5, in Fig. 10 and 13, and by Scardovi and Trovatelli (25).

The type strain, ATCC 25911, was isolated from the intestine of a bee.

***B. globosum* (ex Scardovi, Trovatelli, Crociani, and Sgorbati 1969) sp. nov., nom. rev.** (glo.bo' sum. L. adj. *globosus* globelike, referring to the oval and round cells that are produced in some cultures). Cells of the type strain in peptone-yeast extract-glucose broth cultures are oval rods that are 1.4 to 1.5 by 1.5 to 2.8 μm and occur in pairs, short chains, and clumps. Cells from colonies on the surface of blood agar incubated anaerobically, in air with 10% CO_2, or in candle jars are longer and often club shaped.

Colonies on the surface of blood agar are punctiform, circular, entire, convex, gray or white, shiny, and smooth.

Heavy turbidity and a "breadcrumb" sediment are produced in peptone-yeast extract broth containing a fermentable carbohydrate. The final pH is 4.1 to 4.6. Slight turbidity is produced in peptone-yeast extract broth containing carbohydrates that are not fermented.

Other characteristics of the type strain and other strains of this species are given in Tables 1, 2, and 5, in Fig. 6 and 13, and by Scardovi et al. (28).

The type strain, ATCC 25865, was isolated from bovine rumen contents.

***B. minimum* sp. nov.** (min'i.mum. L. adj. *minimus* least, referring to the small size of the cells). This taxon previously was referred to as the "*minimum*" homology group of Scardovi and Trovatelli (26).

Cells of the type strain in peptone-yeast extract or peptone-yeast extract-glucose broth cultures are 0.5- to 0.6-μm by 0.5- to 2.8-μm, oval, club-shaped, or short rods that occur in short chains and in clumps.

TABLE 5. Fermentation patterns of 999 *Bifidobacterium* strains examined[a]

Substrate	*B. adolescentis*				*B. angulatum*				*B. animalis*				*B. asteroides*	*B. bifidum*				*B. boum*	*B. breve*			
	ATCC 15703 (type)	Other strains			ATCC 27535 (type)	Other strains			ATCC 25527 (type)	Other strains			ATCC 25910 (type)	ATCC 25921 (type)	Other strains			ATCC 27917 (type)	ATCC 15700 (type)	Other strains		
		A+	W	−[b]		A+	W	−		A+	W	−			A+	W	−			A+	W	−
Amygdalin	A	274	8	22	A	1	1	3	−	4	0	3	A	−	0	1	37	−	A	16	6	6
Arabinose	A	147	22	135	A	5	0	0	A	2	1	4	A	−	0	0	38		−	1	1	26
Cellobiose	A	287	3	14	A	5	0	0	−	5	0	2	A	−	1	0	37	−	A	24	4	0
Esculin pH	A	43	44	53[c]	A	4	1	0	−	0	0	7	A[d]	−	0	0	38	−	W	9	11	8
Hydrolysis	+	302	2	0	+	5	0	0	+	3	1	3	+	−	3	2	33	W	+	28	0	0
Glycogen	−	277	1	26	A	4	0	1	−	0	0	7	−	−	0	0	38	A	A	23	1	4
Lactose	A	303	1	0	A	5	0	0	A	7	0	0	A−	A	37	1	0	−	A	28	0	0
Maltose	A	304	0	0	A	5	0	0	A	7	0	0	A−	−	4	4	30	A	A	28	0	0
Mannitol	A	143	2	159	−	3	0	2	−	0	0	7	−	−	0	0	38	−	A	23	0	5
Mannose	W	141	13	150	W	0	0	5	−	6	0	1	A	−	1	1	36	−	A	18	9	1
Melezitose	−	25	2	277	−	0	0	5	−	0	0	7	A	−	0	0	38	−	−	7	1	20
Melibiose	A	298	1	5	A	5	0	0	A	7	0	0	A	A	4	3	31	A	A	27	1	0
Raffinose	A	301	1	2	A	5	0	0	A	7	0	0	A	A	3	0	35	A	A	28	0	0
Rhamnose	−	22	4	278	−	0	0	5	−	0	0	7	−	−	0	0	38	−	−	0	1	27
Ribose	A	139	7	158	W	2	0	3	W	5	0	2	W	−	0	0	38	−	W	15	3	10
Salicin	A	291	4	9	A	5	0	0	−	5	0	2	A	−	0	0	38	−	A	20	1	7
Sorbitol	A	186	3	115	W	4	0	1	−	0	0	7	−	−	0	0	38	−	A	22	0	6
Starch pH	A	281	1	22	A	5	0	0	A	3	1	3	−	−	1	1	36	A	A	25	2	1
Hydrolysis	+	156	5	8[c]	+	5	0	0	−	0	0	7	−	−	0	0	37[c]	+	+	24	0	4
Sucrose	A	285	1	18	A	5	0	0	A	7	0	0	A	−	4	2	32	A	A	28	0	0
Trehalose	−	157	5	142	−	1	0	4	−	0	0	7	−	−	0	0	38	−	−	3	2	23
Xylose	A	199	25	80	A	5	0	0	A	6	0	1	A	−	0	0	38	−	−	1	1	26

TABLE 5. Continued

Substrate	*B. catenulatum*				*B. choerinum*	*"B. coryneforme"*				*B. cuniculi*	*B. dentium*				*"B. globosum"*				*B. indicum*
	ATCC 27539 (type)	Other strains			ATCC 27686 (type)	ATCC 25911 (type)	Other strains			ATCC 27916 (type)	ATCC 27534 (type)	Other strains			ATCC 25865 (type)	Other strains			ATCC 25912 (type)
		A+	W	−[b]			A+	W	−			A+	W	−		A+	W	−	
Amygdalin	W	168	16	12	−	A	2	0	1	A	A	50	8	3	−	0	0	9	A
Arabinose	A	123	11	62	−	A	3	0	0	A	A	60	1	0	A	9	0	0	A
Cellobiose	A	174	6	16	−	A	3	0	0	A	A	60	1	0	−	3	1	5	A
Esculin pH	A	31	54	70[c]	−	W	1	0	2	−	A	28	12	14[c]	−	0	0	9	−
Hydrolysis	+	188	6	2	W	+	2	1	0	+	+	52	3	6	W	5	1	3	+
Glycogen	A	121	1	74	A	−	0	0	3	A	A	59	0	2	A	9	0	0	−
Lactose	A	196	0	0	A	A	3	0	0	−	A	61	0	0	A	9	0	0	A
Maltose	A	196	0	0	A	A	3	0	0	A	A	61	0	0	A	9	0	0	A
Mannitol	−	39	0	157	−	−	0	0	3	−	A	54	1	6	−	0	0	9	−
Mannose	−	106	19	71	−	−	0	0	3	A	A	61	0	0	−	3	1	5	A
Melezitose	−	9	2	185	−	−	1	0	2	−	A	45	8	8	−	0	0	9	−
Melibiose	A	191	3	2	A	A	3	0	0	−	A	61	0	0	A	9	0	0	A
Raffinose	A	194	1	1	A	A	2	0	1	−	A	61	0	0	A	9	0	0	A
Rhamnose	−	1	0	195	−	−	0	0	3	−	W	1	12	48	−	0	0	9	−
Ribose	−	99	16	81	−	−	0	1	2	−	A	53	4	4	W	5	0	4	A
Salicin	A	181	2	13	−	A	2	0	1	A	A	61	0	0	−	3	2	4	A
Sorbitol	A	180	0	16	−	−	1	0	2	−	−	1	6	54	−	0	0	9	−
Starch pH	−	157	23	16	A	−	0	0	3	A	A	61	0	0	A	9	0	0	−
Hydrolysis	−	122	5	47[c]	+	−	0	0	3	+	+	61	0	0	+	9	0	0	−
Sucrose	A	191	0	5	A	A	3	0	0	A	A	61	0	0	A	9	0	0	A
Trehalose	−	134	3	59	−	−	0	0	3	−	A	56	4	1	−	0	0	9	−
Xylose	A	135	9	52	−	A	3	0	0	A	A	61	0	0	A	9	0	0	A

Surface colonies in prereduced anaerobically sterilized agar streak tubes are 1 mm in diameter; circular; entire; convex; translucent; gray, white, or buff; shiny; and smooth.

Cultures in broth with a fermentable carbohydrate produce dense turbidity with a flocculent sediment and a final pH of 4.1 to 4.4. Little or no growth is produced without a fermentable carbohydrate.

Other characteristics of the type strain and other strains of this species are given in Tables 1, 2, and 5, in Fig. 11 and 12, and by Scardovi and Trovatelli (26).

The type strain, ATCC 27538, was isolated from wastewater.

***B. subtile* sp. nov.** (sub'ti.le. L. adj. *subtilis* slender, referring to the slender cells). This taxon previously was referred to as the "*sub-*

TABLE 5. Continued

Substrate	*B. infantis*		*"B. infantis-longum"*	*B. longum*		*B. magnum*		*"B. minimum"*		*B. pseudo-catenulatum*
	ATCC 15697 (type)	Other strains A+ W −	Strains A+ W −	ATCC 15707 (type)	Other strains A+ W −	ATCC 27540 (type)	Other strains A+ W −	ATCC 27538 (type)	Other strain	ATCC 27919 (type)
Amygdalin	−	4 3 12	1 0 4	−	85 36 151	−	0 0 2	−	−	A
Arabinose	−	0 1 18	5 0 0	A	260 5 7	A	2 0 0	−	−	A
Cellobiose	W	3 1 15	0 1 4	−	44 13 215	−	0 0 2	−	−	A
Esculin pH	−	0 1 18	3 1 1	−	29 21 121[c]	−	0 0 2	−	−	A
Hydrolysis	+	12 5 2	2 2 1	+	105 15 152	−	0 0 2	−	−	+
Glycogen	A	0 0 19	0 0 5	−	5 3 264	−	0 0 2	A	A	A
Lactose	A	19 0 0	5 0 0	A	272 0 0	A	2 0 0	−	−	A
Maltose	−	19 0 0	5 0 0	A	271 1 0	A	2 0 0	A	A	A
Mannitol	−	2 0 17	2 0 3	−	11 1 260	−	0 0 2	−	−	−
Mannose	W	16 1 2	5 0 0	W	149 25 98	−	0 0 2	−	−	A
Melezitose	−	0 0 19	0 0 5	A	220 52 0	−	0 0 2	−	−	−
Melibiose	A	19 0 0	5 0 0	A	270 1 1	A	1 1 0	−	−	A
Raffinose	A	19 0 0	5 0 0	A	266 3 3	A	2 0 0	−	−	A
Rhamnose	−	0 0 19	0 0 5	−	3 0 269	−	0 0 2	−	−	−
Ribose	A	7 3 9	3 0 2	A	159 8 105	A	2 0 0	−	−	A
Salicin	A	5 1 13	2 1 2	−	64 8 200	−	0 0 2	−	−	A
Sorbitol	−	2 0 17	0 0 5	−	10 0 262	−	0 0 2	−	−	A
Starch pH	A	3 0 16	1 4 0	A	115 67 90	A	1 1 0	A	A	A
Hydrolysis	+	2 0 17	0 1 4	−	33 8 159[c]	−	0 0 2	+	+	+
Sucrose	A	19 0 0	5 0 0	A	267 5 0	A	2 0 0	−	A	A
Trehalose	−	2 1 16	2 0 3	−	33 8 231	−	0 0 2	−	−	A
Xylose	−	10 0 9	5 0 0	A	267 5 0	A	2 0 0	−	−	A

TABLE 5. Continued

Substrate	*B. pseudo-longum*		*B. pullorum*	*"B. subtile"*		*B. suis*		*B. thermophilum*	
	ATCC 25526 (type)	Other strains A+ W −	ATCC 27685 (type)	ATCC 27537 (type)	Other strains A+ W −	ATCC 27533 (type)	Other strains A+ W −	ATCC 25525 (type)	Other strains A+ W −
Amygdalin	−	0 0 3	−	−	0 0 4	−	0 0 3	−	0 0 15
Arabinose	A	3 0 0	−	−	0 0 4	A	3 0 0	−	0 0 15
Cellobiose	−	2 0 1	A	A	2 0 2	−	0 0 3	−	1 0 14
Esculin pH	−	1 0 2	A	W	0 0 4	W	1 1 1	−	1 0 14
Hydrolysis	+	2 0 1	+	+	2 2 0	+	3 0 0	+	9 0 6
Glycogen	A	2 0 1	−	A	3 0 1	−	0 0 3	A	14 0 1
Lactose	A	3 0 0	−	W−	0 0 4	A	3 0 0	−	1 0 14
Maltose	A	3 0 0	A	A	3 0 1	A	3 0 0	A	15 0 0
Mannitol	−	0 0 3	−	−	0 0 4	−	0 0 3	−	0 0 15
Mannose	−	1 0 2	A	−	0 0 4	A	3 0 0	−	0 0 15
Melezitose	−	0 0 3	A	A	3 0 1	−	0 0 3	−	0 0 15
Melibiose	A	3 0 0	A	A	4 0 0	A	3 0 0	A	15 0 0
Raffinose	A	3 0 0	A	A	4 0 0	A	3 0 0	A	15 0 0
Rhamnose	−	0 0 3	−	−	0 0 4	−	0 0 3	−	0 0 15
Ribose	A	3 0 0	A	A−	1 1 2	−	1 0 2	−	0 0 15
Salicin	A	3 0 0	A	A−	1 1 2	−	0 0 3	A	2 0 13
Sorbitol	−	0 1 2	−	−	4 0 0	−	0 0 3	−	0 0 15
Starch pH	A	3 0 0	−	A	3 0 1	−	0 1 2	A	15 0 0
Hydrolysis	+	2 0 1	−	+	3 0 1	−	0 0 3	+	8 0 7
Sucrose	A	3 0 0	A	A	4 0 0	A	3 0 0	A	15 0 0
Trehalose	−	0 0 3	A	−	1 0 3	−	0 0 3	−	0 0 15
Xylose	A	3 0 0	A	−	0 0 4	A	3 0 0	−	0 0 15

[a] All strains fermented glucose and fructose. None fermented erythritol, produced indole, or digested meat. Nine of nineteen strains of *B. infantis* fermented inositol; no other species fermented inositol. For those characteristics in which variation is shown, the acid production is observed most frequently when Tween-80 is added to the medium (5).

[b] A = pH below 5.5, + = positive, W = pH 5.5–5.9 or weak (hydrolysis), − = negative. [c] Not all strains were tested. [d] Results vary from run to run.

tile" homology group of Scardovi and Trovatelli (26).

Cells of the type strain in peptone-yeast extract-glucose broth cultures are 0.8- by 2.1-μm to 4.5-μm, straight or bent rods that occur singly or in pairs. Longer, branching, bifid, knobbed, and club-shaped forms in tangled masses are produced in semisolid thioglycolate medium. Smaller coccoid and rod-shaped cells that occur in short chains are produced in broth media that do not contain fermentable carbohydrates.

Colonies on the surface of blood agar incubat-

TABLE 6. Key for differentiation of major *Bifidobacterium* species of human origin

Substrate				Suggested species
Melezitose	Glycogen	Xylose	Rhamnose	
+[a]	+	+	A	*(B. adolescentis, B. dentium)*[b]
			–	*B. dentium (B. adolescentis, B. catenulatum)*[c]
		–	A	*(B. adolescentis)*
			–	*(B. adolescentis, B. breve, B. catenulatum)*
	–	+	A	*(B. adolescentis)*
			–	*B. longum (B. adolescentis, B. catenulatum)*
		–	A	*(B. adolescentis)*
			–	*(B. adolescentis, B. breve, B. catenulatum)*
–	+	+	A	*(B. adolescentis, B. dentium)*
			–	*B. adolescentis, B. catenulatum, B. globosum (B. dentium)*
		–	A	*(B. adolescentis)*
			–	*B. breve (B. adolescentis, B. catenulatum)*
	–	+	A	*(B. adolescentis)*
			–	*B. infantis (B. adolescentis, B. catenulatum)*
		–	A	*(B. adolescentis)*
			–	*B. bifidum*,[d] *B. infantis (B. adolescentis, B. breve, B. catenulatum)*

[a] + = Acid or weak acid, A = acid, – = not fermented.

[b] Species in parentheses are less likely possibilities.

[c] Because we were unable to differentiate *B. catenulatum* and *B. pseudocatenulatum* by protein patterns, the reactions listed here and in Table 5 may represent both species. Scardovi et al. (27) indicate that starch- and dextrin-positive strains are *B. pseudocatenulatum*, while starch- and dextrin-negative reactions correlate with strains that are *B. catenulatum* by DNA homology.

[d] Amygdalin, arabinose, and cellobiose all negative = *B. bifidum*.

ed anaerobically are punctiform to 1 mm in diameter, circular, entire, slightly raised, translucent, colorless, shiny, and smooth. Similar (but smaller) colonies are produced on blood agar incubated in a candle jar.

Dense turbidity with a granular or flocculent sediment and a final pH of 4.1 to 4.8 are produced in peptone-yeast extract broth containing fermentable carbohydrates. Slight to moderate turbidity is produced in peptone-yeast extract broth containing carbohydrates that are not fermented.

Other characteristics of the type strain and other strains of this species are given in Tables 1, 2, and 5, in Fig. 11 and 12, and by Scardovi and Trovatelli (26).

The type strain, ATCC 27537, was isolated from wastewater.

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REPRINT REQUESTS

Address reprint requests to: Dr. W. E. C. Moore, Department of Anaerobic Microbiology, Virginia Polytechnic Institute and State University, Blacksburg, VA 24061.

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