Characterization of Growth and Inulinase Production by *Bifidobacterium* spp. on Fructooligosaccharides

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Abstract A total of 19 species of *Bifidobacterium* were screened for the ability to grow with fructooligosaccharides (FOS) as the carbohydrate source. Several strains of animal origin (*B. thermophilum*, *B. minimum*, and *B. cuniculi*) grew significantly better than strains from human source on inulin (degree of polymerization (DP) ≥ 15). These strains grew equally well on inulin from several sources, but were unable to metabolize levan, a polymer of $\beta(2-6)$ -D-fructose. Best growth was obtained on FOS preparations containing a significant proportion of low-molecular-weight (DP ≤ 5) carbohydrates. Strains which grew best on inulin required the presence of this FOS for maximal induction of the *exo*-inulinase. These results suggest that animal strains of bifidobacteria may be used in conjunction with short-chain FOS as a supplement to animal feed.

Key words: bifidobacteria; fructooligosaccharides (FOS); inulinase

The widespread use of antibiotics in animal feed to reduce disease and improve animal performance, and thus profitability, has come under criticism for increasing the antibiotic resistance of many bacteria, including some pathogens. Organisms which cause respiratory and cutaneous infections (*Streptococcus pneumoniae*, *Streptococcus pyogenes* and staphylococci) as well as diarrhea, urinary infection and sepsis (Enterobacteriaceae, Pseudomonas families) are now resistant to many of the older antibiotics (13). This concern, with antimicrobial agent becoming less effective, has prompted many investigations into mechanisms to replace antibiotics with probiotic agents in animal feed rations (7).

The administration of probiotic agents in animal feeds has met with limited success and produced highly variable results (16). Most of the preparations contain lactobacillus preparations, and more recently bifidobacteria species have been added to many of the probiotic preparations. Lactobacilli tend to occupy the more proximal area of the gastrointestinal tract (GI) (16, 18), while bifidobacteria inhabit principally the large intestine or cecal areas (11).

Bifidobacteria inhibit the growth of many undesirable organisms in the lower GI (5) through the production of lactic and acetic acid (15). By controlling intes-

tinal pH it is possible to restrict the growth of pathogens and putrefactive bacteria and in turn reduce the production of phenols, ammonia, steroid metabolites, bacterial toxins as well as vasoconstricting amines (5). To be of benefit to the host, bifidobacteria must receive sources of carbohydrate, termed bifidogenic factors (10), for metabolism in the lower bowel/ceca area. Ideally such compounds should be not be metabolized by the host and arrive intact at the distal end of the small intestine and colon/cecal areas of the GI tract (9).

There are a number of compounds which can survive gastric digestion and be delivered to the lower GI for metabolism by bifidobacteria (10). To date, the polymers of D-fructose, joined by $\beta(1-2)$ linkage, have received the most attention. The short-chained polymers, termed neosugars, consist of glucose (G)-fructose (F) linkages to form GF₂, GF₃ and GF₄ compounds (5, 6). These compounds can be produced by enzymatic means (6) while the longer-chained fructan polymers are obtained from natural sources such as chicory, Jerusalem artichoke tubers or dahlia (10).

The feeding of bifidobacteria and fructooligosaccharides (FOS) to young animals has been suggested as a method for improving weight gain and feed efficiency, as well as providing protection from colonization by *Salmonella* spp.; however, there are limited data describing the effects or benefits of adding FOS to animal feed rations. Bailey et al. (3) observed that when an undefined FOS was fed to chickens, there was a 1 to 2 log reduction of *Salmonella typhimurium* in challenged chicks when the diet was supplemented with 0.75% FOS. When the chicks were given a combination of FOS (0.75%) and an undefined competitive exclusion culture, 81% of the chicks were *Salmonella* free. Chickens treated with FOS had a fourfold reduction in the level of *Salmonella* present in the cecum. Bailey et al. (3) concluded that chickens fed FOS may have an altered intestinal gut flora and this could increase their resistance to *Salmonella* colonization. Others (9) observed that 2% FOS in poultry rations reduced cecum pH by about 0.4 pH units over control treatments but the effect on *Salmonella* colonization was not determined.

There are other studies which indicate that feeding probiotic bacteria alone may be sufficient to competitively exclude pathogens. Schleifer (16) reported some success in poultry experiments, while both Beeman (4) and Wren (22) demonstrated some utility for lactobacilli preparations in calf diets. It is well known that lactobacilli tend to occupy the more proximal areas of the digestive tract of poultry, through attachment to the squamous epithelial tissue (19). Bifidobacteria do not appear to live in close association with the epithelium tissue, and are generally found in the most distal area of the digestive tract and reside in the gut lumen (20).

Metabolism of FOS by bifidobacteria is made possible by the splitting off of fructose moieties by a cell-bound inulinase or 2,1- β -D-fructan-fructanohydrolases (EC 3.2.1.7) (8). This enzyme has been previously characterized and shown to be constitutive in human, and inducible in animal strains. The inulinase from all strains studied was repressed by monosaccharides (8). There have been few reports of characterization of inulinases from bifidobacteria (12) and other bacteria (1, 2, 21).

Some intestinal pathogens can metabolize short-chain FOS; thus in order to maximize the efficient use of FOS, it is necessary to determine which *Bifidobacterium* spp. metabolize FOS, and what is the most efficient chain length and source of this polysaccharide. The purpose of the present study is to (1) select strains of bifidobacteria which can metabolize long-chain FOS such as inulin and (2) characterize the influence of environmental factors on growth and induction of inulinase.

MATERIALS AND METHODS

Strains and culture conditions. Strains of bifidobacteria used in this study are described in Table 1. Cells were routinely cultured in 10 ml of TPY broth (8) anaerobically under CO₂/H₂ (Scott Anapak, Scott Laboratories, Fiskeville, RI) at 37°C, and transferred every 72 hr.

Carbohydrate source. The following FOS were used: raffinose, inulin from dahlia tubers, inulin from chicory root, and inulin from Jerusalem artichoke tubers (all from Sigma Chemical Company, St. Louis, MO); purified neosugars (P) from Meiji Seika Kaisha; Fibruline (see Table 2 for composition) from Cosucra, Momale, Belgium; and levan (a polymer of $\beta(2-6)$ -D-fructose) kindly provided by Dr. P.J. Wan, SRRC/USDA, New Orleans, LA. Inulin from dahlia tubers (Sigma) was used at 0.5% (w/v) as the carbohydrate source unless specified otherwise.

HPLC procedures. The oligo- and polysaccharides were separated on a 4 $\times$ 250 mm Dionex Carbopac PA-100 column fitted with a Carbopac PA-100 guard column. Samples were applied from a manual Rheodyne #7161 injector with a 200 μ l sample loop. The eluting gradient was provided from a Waters 600 pump and control system, the flow rate was 1 ml/min and the gradient was as follows: with constant 100 mM NaOH throughout the program, the initial concentration of Na Acetate was 100 mM increasing to 550 mM over 80 min, then returning to 100 mM over 5 min to equilibrate for 5 min before injecting the next sample. The detector was a Waters 464 Pulsed Electrochemical Detector provided with a post column addition pump to add 0.5 M NaOH, 1.0 ml/min to the column effluent immediately before entering the detector cell. The detector pulse potentials and durations were as follows: E₁ = -100 mv, (216 ms); E₂ = +550 mv, (216 ms); E₃ = -750 mv, (299 ms). All data were collected and processed using the Waters 991 Powerline system.

Cell fractionation. Cells were centrifuged at 6000 $\times g$ for 10 min, washed in 50 mM TES buffer (Sigma) pH 7.5, and resuspended in 5 ml of TES. Cells were broken by sonication for 5 min at maximum power on low setting using a Sonic 2000 (Braun, Bethlehem, PA) fitted with a needle probe. Unbroken cells were removed by centrifugation at 480 $\times g$ for 5 min.

Growth experiments. Cells from a TPY stock culture were inoculated (1% v/v) in fresh TPY and incubated anaerobically at 37°C for 48 hr, then inoculated (1%) into 10 ml of TPY containing 0.5% (w/v) of carbohydrate. After a further 48 hr of anaerobic incubation at 37°C, growth was determined by measuring the increase in absorbance at 600 nm of an appropriate dilution with a Spectronic 2000 (Bausch

Table 1. *Bifidobacterium* spp. used in the present study

CFAR #	Strain	Source
61	<i>Bifidobacterium adolescentis</i>	ATCC 15703
63	<i>Bifidobacterium longum</i>	Commercial
66	<i>Bifidobacterium infantis</i>	ATCC 15697
67	<i>Bifidobacterium breve</i>	ATCC 15700
68	<i>Bifidobacterium longum</i>	ATCC 15707
69	<i>Bifidobacterium longum</i>	ATCC 15708
70	<i>Bifidobacterium thermophilum</i>	ATCC 25525
115	<i>Bifidobacterium bifidum</i>	Commercial
116	<i>Bifidobacterium bifidum</i>	Commercial
117	<i>Bifidobacterium bifidum</i>	Commercial
118	<i>Bifidobacterium breve/longum</i>	Commercial
119	<i>Bifidobacterium</i> species	Commercial
120	<i>Bifidobacterium</i> species	Commercial
121	<i>Bifidobacterium</i> species	Food isolate
122	<i>Bifidobacterium longum</i>	Commercial
123	<i>Bifidobacterium longum</i>	Commercial
124	<i>Bifidobacterium bifidum</i>	ATCC 29521
169	<i>Bifidobacterium bifidum</i>	Commercial
170	<i>Bifidobacterium bifidum</i>	Commercial
171	<i>Bifidobacterium bifidum</i>	Commercial
172	<i>Bifidobacterium thermophilum</i>	Commercial
319	<i>Bifidobacterium gallinarum</i>	St. Hyacinthe
320	<i>Bifidobacterium pullorum</i>	St. Hyacinthe
321	<i>Bifidobacterium globosum</i>	St. Hyacinthe
322	<i>Bifidobacterium animalis</i>	St. Hyacinthe
323	<i>Bifidobacterium thermophilum</i>	St. Hyacinthe
324	<i>Bifidobacterium cuniculi</i>	St. Hyacinthe
325	<i>Bifidobacterium choerinum</i>	St. Hyacinthe
326	<i>Bifidobacterium minimum</i>	St. Hyacinthe
327	<i>Bifidobacterium boum</i>	St. Hyacinthe
328	<i>Bifidobacterium pseudolongum</i>	St. Hyacinthe
329	<i>Bifidobacterium suis</i>	St. Hyacinthe
330	<i>Bifidobacterium magnum</i>	St. Hyacinthe
331	<i>Bifidobacterium subtilis</i>	St. Hyacinthe
332	<i>Bifidobacterium bifidum</i>	H & W #781
333	<i>Bifidobacterium infantis</i>	H & W #782
334	<i>Bifidobacterium breve</i>	H & W #783
335	<i>Bifidobacterium adolescentis</i>	H & W #784
336	<i>Bifidobacterium longum</i>	H & W #785
337	<i>Bifidobacterium cuniculi</i>	ATCC 27916
338	<i>Bifidobacterium ruminantium</i>	ATCC 49390
339	<i>Bifidobacterium merycicum</i>	ATCC 49391
347	<i>Bifidobacterium thermophilum</i>	ATCC 25866
348	<i>Bifidobacterium thermophilum</i>	ATCC 25867

Table 2. Composition of commercial inulin preparations

Degree of polymerization	Fibruline ^a	Inulin from dahlia	Inulin from JA	Inulin from chicory
	Peak area (%).....			
1+2	7.8	<0.1	<0.1	<0.1
3	5.1	<0.1	<0.1	<0.1
4	4.6	<0.1	<0.1	<0.1
5	4.0	<0.1	<0.1	<0.1
6	4.2	<0.1	<0.1	<0.1
7	4.1	<0.1	<0.1	<0.1
8	4.0	<0.1	<0.1	<0.1
9	4.0	0.1	<0.1	<0.1
10	3.9	0.3	0.1	0.2
11	3.7	0.6	0.4	0.4
12	3.3	1.1	0.8	0.7
13	3.1	1.7	1.3	1.3
14	2.9	2.4	1.8	1.9
15	2.7	3.0	2.3	2.5
16	2.6	3.5	2.6	2.9
17	2.6	4.5	2.8	3.7
18	2.4	4.8	3.0	4.0
19	2.3	4.9	3.0	4.1
20	2.2	4.9	3.0	4.2
21	2.1	4.9	3.0	4.2
22	1.9	4.7	3.0	4.2
23	1.8	4.6	3.0	4.1
24	1.7	4.2	3.0	4.0
25	1.5	3.6	3.0	3.7
26-30	5.8	15.1	16.1	15.6
31-35	4.2	13.5	16.3	13.1
36-40	2.6	8.0	12.6	9.2
Total	91.1	90.4	81.1	84.0
<15	39.67			

^a Produced from chicory by Cosucra (Momale, Belgium).

and Lomb, Rochester, NY). When the influence of pH, temperature and atmosphere were being determined, the carbohydrate was 0.5% (w/v) inulin from dahlia tubers. When the effect of pH was being determined, pH of TPY was adjusted to 6.0-9.0 using HCl prior to sterilization. When the effect of atmosphere was being considered, cultures were incubated in the presence and absence of cysteine hydrochloride (0.05% w/v), either aerobically, anaerobically, or in a CO₂ chamber (Napco Model 6200, Napco Scientific Company, Tualatin, OR) under ambient air+5% (v/v) CO₂.

Inulinase assay. Inulinase activity was determined as described by McKellar and Modler (8) using neosugar (0.55% w/v) as the substrate. Activity was expressed as μ moles fructose or glucose released per minute per milligram protein.

Protein determination. Protein was determined by the method of Lowry et al. as modified by Peterson (14).

RESULTS AND DISCUSSION

Metabolic Studies

A total of 19 species of *Bifidobacterium* (43 strains total) (Table 1) were screened for ability to grow on various carbohydrate sources. Average growth for all strains was significantly ($p < 0.05$) better on glucose and sucrose as compared to the other carbon sources (Table 3). Inulin was the poorest carbon source tested. There were no significant differences between strains with respect to ability to grow on glucose ($p = 0.1508$) or sucrose ($p = 0.2904$); however, some strains grew significantly ($p \leq 0.05$) better on fructose, neosugar and inulin (Table 3).

These results agree with previous studies which showed that inulin was a poor carbon source for *Bifidobacterium* spp. (8). In contrast, Allais et al. (1) found that thermophilic bacilli grew equally well on all carbon sources including inulin.

All strains tested grew on neosugar as evidenced by increases in absorbance over control lacking carbohydrate of 0.256 to 2.13 (Table 4). The differences between strains were slight; however, the best growth was with *B. minimum*, *B. cuniculi* and *B. thermophilum*. The latter genus grew significantly better than all other strains (Table 4). With inulin as the carbohydrate source, 10 strains grew poorer than the control. The same three genera produced the best growth on inulin, with *B. minimum* giving the highest growth yield. Strains which grew best on neosugar also grew well on inulin. Simple linear regression gave an r^2 of .52 for the relationship between these two carbohydrates (Fig. 1).

Several strains which gave the greatest yield on inulin were selected for further study. These included the 4 strains of *B. thermophilum* (CFAR 70, 323, 347, 348), *B. minimum*, and *B. cuniculi*. Table 5 shows the ability of these selected strains to grow on different sources of FOS. There was no significant difference between strains with respect to ability to grow on neosugar or levan (Table 5). With inulin from Jerusalem artichoke, dahlia, and chicory, two of the *B. thermophilum* spp. (CFAR 347 and 348) gave significantly lower growth yields compared to the other strains. When grown on Fibruline, CFAR 70 gave a significantly greater growth response than the other strains (Table 5).

Table 3. Effect of carbohydrate on average growth of *Bifidobacterium* spp. ($n=43$)

Sugar	Average growth ¹ (A_{600})	p -Value ²	Pooled SE ³
Glucose	1.704 ^c	0.1508	0.0489
Fructose	1.208 ^b	0.0076	0.0767
Sucrose	1.767 ^c	0.2904	0.0643
Neosugar	1.258 ^b	0.0000	0.0324
Inulin	0.0937 ^a	0.0059	0.232

¹ Average growth yield for total of 43 strains.

² Level of significance for between-species differences.

³ Pooled standard error for between-species differences.

^{a,b,c} Means not followed by the same letter are significantly ($p \leq 0.05$) different.

Table 4. Growth of various *Bifidobacterium* spp. on neosugar and inulin

Strain	n	Neosugar (A ₆₀₀)	Inulin (A ₆₀₀)
<i>B. boum</i>	1	0.256 ^a	0.024 ^{ab}
<i>B. pseudolongum</i>	1	0.542 ^{ab}	-0.051 ^a
<i>B. globosum</i>	1	0.659 ^{abc}	-0.092 ^a
<i>B. pullorum</i>	1	0.774 ^{abcd}	-0.068 ^a
<i>B. ruminantium</i>	1	0.889 ^{bcde}	-0.106 ^a
<i>B. choerinum</i>	1	0.900 ^{bcde}	-0.214 ^a
<i>B. animalis</i>	2	0.957 ^{bcde}	-0.016 ^a
<i>B. gallinarum</i>	1	1.09 ^{bcdef}	0.071 ^{abcdef}
<i>B. bifidum</i>	8	1.10 ^{cdefg}	-0.032 ^a
<i>B. breve</i>	2	1.13 ^{cdefgh}	-0.070 ^a
<i>B. longum</i>	6	1.18 ^{defghi}	0.002 ^a
<i>B. species</i>	4	1.21 ^{defghij}	0.045 ^{abcde}
<i>B. suis</i>	1	1.22 ^{cdefghij}	-0.051 ^a
<i>B. breve/longum</i>	1	1.28 ^{cdefghijk}	0.029 ^{ab}
<i>B. merycicum</i>	1	1.43 ^{efghijkl}	0.042 ^{abcd}
<i>B. magnum</i>	1	1.47 ^{efghijkl}	-0.051 ^a
<i>B. adolescentis</i>	2	1.54 ^{fghijkl}	0.041 ^{abc}
<i>B. infantis</i>	2	1.57 ^{fghijkl}	0.300 ^{bcdfg}
<i>B. minimum</i>	1	1.85 ^{klm}	0.670 ^h
<i>B. cuniculi</i>	1	2.05 ^{lm}	0.578 ^{gh}
<i>B. thermophilum</i>	4	2.13 ^m	0.390 ^{gh}
Total	43		

^{a-m} Values in the same column not followed by the same letter are significantly different ($p \leq 0.05$).

Values are the means of two trials.

Fig. 1. Relationship between growth on neosugar and inulin for all *Bifidobacterium* spp. ($n=43$). Values are the means of two trials.

Table 5. Growth of selected *Bifidobacterium* spp. on various sources of fructooligosaccharides

Species	Levan	JA	Dahlia	Chicory	Fibruline	Neosugar
				A ₆₀₀		
CFAR 70	0.137 ^a	0.760 ^b	0.843 ^b	0.94 ^b	2.05 ^c	2.22 ^a
CFAR 323	0.137 ^a	0.994 ^b	0.852 ^b	0.872 ^b	1.78 ^b	2.23 ^a
CFAR 347	0.057 ^a	0.279 ^a	0.361 ^a	0.357 ^a	1.36 ^a	2.13 ^a
CFAR 348	0.081 ^a	0.295 ^a	0.377 ^a	0.434 ^a	1.41 ^a	1.94 ^a
<i>B. minimum</i>	0.092 ^a	0.747 ^b	0.843 ^b	0.875 ^b	1.46 ^a	1.11 ^a
<i>B. cuniculi</i>	0.203 ^a	0.827 ^b	0.959 ^b	1.08 ^b	1.92 ^{bc}	2.29 ^a

JA, inulin from Jerusalem artichoke tubers; Dahlia, inulin from dahlia tubers; Chicory, inulin from chicory; Fibruline, fibruline; Neosugar, purified neosugars (P).

^{a,b,c} Values in the same column not followed by the same letter are significantly different ($p \leq 0.05$).

Values are the means of two trials.

Fig. 2. Effect of source of fructooligosaccharide on growth of *Bifidobacterium* species.

JA, Jerusalem artichoke flour; Dahlia, inulin from dahlia tubers; Chic, inulin from chicory; Fib, fibruline; Neo, neosugar. Data from strains CFAR 70, 323, 347, 348, *B. minimum*, and *B. cuniculi* were pooled. Bars represent standard deviation (means of two trials).

Neosugar was the best overall carbohydrate source, giving a significantly greater cell yield for all the strains combined (Fig. 2). Growth on levan was poor, and the source of inulin seemed not to be significant. Fibruline supported almost the same level of growth as neosugar. This is not surprising since 24.4% of the carbohydrate in fibruline has a DP of ≤ 5 (Table 2).

Earlier work demonstrated that animal strains grew best on inulin (8), and this was confirmed by the present study. The relationship between growth on neosugar and inulin suggests that the same enzyme is responsible for metabolism of these two substrates; absence of complete correlation may be related to the observed ability of some strains to grow on neosugar but not inulin, due likely to stearic hindrance (8).

Fig. 3. Effect of fructooligosaccharide on induction of inulinase activity by *Bifidobacterium* species. Control, no fructooligosaccharide; Raff, raffinose; Inulin, inulin from dahlia tubers. Data from strains CFAR 70, 323, 347, 348, *B. minimum*, and *B. cuniculi* were pooled. Bars represent standard deviation (means of two replicates from two trials).

Table 6. Inulinase activity of selected species of *Bifidobacterium* after growth on various carbohydrates

Species	Control	Raffinose	Inulin
	μmol/min/mg protein.....		
CFAR 70	0.211 ^c	0.063 ^c	1.39 ^{bc}
CFAR 323	0.040 ^a	0.016 ^a	0.680 ^a
CFAR 347	0.081 ^{ab}	0.025 ^a	1.02 ^{abc}
CFAR 348	0.047 ^a	0.020 ^a	0.984 ^{ab}
<i>B. minimum</i>	0.062 ^{ab}	0.021 ^a	1.01 ^{ab}
<i>B. cuniculi</i>	0.140 ^{bc}	0.046 ^b	1.50 ^c

^{a,b,c} Values in the same column not followed by the same letter are significantly different ($p \leq 0.05$).

Values are the means of two replicates of two trials.

Levan was not a good substrate for growth of animal strains of bifidobacteria. Muramatsu et al. (12) also showed that bifidobacteria from human origin failed to utilize levan. Fibruline, consisting of a large proportion of short-chain FOS, was the best inulin preparation, confirming previous observations that bifidobacteria preferred short-chain FOS to inulin (8, 12).

The ability of raffinose and inulin to induce inulinase production is shown in Fig. 3. Significant inulinase was only produced with inulin as carbohydrate source. When inulinase production by individual strains was compared, *B. cuniculi* gave higher activity than CFAR 323, CFAR 348 and *B. minimum* (Table 6). CFAR 70 gave greater activities than CFAR 323, and also produced higher levels of inulinase on raffinose and in the absence of carbohydrate (Table 6).

Fig. 4. Effect of pH on induction of inulinase activity (○) and growth (●) of strain CFAR 70. Values are the means of two replicates of two trials.

Table 7. Effect of atmosphere and cysteine HCl addition on growth and inulinase production by strain CFAR 70

Conditions	Cysteine	Activity (μmol/min/mg)	Growth (A ₆₀₀)
O ₂	—	1.07 ^a	0.375 ^a
O ₂	+	1.19 ^a	1.01 ^{b,c}
CO ₂	—	1.22 ^a	0.480 ^a
CO ₂	+	1.49 ^a	1.30 ^c
AnO ₂	—	1.64 ^a	0.798 ^{a,b}
AnO ₂	+	2.40 ^a	1.18 ^{b,c}

O₂, aerobic incubation; CO₂, aerobic incubation with 5% CO₂; AnO₂, anaerobic incubation.

^{a,b,c} Values in the same column not followed by the same letter are significantly different ($p \leq 0.05$).

Values are the means of two replicates of two trials.

With all strains tested, inulin was required for maximum production of inulinase. Raffinose, previously shown to induce inulinase of *Kluyveromyces fragilis* (17), failed to induce significant levels of the enzyme. These results confirm and extend the previous observations (8) that *B. thermophilum* CFAR 70 enzyme was induced by inulin while animal strains (which grew poorly on inulin) were constitutive, suggesting a possible link between inducibility and access of the physiological inducer (inulin) to the active site (8). Allais et al. (1), in a study of inulinase produced by *Flavobacterium* spp., found that maximum levels of inulinase were found with inulin as the carbon source.

All further studies were conducted with CFAR 70. Inulinase production by CFAR 70 was affected by initial pH of the medium (Fig. 4). The activity at pH 8 and 9 was significantly ($p \leq 0.05$) poorer than was found at 8.5. Growth at pH 9.0 was significantly ($p \leq 0.05$) poorer than any of the other conditions. Significantly

Fig. 5. Effect of temperature on induction of inulinase activity (○) and growth (●) of strain CFAR 70. Values are the means of two replicates of two trials.

($p \leq 0.05$) less growth was also found at 6.5 compared to 6.0, 7.5, and 8.0 (Fig. 4).

The effect of atmosphere and cysteine on growth and inulinase production by CFAR 70 was examined (Table 7). Better inulinase production was noted with anaerobic conditions with cysteine; however, this activity was not significantly greater than the other conditions. The best growth was found anaerobically, and addition of cysteine increased growth under the other conditions (Table 7).

Temperature also had a significant effect on growth and inulinase production by CFAR 70 (Fig. 5). Growth was poorest at 32°C; however, activity at this temperature was significantly greater than found at 43°C.

Animal Applications

Marginal reductions in intestinal pH, upon administration of FOS to animal diets suggests the lack of sufficient numbers of bifidobacteria to alter intestinal acidity, through the production of lactic and acetic acid. This situation may have evolved as the result of farming practices (isolation rearing of the young) or feed composition (lack of FOS components). Methods of improving the effectiveness of bifido-containing probiotics may include:

- Early administration of bifidobacteria to the host e.g. at birth;
- Incorporation of FOS into the feed components;
- Selection of animal strains of *Bifidobacterium* which can readily metabolize long-chained FOS components e.g. *B. thermophilum*, *B. minimum* and *B. cuniculi*;

In terms of animal application, it may be advantageous for probiotic preparations to contain both lactobacilli, for attachment to gut epithelium tissue, and bifidobacteria, to provide luminal acidification. Most diets contain sufficient simple carbohydrates to support the growth of lactobacilli in the proximal areas of the digestive tract while complex carbohydrates, such as FOS, resist gastric digestion and can serve as bifidogenic factors in the lower gut.

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