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Bifidobacterium suis n. sp.: a new species of the genus *Bifidobacterium* isolated from pig feces[1])

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A lot of strains of bifidobacteria isolated from pig feces were tested for morphological, physiological, biochemical, and enzymological characters. Nutrition, DNA base composition and DNA homology were also examined. The phenotypic characters of the bacterial group isolated from pig feces and its genetic homology warranted the proposal of the creation of the new species *Bifidobacterium suis*.

The bifidobacteria are a group of gram-positive, anaerobic bacteria clearly differentiated on biochemical grounds from other forms with similar morphology as anaerobic corynebacteria or other branching bacteria: they ferment glucose via a specific pathway wherein the key reaction is a phosphoketolase cleavage of fructose-6-phosphate into acetylphosphate and erytrose-4-phosphate (SCARDOVI and TROVATELLI 1965, DE VRIES and GERBRANDY 1967). The bifidobacteria moreover differ from the species of the genera *Corynebacterium* and *Propionibacterium* for their DNA base composition (GC %) (SEBALD *et al.* 1965).

Many investigators focused their attention on the bifidobacteria of human origin and REUTER (1963) proposed to distinguish eight species in the genus *Bifidobacterium*. The bifidobacteria are, however, present in other habitats as the alimentary tract of the honey-bee (SCARDOVI and TROVATELLI 1969), sheep and calf rumen (WASSERMAN *et al.* 1953, BAUMANN and FOSTER 1956, GIBBONS and DOETSCH 1959, CLARKE 1959, PHILLIPSON *et al.* 1962, KROGH 1963, SCARDOVI *et al.* 1969) and feces of several vertebrates like guinea-pig, rabbit, rat and hen (HAENEL and MÜLLER-BEUTHOW 1956, OCHI *et al.* 1964). UCHIDA *et al.* (1965) found that bifidobacteria and lactic acid bacteria predominate in the fecal microflora of the feces of pigs within few weeks after birth. MITSUOKA (1969) isolated about 300 strains of bifidobacteria from the feces of several animals like swine, cow, sheep, calf, mice, rat, guinea-pig, and chicken and concluded that the "animal" strains of bifidobacteria can be distinguished from those from man on account of their fermentative behaviour. MITSUOKA (1969) proposed the creation of two new species, *Bifidobacterium thermophilum* and *Bifidobacterium pseudolongum* and of a variety *animalis* of the species *Bifidobacterium longum*.

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The present paper concerns the study of the characters of a group of strains of bifidobacteria isolated from pig feces.

Material and methods

Isolation: The feces of 52 pigs were examined. These 52 samples were taken from 19 different farms. Most pigs were 2—6 weeks old except some 1—2 years old. Immediately, after the feces were voided a sample of them was taken to the laboratory, mixed with little saline and plated with TPG medium. The TPG medium contained per 100 ml: trypticase (BBL) 1.0 g, phytone (BBL) 0.5 g, glucose 1.5 g, yeast extract (DIFCO) 0.25 g, cysteine HCl 0.05 g, dipotassium phosphate 0.15 g, magnesium chloride 0.05 g, zinc sulphate 0.025 g, traces of ferric chloride. Zinc sulphate and ferric chloride were dissolved separately. The plates were incubated anaerobically at 37°C under carbon dioxide. After 3—4days colonies formed by cells of irregular shape were picked off and transferred in stab cultures of TPG medium with 0.5% agar. Subcultures were made every ten days in the same medium and, after development, kept at 3—5 °C under carbon dioxide. All isolates were liophilyzed and maintained in the collection of the Institute.

Bacterial strains: A total of 89 strains were isolated, checked for morphology and purity and studied. The characters of our strains were compared with those of same representatives of MITSUOKA's species: *B. thermophilium* type a) strain P2—91, type b) strain 14—44, typec) strain P 16—6, type d) strain Nissin, *B. pseudolongum* type a) strain PNC-2-9G, type c) strain 29 SrT, type d) strain Mo2—10, *B. longum var. animalis* type a) strain R101—8, type b) strain C10—45.

Physiology: Oxygen relationships and need for CO_2 were recorded by observing the growth in stabs and slants incubated as follows: in full air, in nitrogen, in 10% CO_2-nitrogen and in 10% CO_2-air. Fermentation tests were performed in TPG medium without glucose and with bromocresol purple as indicator and after 7 days of incubation under low CO_2 pressure. Catalase was tested by flooding agar slopes incubated both under nitrogen and under 10% CO_2-air, with 10 vls hydrogen peroxide and examining for gas evolution. The temperature relationships were determined with the temperature gradient incubator of OPPENHEIMER and DROST-HANSEN (1960). The reduction of nitrate to nitrite was tested with sulfanilic-naphtylamine reagent in liquid cultures. The behaviour in litmus milk was determined by inoculating litmus milk (DIFCO) and incubating for 1 week in anaerobiosis. The formation of indole and acetyl-methyl carbinol was determined according to routine standard procedures (Manual of Microbiological Methods 1957). In order to ascertain the kind of acids produced in glucose fermentation and the ratio between lactic and acetic acids, experiments were made with the procedure of SCARDOVI and TROVATELLI (1969).

Enzyme assay: Cells grown in trypticase-phytone broth were centrifuged, washed with phosphate buffer at pH 6.5 and disrupted mechanically with a NOSSAL disintegrator and glass beads. Cell-free extracts, clarified by high-speed centrifugation usually contained 5—12 mg of protein per ml, as determined by the method of LOWRY *et al.* (1951). Spectrophotometric assays were performed with a BECKMAN DB-G. The determination of fructose-6-phosphate phosphoketolase, enzyme forming acetyl-phosphate from fructose-6-phosphate, was performed according to SCHRAMM *etal.* (1958). Aldolase was determined according to SIBLEY and LEHNINGER (1949) as modified by DOUNCE *et al.* (1950). Glucose-6-phosphate and 6-phosphogluconate dehydrogenases were assayed by following the variation in absorbance at 340 μm due to the reduction of either nicotinamide adenine dinucleotide (NAD) or nicotinamide adenine dinucleotide phosphate (NADP). The assay mixture contained: 2 ml of Tris buffer pH 7.4, 30 μmoles of substrate, 0.3 μmoles of NAD^+ or $NADP^+$ and water to 3 ml of final volume.

Nutrition: The "complete" basal medium of GYLLENBERG and CARLBERG (1958), slightly modified, was used, The composition of this medium was as follows (per 100 ml of medium): glucose 1.0 g, sodium acetate 1.0 g, ammonium sulphate 0.4 g, bipotassium phosphate 0.5 g, vitamin free casamino acids (DIFCO) 0.5 g, ascorbic acid 0.1 g, cysteine HCl 0.05 g, salt solution 0,5 ml ($MgSO_4$ 10 g, $FeSO_4$ 0.5 g, $MnSO_4$ 0.4 g, NaCl 0.5 g l n 250 ml of distilled water), alanine and tryptophan 0.02 g each, adenine, guanine, xantine and uracil 0.5 mg each, pantothenic acid and riboflavine 0.1 mg each, pyridoxine, nicotinic acid and thiamine 0.2 mg each, *p*-aminobenzoic acid and biotine 5 mg each, folic acid 1 mg and Tween 80 0.025 ml. The pH of the medium is adjusted to 7.5. After sterilization the tubes were cooled and immediately inoculated with one drop of actively growing

cultures. The tubes were incubated anaerobically at 37 °C in glass container under low CO_2 pressure. Several serial transfer (5—7) were made and visual observations of growth in each serial transfer were made.

DNA base composition: For DNA extraction the cells were grown in the usual TPG medium and DNA was extracted according to MARMUR (1961). The GC% was determined with the thermal denaturation method of MARMUR and DOTY (1962) and calculated as reported by SILVESTRI and HILL (1965).

DNA × DNA hybridization: The single-point competition procedure was adopted all throughout in DNA × DNA hybridization experiments, under the conditions suggested by JOHNSON and ORDAL (1968) that were already used in previous work with bifidobacteria (SCARDOVI *et al.* 1970)

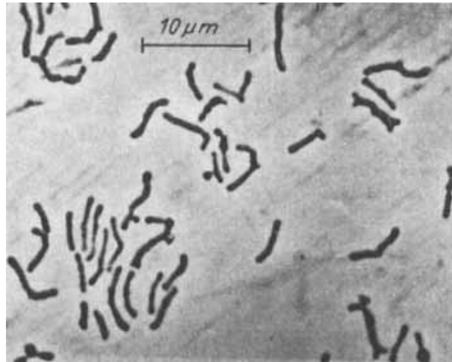

Fig. 1. *Bifidobacterium suis*, type strain Su 859 in stab of TPG medium. Observation in phase contrast

Results

All the strains isolated from pig feces were scored for their morphology and fermentation patterns: 40 strains were recognized to form an apparently homogenous group: therefore this group was submitted to a closer investigation.

Morphological and cultural characters

In solid medium slender rods 2—6 µ long with rounded ends, single, with rare branchings or clubs (see Fig. 1). This morphology does not change considerably with the conditions of growth *i. e.* in liquid medium, in slopes incubated in air-CO_2 or in BREWER thioglycollate medium (DIFCO). Non motile. Gram-positive. Liquid cultures are of uniform turbidity and the sediment formed later is easily dispersable at gentle agitation.

Colonies on plates or in slants incubated in air-CO_2 are soft, smooth, with entire margins. Abundant growth is obtained in stab of TPG medium incubated anaerobically with or without CO_2: most strains grow weakly also in air-CO_2 slopes. No growth occurs in aerobic slants.

Physiological characters

All the 40 strains examined gave identical results in fermentation tests (see Table 2). They ferment: arabinose, xylose, glucose, fructose, galactose, mannose, maltose, sucrose, melibiose, lactose, raffinose; the final pH is about 3.5. Rham-

nose, melezitose, threalose, cellobiose, dextrin, starch, inulin, mannitol, sorbitol, glycerol, salicin, lactate and gluconate were never fermented. The fermentation tests were repeated about 12 months after the isolation with the same results.

The other physiological tests *i.e.* nitrate reduction, indole, and acetyl-methylcarbinol production, were negative. Litmus milk is coagulated. Catalase tests were negative also in slopes developed under CO_2-air in hemin-enriched medium.

In glucose fermentation all strains produce acetic and lactic acids in the ratio 1:1.7–1:2.

The optimum pH growth lies between pH 7.0 and 8.0; growth occurs between pH 5.3 and 9.4.

The cultures behave similarly towards temperature: the optimum temperature for growth lies between 38° and 39 °C; no growth occurs at 44–44.5 °C or at 20 °C after 2–3 weeks of incubation. No strain survives heating at 60 °C for 30 min.

Nutritional characters

Ten strains were studied for vitamins requirements: some of these strains stopped growing after two or three transfers in the "complete" basal medium; the medium employed was not suitable evidently for all the isolates. Only three

Table 1
Relative similarity values[1]) of DNA from competitor strains to reference organisms DNA

Competitor strains	Reference strain Su 859
B. thermophilum a (MITSUOKA P2–9 I)	20
B. thermophilum b (MITSUOKA 14–44)	26
B. thermophilum c (MITSUOKA P1 6–6)	12
B. *thermophilum* d (MITSUOKA – NISSIN)	23
B. pseudolongum a MITSUOKA PNC-2-9G)	25
B. pseudolongum c (MITSUOKA 29 SrT)	9
B. pseudolongum d (MITSUOKA Mo 2–I0	26
B. longum var. animalis a (MITSUOKA R 101–8)	27
B. longum var. animalis b (MITSUOKA C 10–45)	II
Strain Su 859	100
Strain Su 864	100
Strain Su 868	100
Strain Su 915	100

[1]) Similarities are expressed in percent of binding depression in respect to the depression in the homologous system (= 100)

Table 2
Differential characteristics of the species of bifidobacteria isolated from animals

Species and variants of *Bifidobacterium*		Source	Growth at 46.5 °C	Coag. of skim milk	Fermentation patterns											
					Arabinose	Xylose	Mannose	Fructose	Cellobiose	Lactose	Trehalose	Melizitose	Dextrin	Starch	Inulin	Salicin
B. globosum		rumen	−	+	□	−	−	+	−	+	□	−	+	+	n.t.	n.t.
B. ruminale		rumen	−	−	□	−	−	+	−	−	□	−	+	+	n.t.	n.t.
B. thermophilum	a	feces	+	−	−	−	−	+	>	−	>	−	+	+	△	(+)
B. thermophilum	b	feces	+	−	−	−	−	+	+	−	−	+	+	+	−	+
B. thermophilum	c	feces	+	+	−	−	−	+	>	+	+	−	+	+	+	+
B. thermophilum	d	feces	+	+	−	−	−	+	+	+	+	+	+	+	+	+
B. pseudolongum	a	feces and rumen	−	+	+	+	−	+	±	+	−	−	+	+	−	±
B. pseudolongum	b	feces	−	+	+	+	+	+	+	+	−	−	+	+	−	+
B. pseudolongum	c	feces	−	+	+	+	+	+	+	+	−	+	+	+	−	±
B. pseudolongum	d	feces	−	−	+	+	+	+	+	−	−	−	+	+	−	+
B. longum var. animalis	a	feces	−	>	+	+	−	+	−	+	−	−	±	±	−	±
B. longum var. animalis	b	feces	−	+	+	+	+	+	−	+	−	−	±	±	−	±
B. suis		feces	−	+	+	+	△	△	−	+	−	−	□	−	−	−

All strains ferment glucose, galactose, sucrose, maltose, melibiose, and raffinose. None ferments rhamnose, sorbitol, mannitol, and glycerol. + = fermented; − = not fermented; △ = sometimes not fermented; □ = sometimes fermented; > = variable; (+) = fermented slowly; ± = most strains negative, some slowly fermenting. The indicative signs and meanings are taken from Mitsuoka (1969).

strains (SU 901, SU 932, SU 934) grew abundantly in this medium (see under Methods). These strains require riboflavine: in absence of this growth factor the growth ceased after two or three transfers.

Enzymology

The cell-free extracts were tested for the presence of aldolase, fructose-6-phosphate, phosphoketolase, glucose-6-phosphate and 6-phosphogluconate dehydrogenases. Homo and heterofermentative strains of lactic acid bacteria were tested as negative reference. The fructose-6-phosphate phosphoketolase tests were positive in accordance with the results obtained by SCARDOVI and TROVATELLI (1965) and DE VRIES and GERBRANDY (1967). Aldolase is present like in the other species isolated from animals (SCARDOVI *et al.*, unpublished). Our strains possess moreover the HMP dehydrogenases.

DNA base composition and DNA × DNA hybridization

The GC % of three selected strains, SU 850, SU 859, SU 868, is respectively, 62.1, 62.3 and 62.1.

The data concerning the DNA homologies between a typical strain SU 859 and the representatives types and varieties of the species suggested by MITSUOKA are reported in Table 1; as competitor strains we tested three additional strains of our bacterial group. The relative levels of the competitive action exerted by the various competitor DNA upon the homologous annealing reaction are self evident.

Discussion

The taxonomy of the bifid bacteria is still largerly provisional, although, as a group, they can be well characterized on biochemical grounds because of their unique pattern of carbohydrate degradation process *i. e.* the fructose-6-phosphate shunt (cfr. SCARDOVI and TROVATELLI 1965, DE VRIES and GERBANDY 1967). The separation of many nomen-species in the genus *Bifidobactreium* was suggested mainly on the basis of differences in the fermentation patterns and in morphology, whose value is sometimes vanishing.

A promising start was recently made in this field (SCARDOVI *et al.* 1970) with the adoption of the DNA × DNA hybridization technique: species like *B. globosum* and *B. ruminale* or those isolated from honey bees intestine were sharply distinguishable for their almost complete genetic unrelatedness (SCARDOVI *et al.* 1970).

It is undeniable that the new species we propose here could hardly be distinguished from *B. longum var. animalis* b suggested by MITSUOKA (1969) on the guideline of the fermentation tests alone (cfr. Table 2). The only phenotypic distinguishing character can be found in the cellular morphology as clearly depicted in Fig. 2; it should be mentioned at this point that DNA × DNA hybridization experiments indicated (SCARDOVI *et al.*, 1971) that the varieties of the species *B. longum* suggested by MITSUOKA are close genetically to the species *B. globosum*, characterized by the shortness and globular form of its cells.

Morphology should, however, be taken with caution in the taxonomy of bifid bacteria, except for few cases (SCARDOVI and TROVATELLI 1969), because of its rather large variability (KOJIMA *et al.* 1968). The evaluation of the genetic

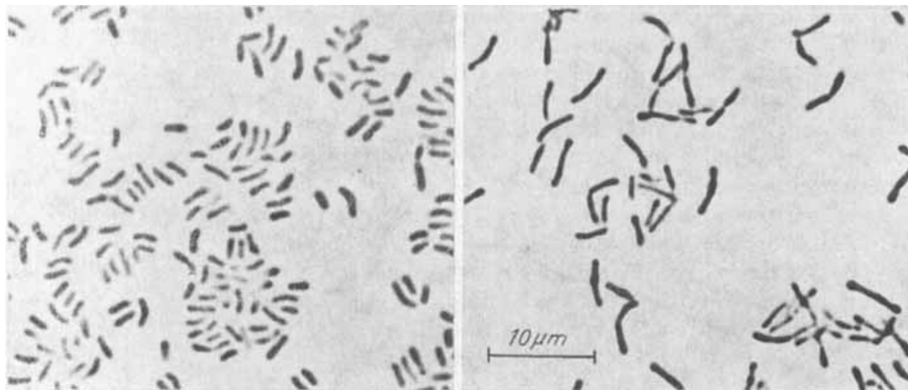

Fig. 2. Liquid cultures for DNA extraction. Left: *Bifidobacterium longum var. animalie* type b, strain CIO-45; right: *Bifidobacterium suis*, type strain Su 859. Observation in phase contrast

relatedness, eventually by DNA × DNA hybridization in the competition procedure, seems therefore the method of choice to judge about the validity of the proposed specific taxa of the genus *Bifidobacterium* in the present status of a still general paucity of known phenotypic distinguishing characters.

The typical strain among our isolates (strain Su 859) was already shown by SCARDOVI *et al.* (1970) bearing no genetic relatedness with *B. globosum* and *B. ruminale*, species inhabiting the rumen of cattle (SCARDOVI *et al.* 1969), with *B. asteroides* and *B. indicum*, species found in the intestine of honey bees (SCARDOVI and TROVATELLI 1969) or with species like *B. bifidum*, *B. longum* and *B. breve*, fecal bifidobacteria of man (REUTER 1963, MITSUOKA 1969). Our additional results on the genetic relatedness between our strains and those found by MITSUOKA in the feces of several animals (see Table 1) and the results reported above, leave no doubt about the genetic unrelatedness between the bifids we isolated from pig feces and anyone of the species previously proposed either of "animal" or "human" habitats, and warrant the proposal to create the new species *Bifidobacterium suis* for the bifidobacteria we isolated from the feces of pigs.

Bifidobacterium suis n. sp.

Slender cells elongated (2—6µ long) with rare terminal bifurcations of clubs. Non motile. Gram-positive. Colonies circular, soft, smooth, white with entire margins. Liquid cultures are at first turbid, after 24—36 hours became clear with sediment dispersable at agitation. Anaerobic. Catalase negative.

Temperature relations: optimum, 38—39 °C; minimum, 19—20 °C; maximum 44.5—45 °C.

Optimum pH: 7—8. No growth at pH 5.0 or 9.5.

Sugar fermented: arabinose, xylose, glucose, fructose, mannose, galactose, maltose, sucrose, lactose, melibiose, raffinose. Never fermented: rhamnose, melezitose, cellobiose, trehalose, dextrin, starch, inulin, sorbitol, mannitol, glycerol, salicin, gluconate and lactate.

No growth in carbohydrate-free media.

Lactic and acetic acids (in the ratio 1.0:1.7—1.0:2.0) are the end products of glucose fermentation. Glucose is fermented via "fructose-6-phosphate" shunt. Cell-free extracts possess fructose-6-phosphate phosphoketolase, aldolase and HMP dehydrogenases.

Nitrites not produced from nitrates. Indole and acetyl-methyl-carbinol not produced.

Skim milk: acidification followed by coagulation in 1—2 days.

Riboflavine is the only growth factor required for growth.

DNA base composition: 62%.

DNA homology: genetically not related with the other nomen-species of bifidobacteria isolated from man and animals.

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References

BAUMANN, M. E. and FOSTER, E. M., 1956. Characteristics of organisms isolated from the rumen of cows fed high and low roughage rations. J. Bacteriol., **71**, 333.

CLARKE, R. T. J., 1959. A dextran-fermenting organism from the rumen closely resembling *Lactobacillus bifidus*. J. gen. Microbiol., **20**, 549.

DOUNCE, A. L., BARNETT, S. R. and BEYER, G. T., 1950. Further studies in the kinetics and determination of aldolase. J. biol. Chemistry, **185**, 769.

GIBBONS, R. J. and DOETSCH, R. N., 1959. Physiological study of an obligately anaerobic ureolytic bacterium. J. Bacteriol., **77**, 417.

GYLLENBERG, H. and CARLBERG, G., 1958. The nutritional characteristics of the bifid bacteria (*Lactobacillus bifidus*) of infants. Acta pathol. microbiol. scand., **44**, 287.

HAENEL, H. and MÜLLER-BEUTHOW, W., 1956. Vergleichende quantitative Untersuchungen über Keimzahlen in den Feces des Menschen und einiger Wirbeltiere. Zbl. Bakteriol., I. Abt., Orig., **167**, 123.

JOHNSON, J. L. and ORDAL, E. J., 1968. Deoxyribonucleic acid homology in bacterial taxonomy: effect of incubation temperature on reaction specificity. J. Bacteriol., **95**, 893.

KROGH, 1963. Identification of the gram-positive rumen flora of cattle and sheep in clinical cases of acute indigestion. Acta vet. scand., **4**, 41.

KOJIMA, M., SUDA, S., HOTTA, S. and HAMADA, K., 1968. Induction of pleomorphism in *Lactobacillus bifidus*. J. Bacteriol., **95**, 710.

LOWRY, O. H., ROSENBROUGH, N. J., FARR, A. L. and RANDAL, R. L., 1951. Protein measurement with the Folin-phenol-reagent. J. biol. Chemistry, **193**, 265.

Manual of Microbiological Methods., McGraw-Hill Comp., Inc., New York, 1957.

MARMUR, J., 1961. A procedure for the isolation of deoxyribonucleic acid from microorganisms. J. molecular Biol., **3**, 208.

MARMUR, J. and DOTY, P., 1962. Determination of the base composition of deoxyribonucleic acid from its thermal denaturation temperature. J. molecular Biol., **5**, 109.

MITSUOKA, T., 1969. Vergleichende Untersuchungen über die Bifidobakterien aus dem Verdauungstrakt von Menschen und Tieren. Zbl. Bakteriol., I. Abt., Orig., **210**, 52.

OCHI, Y., MITSUOKA, T. und SEGA, T., 1964. Untersuchungen über die Darmflora des Huhnes. III. Mitteilung: Die Entwicklung der Darmflora von Kücken bis zum Huhn. Zbl. Bakteriol., 1 Abt., Orig., **193**, 80.

OPPENHEIMER, C. H. and DROST-HANSEN, W. A., 1960. A relationship between multiple temperature optima for biological systems and the properties of water. J. Bacteriol., **80**, 21.

PHILLIPSON, A. T., DOBSON, N. J., BLACKBURN, T. H. and BROWN, M., 1962. The assimilation of ammonia nitrogen by bacteria of the rumen of sheep. Brit. J. Nutr., **16**, 151.

REUTER, G., 1963. Vergleichende Untersuchungen über die Bifidus-Flora im Säuglings- und Erwachsenenstuhl. Zbl. Bakteriol., I Abt., Orig., **191**, 486.

SCARDOVI, V. and TROVATELLI, L. D., 1965. The fructose-6-phosphate shunt as peculiar pattern of hexose degradation in the genus *Bifidobacterium*. Annu. Rev. Microbiol., **15**, 19.
SCARDOVI, V. and TROVATELLI, L. D., 1969. New species of bifid bacteria from *Apis mellifica* L. and *Apis indica* F. A contribution to the taxonomy and biochemistry of the genus *Bifidobacterium*. Zbl. Bakteriol., II. Abt., **123**, 64.
SCARDOVI, V., TROVATELLI, L. D., CROCIANI, F. and SGORBATI, B., 1969. Bifid bacteria in bovine rumen. New species of the genus *Bifidobacterium*: *B. globosum n. sp.* and *B. ruminale n. sp.* Arch. Mikrobiol., **68**, 278.
SCARDOVI, V., ZANI, G. and TROVATELLI, L. D., 1970. Deoxyribonucleic acid homology among the species of the genus *Bifidobacterium* isolated from animals. Arch. Mikrobiol., **72**, 218.
SCARDOVI, V., TROVATELLI, L. D., ZANI, G., CROCIANI, F. and MATTEUZZI, D., 1971. Deoxyribonucleic acid homology relationships in the genus *Bifidobacterium*. Int. J. syst. Bacteriol., in press.
SCHRAMM, M., KLYBAS, V. and RACKER, F., 1958. Phosphorolytic cleavage of fructose-6-phosphate by fructose-6-phosphate phosphoketolase from *Acetobacter xylinum*. J. biol. Chemistry, **177**, 859.
SEBALD, M., GASSER, F. et WERNER, H., 1965. Teneur GC % et classification. Application au groupe des bifidobactéries et à quelches genres voisins. Ann. Inst. Pasteur, **109**, 251.
SIBLEY, J. A. and LEHNINGER, A. L., 1949. Determination of aldolase in animal tissue. J. biol. Chemistry, **177**, 859.
SILVESTRI, L. G. and HILL, L. R., 1965. Agreement between deoxyribonucleic acid base composition and taxometric classification of gram-positive cocci. J. Bacteriol., **90**, 136.
UCHIDA, K., KATAOKA, K., MITSUOKA, T., SHINJO, T. and OGATA, M., 1965. Studies on the intestinal flora of pigs. I. The fecal bacterial flora of the healthy pig. Jap. J. vet. Sci., **27**, 215.
VRIES, W. DE and GERBRANDY, S. J., 1967. Carbohydrate metabolism in *Bifidobacterium bifidum*. Biochim. biophysica Acta, **136**, 415.
WASSERMANN, R. H., SEELEY, H. W. and LOSLI, J. K., 1953. The physiology and nutrition of a rumen *Lactobacillus bifidus*. J. animal Sci., **12**, 935.

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