Plasmids in the Genus *Bifidobacterium*

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A total of 1461 bacterial isolates, representing 24 different species of the genus *Bifidobacterium*, were examined for the presence of plasmid DNA. Approximately 20% of the isolates contained detectable plasmids, but only four species were represented: *B. longum*, the predominant bifid species in the human intestine; *B. globosum*, the most common in animals; and *B. asteroides* and *B. indicum*, species found exclusively in the intestines of western and asiatic honey bees, respectively. Multiple plasmids were common among isolates of *B. longum* and *B. asteroides*, while all plasmid-bearing isolates of *B. globosum* and 60% of *B. indicum* isolates contained only one plasmid each. Certain multiple plasmid profiles were predominant among the *B. longum* and *B. asteroides* isolates.

INTRODUCTION

Plasmids have been observed in representatives of virtually every bacterial genus examined for their presence, and contribute to the genetic and evolutionary potential of these microorganisms. Several categories of phenotypic trait, such as antibiotic resistance, toxin and bacteriocin production, modification of bacterial virulence and an ever-increasing number of metabolic capabilities, are often associated with plasmids (Falkow, 1975; Broda, 1979). These extrachromosomally determined properties can be widely disseminated among bacterial populations by natural transfer mechanisms, such as transformation, transduction and conjugation (Falkow, 1975; Broda, 1979). Exclusive of their role as carriers and transporters of genetic information, plasmids can also provide a means of strain typing (Davies *et al.*, 1981), or molecular fingerprinting, for following the flow of specific bacteria through host populations.

The presence of plasmids is common among natural isolates of bacterial species which inhabit the intestinal tracts of humans and animals. In fact, the isolation of *Enterobacteriaceae* or enterococci which are plasmid-less is rare, and most natural isolates have multiple plasmids. Recent reports have described the presence of plasmids in the genus *Bacteroides*, a major component of the intestinal flora (Privitera *et al.*, 1979; Tally *et al.*, 1979; Welch *et al.*, 1979). The bifidobacteria are also normal constituents of the intestinal flora of man, various animals and honey bees (Mitsuoka & Kaneuchi, 1977; Scardovi, 1981). Most species are found almost exclusively in the intestinal tract of the host organism, where their presence in high numbers (10^9 – 10^{10} in humans; Mitsuoka & Kaneuchi, 1977) is thought to be beneficial (Poupard *et al.*, 1973). To our knowledge, there is no published information regarding plasmids in this genus.

We have surveyed the genus *Bifidobacterium* for the presence and distribution of plasmids. A total of 1461 strains, from the Collection of the Institute for Agricultural Microbiology of Bologna University, representing 22 different species and two DNA homology groups were examined. The results of this survey, as well as some preliminary data on the potential taxonomic and ecological significance of plasmid profiles in some of the species, are presented in this communication.

METHODS

Bacterial strains and culture conditions. Strains of *Bifidobacterium* spp. were isolated from a variety of sources and have been identified by DNA-DNA hybridization, except for a few from honey bees. Most isolates, nearly 1200, have also been characterized by isozyme electrophoresis (Scardovi *et al.*, 1979). A large number of fermentative biotypes were included among the strains of most species, as well as urease-positive and urease-negative strains of the species *B. suis* (Matteuzzi & Crociani, 1973). Because of the possible loss of plasmids by repeated subculturing, freeze-dried preparations from the first cultures following isolation were used in the plasmid analyses.

Most species were grown on trypticase/phytone/yeast extract medium of the following composition: trypticase (BBL), 0.8 g; phytone (BBL), 0.3 g; yeast extract (Difco), 0.25 g; glucose, 1.0 g; K_2HPO_4 , 0.15 g; $MgCl_2$, 0.05 g; cysteine.HCl, 0.05 g; Tween 80, 0.1 ml; distilled water, 100 ml; pH adjusted to 6.5–6.7. *Bifidobacterium pullorum* and *B. coryneforme* isolates were grown on MRS medium (DeMann *et al.*, 1960).

Cylindrical vessels (35 × 160 mm) were filled with medium (90 ml), sterilized at 110 °C for 20 min, and inoculated with culture (10 ml). Incubation was at 38–39 °C under a CO_2/N_2 atmosphere with a palladium catalyst (BBL, Gas-Pak). One or two culture vessels were used for each screening, depending on cell yield. When large quantities of cells were required, flasks (1 litre) almost completely filled with medium were inoculated as soon as possible after autoclaving. Sodium bicarbonate solution (10%, w/v) was filter-sterilized and added to the medium (3 ml l⁻¹), and the flasks incubated under air. Cells were harvested in exponential or early stationary phase.

Plasmid analyses. The rapid screening technique of LeBlanc & Lee (1979), developed for streptococci, was suitable for bifidobacteria and used here for all plasmid analyses. Agarose gel electrophoresis was performed according to Meyers *et al.* (1976) using vertical slab gels (14 × 12 × 0.3 cm) with 0.8% (w/v) agarose (BioRad, standard 10w-m), using combs giving 4 or 8 mm wells. Plasmid DNA from *Escherichia coli* strain V517 (Macrina *et al.*, 1978), isolated by the above procedure, was used as a source of eight covalently closed circular (CCC) size markers of 1.4, 1.8, 2.0, 2.6, 3.4, 3.7, 4.8 and 36 MDal. Alternatively, a plot of *E. coli* V517 CCC DNA on log-log paper was used to determine the CCC equivalents of linear *Hind*III-digested λ DNA fragments. This provided six additional CCC size markers at 1.9, 2.2, 3.5, 4.4, 5.5 and 8.5 MDal.

RESULTS AND DISCUSSION

We have examined nearly 1500 bacterial isolates belonging to the genus *Bifidobacterium* for the presence of plasmid DNA. Approximately 21% of the plasmid-enriched lysates from these isolates produced one or more extrachromosomal DNA bands when electrophoresed in agarose gels. However, among the 303 plasmid-containing isolates only four species of *Bifidobacterium* were represented, as shown in Table 1. The variety of plasmid profiles observed among these four species is presented schematically in Figs 1, 2 and 5. Failure to detect plasmids in the remaining species and homology groups may be due to (1) the absence of plasmids in certain species, (2) the inability to isolate certain classes of plasmids by the procedure employed, or (3) examination of too few representatives of some species.

Our screening results suggested that particular DNA banding profiles appeared to predominate within each of the four species harbouring detectable plasmids. Since plasmid profiles may have taxonomic value as well as providing useful ecological and evolutionary information, we have begun a systematic analysis of the plasmids in these species.

Plasmid profiles of B. longum isolates

Seven species of *Bifidobacterium* are listed in Table 1 as having humans as their primary host, and three of these, *B. longum*, *B. breve* and *B. infantis*, constitute the major portion of the bifidobacterial flora of the faeces of newborn infants. Plasmid bands were detected among isolates of only one of these species, *B. longum*. The above three species are very closely related: DNA homology between *B. longum* and *B. infantis* is 65–80%, and *B. breve* shows 50–75% homology with both (Scardovi *et al.*, 1979; Sgorbati, 1979). Furthermore the boundaries between *B. longum* and *B. infantis* seem to vanish if one considers the strains termed 'intermediates' (Table 1) isolated from calf faeces, which, being more than 80% related by DNA homology to both *B. infantis* and *B. longum*, cannot be assigned to either species (Scardovi *et al.*, 1979). Furthermore, most of the strains of *B. longum* and *B. infantis* studied here have been isolated from the same faecal specimens. From 186 isolates of *B. longum* obtained from human adult and infant faeces, plasmids were observed in 127 (about 70%), all from infants, and 125 of these were examined in detail. Thirteen different profiles, each consisting of two to seven plasmid bands,

Table 1. *Species of Bifidobacterium examined for the presence of plasmid DNA*

Host	Species	Sources of isolates studied*	No. of isolates:	
			Studied	Containing plasmids
Humans	<i>B. longum</i>	Infant and adult faeces	186	127
	<i>B. dentium</i>	Dental caries; infant and adult faeces; vagina; rabbit faeces; lung abscesses, pleural fluid and appendices of humans	140	0
	<i>B. pseudocatenulatum</i>	Infant and calf faeces, sewage	120	0
	<i>B. breve</i>	Infant faeces; human vagina; calf faeces	106	0
	<i>B. infantis</i>	Infant faeces; human vagina	63	0
	<i>B. catenulatum</i>	Infant and adult faeces; sewage	41	0
	<i>B. bifidum</i>	Infant and calf faeces	39	0
	<i>B. globosum</i> †	Rat, rabbit and calf faeces; rumen of cattle; chicken, lamb and piglet faeces; sewage; infant faeces	129	29
Animals	<i>B. thermophilum</i>	Rumen of cattle; sewage; piglet and calf faeces	74	0
	<i>B. animalis</i>	Rabbit, rat and chicken faeces; sewage	24	0
	<i>B. suis</i>	Piglet faeces	22	0
	<i>B. boum</i>	Rumen of cattle; piglet faeces	22	0
	<i>B. magnum</i>	Rabbit faeces	16	0
	<i>B. choerinum</i>	Piglet faeces; sewage	15	0
	<i>B. cuniculi</i>	Rabbit faeces	7	0
	<i>B. pullorum</i>	Chicken faeces	3	0
	<i>B. pseudolongum</i>	Pig, chicken and mouse faeces	3	0
	<i>B. adolescentis</i>	Sewage; rumen of cattle; adult human faeces	54	0
	<i>B. infantis</i> - <i>B. longum</i> 'intermediates'	Calf faeces	45	0
	<i>B. angulatum</i>	Sewage; adult human faeces	6	0
	<i>B. adolescentis</i>	Sewage; adult human faeces	6	0
Animals and humans	<i>B. adolescentis</i>	Sewage; adult human faeces	6	0
	<i>B. infantis</i> - <i>B. longum</i> 'intermediates'	Calf faeces	45	0
	<i>B. angulatum</i>	Sewage; adult human faeces	6	0
	<i>B. adolescentis</i>	Sewage; adult human faeces	6	0
	<i>B. infantis</i> - <i>B. longum</i> 'intermediates'	Calf faeces	45	0
	<i>B. angulatum</i>	Sewage; adult human faeces	6	0
Honey bees	<i>B. asteroides</i>	Bee intestine	225	74
	<i>B. indicum</i>	Bee intestine	106	73
	<i>B. coryneforme</i> †	Bee intestine	8	0
	DNA homology groups‡			
	'subtile'	Sewage	5	0
	'minimum'	Sewage	2	0

* The sources of isolates are indicated in order of decreasing number of strains isolated therefrom.

† Species not included in the Approved Lists of Bacterial Names (Skerman *et al.*, 1980).

‡ Groups of strains distinguished primarily from all other bifidobacteria on the basis of their DNA homology, but not currently proposed as new species (Scardovi & Trovatielli, 1974).

within a size range of 1.25 to 9.5 MDal, were observed (Fig. 3). Three of these, A1, A2 and A3, were produced by 65% of the plasmid-containing isolates (Table 2 and Fig. 1). In several instances where the profiles appeared to be identical, plasmid preparations were electrophoresed side by side on the same gel to confirm their identical sizes (data not shown). With the exception of profiles A1, A2 and A3, together with profile E, which was common to isolates obtained from two different faecal samples, each of the remaining profiles appeared in isolates from only one sample.

Profiles E and A1, each with two plasmid bands, and A2 and A3, each with four bands, were observed in isolates from 2, 7, 9 and 6 different faecal samples respectively. Given the size of the plasmids in these isolates, and the number in each profile (particularly profiles A2 and A3), it seems unlikely that they have been transferred to the strains from each of the different faecal samples. More probably, these data suggest that a limited number of *B. longum* strains have been disseminated among the various infants from which the samples were obtained.

For the most part, the isolates from each faecal sample produced only one of the 13 plasmid profiles common to this species. There were only three exceptions among the 30 samples

Fig. 1. Diagrammatic representation of the plasmid profiles most frequently found in four species of the genus *Bifidobacterium*. The letters at the bottom refer to the different plasmid profiles and percentage values represent the proportion of the strains in which each profile was found.

Fig. 2. Diagrammatic representation of the plasmid profiles (indicated by letters) less frequently found in *B. longum*.

examined, samples 1, 5 and 6, each of which yielded isolates whose plasmid profiles fell into two different groups (Table 2).

Plasmids in *B. globosum*

Of the *Bifidobacterium* spp. listed in Table 1, 10 are found almost exclusively in the intestines of animals. Plasmids were detected in only one of these, *B. globosum*. However, with the possible

Fig. 3. Agarose gel electrophoresis of plasmids from strains of *B. longum*. Letters above the lanes refer to plasmid profiles used in Table 2 and Figs 1 and 2. The strains used were: *B. longum* E, B2192; A1 (left), B2352; A1 (right), B1678; A3, B2399; A2, B2088; F, B1433; G, B2640; H, B2349; I, B1835; J, B1393; K, B1990; L, B1319; M, B1694; N, B2577. λ , lanes containing *Hind*III-restricted phage λ DNA. Residual chromosomal DNA is arrowed.

exception of *B. thermophilum*, it is likely that too few isolates of the other species were examined to permit any conclusions regarding the presence or absence of plasmid DNA.

The species *B. globosum* (Scardovi *et al.*, 1969) is not included in the Approved Lists of Bacterial Names (Skerman *et al.*, 1980), having been considered by Rogosa (1974) as identical with *B. pseudolongum* (Mitsuoka, 1969). Scardovi *et al.* (1979) are of the opinion that, on account of differences in G + C content of their DNA (about 3%) and extent of DNA homology (about 70%), the two species should be considered distinct.

A number (22%) of the *B. globosum* isolates examined contained extrachromosomal DNA. Unlike the profiles observed in *B. longum*, which exhibited between two and seven plasmid bands, only a single band was detectable in each of the 29 plasmid-containing isolates of *B. globosum*, and a total of only three different sizes were seen (D1, D2 and D3; Table 3, Figs 1 and 4). A 46 MDal plasmid was present in 11 isolates obtained from faecal samples of rats, rabbits and chickens, the rumen of cattle, and sewage. Thirteen isolates from rat, calf and lamb faeces, and sewage, harboured a single 24.5 MDal species. One isolate each from rat, calf and lamb faeces, and from cattle rumen, had a 13.5 MDal plasmid.

It is not known whether plasmids of the same size are identical, nor whether there is any homology between the plasmids of different sizes. All three of the plasmid classes in *B. globosum* are large enough to code for conjugative functions. Thus, it will be difficult to determine whether specific strains, or their plasmids, are being disseminated among the various animal species.

The species *B. longum* and *B. globosum* represent the predominant species isolated from the intestinal tracts of humans and animals respectively (Reuter, 1963–64; Mitsuoka & Kaneuchi, 1977; Scardovi, 1981). The distinction between 'human' and 'animal' species has been based on ecological and immunological data (Sgorbati, 1979). It appears that *B. longum* and *B. globosum* can also be readily distinguished on the basis of their plasmid complements.

Table 2. *Plasmid profiles of B. longum isolates*

All isolates were obtained from 30 samples of infant faeces. Plasmid profiles are shown in Figs 1, 2 and 3.

Sample no.	No. of plasmid-containing isolates	No. of isolates with profile:													
		A1	A2	A3	E	F	G	H	I	J	K	L	M	N	
1	7	1	6	.	.	.	.	.	.	.	.	.	.	.	
5	6	1	5	.	.	.	.	.	.	.	.	.	.	.	
6*	4	2	.	.	.	.	.	2	.	.	.	.	.	.	
2	2	2	.	.	.	.	.	.	.	.	.	.	.	.	
3*	2	2	.	.	.	.	.	.	.	.	.	.	.	.	
4*	2	2	.	.	.	.	.	.	.	.	.	.	.	.	
7*	6	6	.	.	.	.	.	.	.	.	.	.	.	.	
8	2	.	2	.	.	.	.	.	.	.	.	.	.	.	
9	1	.	1	.	.	.	.	.	.	.	.	.	.	.	
10	1	.	1	.	.	.	.	.	.	.	.	.	.	.	
11	6	.	6	.	.	.	.	.	.	.	.	.	.	.	
12	4	.	4	.	.	.	.	.	.	.	.	.	.	.	
13	9	.	9	.	.	.	.	.	.	.	.	.	.	.	
14	1	.	1	.	.	.	.	.	.	.	.	.	.	.	
15	3	.	.	3	.	.	.	.	.	.	.	.	.	.	
16	6	.	.	6	.	.	.	.	.	.	.	.	.	.	
17	9	.	.	9	.	.	.	.	.	.	.	.	.	.	
18	3	.	.	3	.	.	.	.	.	.	.	.	.	.	
19*	4	.	.	4	.	.	.	.	.	.	.	.	.	.	
20*	5	.	.	5	.	.	.	.	.	.	.	.	.	.	
21*	4	.	.	.	4	.	.	.	.	.	.	.	.	.	
22	2	.	.	.	2	.	.	.	.	.	.	.	.	.	
23	7	.	.	.	.	7	.	.	.	.	.	.	.	.	
24	10	.	.	.	.	.	10	.	.	.	.	.	.	.	
25	1	.	.	.	.	.	.	.	1	.	.	.	.	.	
26	1	.	.	.	.	.	.	.	.	1	.	.	.	.	
27*	3	.	.	.	.	.	.	.	.	.	3	.	.	.	
28	5	.	.	.	.	.	.	.	.	.	.	5	.	.	
29	6	.	.	.	.	.	.	.	.	.	.	.	6	.	
30	3	.	.	.	.	.	.	.	.	.	.	.	.	3	

* Samples obtained from artificially fed infants.

Table 3. *Plasmids in isolates of B. globosum*

Plasmid profiles are shown in Figs 1 and 4.

Source of sample	No. examined		No. of isolates with plasmid profile:		
	Samples	Isolates	D1	D2	D3
Rat faeces	24	42	2	10	1
Calf faeces	8	14	0	1	1
Rabbit faeces	10	14	4	0	0
Lamb faeces	7	10	0	1	1
Chicken faeces	12	13	3	0	0
Piglet faeces	5	9	0	0	0
Infant faeces	2	3	0	0	0
Rumen of cattle	6	14	1	0	1
Sewage	10	10	1	1	0
Totals	84	129	11	13	4

Plasmids in bifidobacteria isolated from honey bees

The two remaining species in which plasmids were detected, *B. asteroides* and *B. indicum*, are specific inhabitants of the hind-gut of honey bees; the former is isolated almost exclusively from the intestines of western honey bees, while the latter is found only in the hind-gut of asiatic bees

Fig. 6. Agarose gel electrophoresis of plasmids from strains of *B. asteroides* and *B. indicum*. Letters above the lanes refer to plasmid profiles used in Tables 4 and 5 and Figs 1 and 5. The strains used were: *B. asteroides* B1, C350; B2, C503; O, C359; P, C367/1; Q, C365/1; R, C236; S, C658; T, C212; U, C333; V, C690; W, C353; X, C682; *B. indicum* C2, C576; C1, C515; B2, C503; O, C450. λ , lanes containing *Hind*III-restricted phage λ DNA. Residual chromosomal DNA is arrowed.

(Scardovi & Trovatielli, 1969). These species do not have any significant homology with any other species of the genus (Scardovi *et al.*, 1971) and are evolutionarily quite divergent from them, as suggested by the immunological properties of their transaldolases (Sgorbati, 1979; Sgorbati & London, 1982). The two species display plasmid profiles which are distinct from those of *B. longum* and *B. globosum*. In addition, the profiles of *B. indicum* are, for the most part, different from those of *B. asteroides* (Fig. 1).

Strains of *B. asteroides* were isolated from bees received from 15 countries, and among the 74 plasmid-containing isolates studied, 14 different plasmid profiles were observed (Figs 1, 5, and 6). The ladder effect seen in some lanes of Fig. 6 is probably due to the activity of a topoisomerase (Cozzarelli, 1980), with the resulting faint bands representing different degrees of supercoiling of the same plasmid. These bands were therefore omitted from the plasmid profiles (Figs 1 and 5).

The occurrence and distribution of the different plasmid profiles in *B. asteroides* isolates did not correlate with the geographical areas in which the host bees were raised, as seen in Table 4. Four profiles predominated, accounting for over 75% of the plasmid-containing isolates. Profiles W, B1, B2 and B3 were observed among isolates from four, five, six and seven different countries, respectively. These results may be interpreted to suggest that the profiles W, B1, B2 and B3 identify four strains of *B. asteroides* which have been widely disseminated throughout the world. Alternatively, particularly with regard to profile B3, the plasmids may be widely disseminated among strains of *B. asteroides*.

The 73 plasmid-containing isolates of *B. indicum* were all obtained from two species of honey

Table 4. *Plasmids in B. asteroides*

Plasmid profiles are shown in Figs 1 and 6.

Honey bee	Country	Studied	No. of isolates:													
			With plasmid profiles:													
			B1	B2	B3	O	P	Q	R	S	T	U	V	W	X	Y
<i>A. mellifera ligustica</i>	Italy	33	.	3	1	.	.	.	.	.	.	.	.	2	.	.
<i>A. mellifera mellifera</i>	France	2	.	.	.	.	.	.	.	.	.	.	.	.	.	.
	Norway	10	.	.	.	.	.	.	.	.	.	.	.	.	.	.
<i>A. mellifera caucasica</i>	Denmark	5	.	.	1	.	.	.	.	.	.	.	.	.	.	1
	Bulgaria	8	.	.	.	.	.	.	.	.	.	1	.	.	.	.
	Soviet Union	14	2	6	.	1	1	1	.	.	.	.	.	2	.	.
<i>A. cerana</i>	Philippines	9	.	4	.	.	.	.	.	.	.	.	.	.	.	.
<i>A. mellifera</i>	Scotland	5	.	4	.	.	.	.	.	.	.	.	.	.	.	.
	England	24	2	1	1	.	.	.	.	.	.	.	.	.	.	.
	Germany	28	10	2	1	.	.	.	.	.	.	.	.	.	.	.
	Norway	8	1	.	.	.	.	.	1	.	2	.	.	.	.	.
	Belgium	2	.	.	.	.	.	.	.	.	.	.	.	.	.	.
	Poland	8	.	.	3	.	.	.	.	.	.	.	.	.	.	.
	Yugoslavia	21	.	.	5	.	.	.	.	.	.	.	.	.	.	1
	Greece	6	1	.	1	.	.	.	.	.	.	.	.	.	.	.
	Soviet Union	4	.	.	.	.	.	.	.	.	.	.	.	.	.	.
	Czechoslovakia	4	.	.	.	.	.	.	.	.	.	.	.	1	.	.
	Philippines	34	.	.	.	1	.	.	.	3	.	.	4	2	1	.
Total no. of isolates		225	16	20	13	2	1	1	1	3	2	1	4	7	1	2
No. of countries represented by profile			5	6	7	2	1	1	1	1	1	1	1	4	1	2

Table 5. *Plasmids in B. indicum*

Plasmid profiles are shown in Figs 1 and 6.

Honey bee	Country	Studied	No. of isolates:			
			With plasmid profiles:			
			C1	C2	B2	O
<i>A. dorsata</i>	Philippines	53	15	4	3	4
<i>A. cerana</i>	Philippines	48	9	38	0	0
	Malaysia	4	0	0	0	0
	Japan	1	0	0	0	0
Totals		106	24	42	3	4

bee, *Apis dorsata* and *A. cerana*, received from the Philippines. Nine of the plasmid-containing isolates obtained from *A. cerana* had profile C1, composed of three small plasmids, and 38 of these isolates harboured only a single 22 MDal plasmid (C2; Table 5, Figs 1 and 6). Of the 26 plasmid-containing isolates from *A. dorsata*, 19 had either the C1 or the C2 plasmid complement. Seven of the *B. indicum* isolates from *A. dorsata* had plasmid profiles common among *B. asteroides* strains, namely B2 (three isolates) and O (four isolates).

The species *B. indicum* is distinguished from *B. asteroides* on the basis of DNA homology, morphology and transaldolase and 6-phosphogluconate dehydrogenase zymograms (Scardovi *et al.*, 1979). The plasmid profiles of 90% of the *B. indicum* isolates were different from those of *B. asteroides*.

The plasmid complements of bacterial isolates, particularly the multi-plasmid profiles observed among many isolates of *B. longum*, *B. asteroides* and *B. indicum*, can serve as molecular markers for the identification of specific strains. Such profiles may help to establish the geo-

graphical distribution of specific strains of *B. asteroides*, or the dissemination of particular strains of *B. longum* within a human population. The validity of plasmid profiles as taxonomic markers is dependent on the demonstration of homology between plasmids of the same size in different isolates, and this will be the subject of further investigations.

In addition to establishing the identity of plasmids within a profile, the homology studies are expected to identify identical plasmids in different profiles. This type of information may be invaluable in establishing evolutionary relationships and distances among strains of the same, as well as closely related species.

No phenotypic properties have been correlated with the presence of any of the plasmids in the four plasmid-containing species. There does not appear to be any correlation between carbohydrate fermentation ability and the presence of plasmids in isolates of *B. longum* or *B. globosum*. Biotypes of *B. longum* differing in their ability to ferment ribose, melezitose and gluconate were included in this study as well as *B. globosum* strains capable of fermenting arabinose and xylose but not mannitol.

Since temperate phages may persist in the cell in an extrachromosomal state, so as to mimic plasmids (Lieb, 1970; Novick *et al.*, 1976), one of us attempted to detect phages after UV or mitomycin C treatment in a number of plasmid-containing and plasmid-free strains of *B. longum*. Many strains did liberate phage particles but no correlation with their plasmid complement was evident (B. Sgorbati, unpublished results).

Preliminary results seem to indicate a link between plasmids and tetracycline resistance in some strains of *B. globosum*. The existence of R plasmids in this species may be useful in establishing a genetic transfer system.

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