Research Paper Medical Science

Isolation of Bifidobacteria from Infant Faeces

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ABSTRACT

Bifidobacteria have been in the spotlight of scientific research during the past two decades. This attention has to do with the health promoting effects of these organisms in humans. The isolation of Bifidobacterium species from faeces has assumed considerable importance as a consequence of interest in the potential health promoting properties. They play a significant in controlling pH of the large intestine through liberation of lactic acid and acetic acid thereby restricting the growth of many potential pathogens and putrefactive bacteria. This paper discusses the isolation and identification of bifidobacterial species from infant faeces by phenotypic methods. A study was conducted to isolate and identify bifidobacterial species from the faeces of 46 breast fed infants. Identification of isolates to the genus was based on phenotypic characters like the unique pleomorphic morphology and carbohydrate fermentation profile. A total of 4 isolates were confirmed namely Bifidobacterium longum (Isolate code: IB10 and IB12) Bifidobacterium breve (Isolate code: IB39) and Bifidobacterium bifidum (Isolate code: IB42) .

Keywords : Faecal Bifidobacteria, Bifidobacteria isolation

Introduction

Bifidobacterial species are common members of the infant gut where they form up to 91 per cent of the total micro flora in breast-fed babies and up to 75 per cent in formula fed infants (MiloudHadadji *et al*., 2005). Tissier's discovery of *Bifidobacteria* in breast-fed infants played a key role in establishing the concept that specific bacteria take part in maintaining health. There are about 29bifidobacterial species that have been identified and among them eleven species have been isolated from infant faeces. The most frequently isolated *Bifidobacterium* species in infant faeces are *B.bifidum, B.longum, B.infantis* and *B.breve* (Matsuki *et al.,* 2003). These organisms which are gram positive, non motile, non spore forming anaerobic pleomorphic rods play a significant role as probiotics in controlling the pH of the large intestine through production of lactic and acetic acid thereby restricting the growth of many potential pathogens and putrefactive bacteria (Sullivan and Nord, 2002)

Recently the isolation of *Bifidobacterium* species from infant faeces has assumed considerable importance, as a consequence of interest in the potential health promoting ability of this genus (Arunachalam *et al*., 2000 and Suresh, 2000). In the current study, bifidobacterialspecies were isolated and identified from infant faecal sample by conventional methods.

Materials and Methods

Forty six fresh faecal samples from clinically healthy newborn infants of both the sexes born through normal delivery were examined. The infants were solely breast fed and ranged in the age group from 3 to 90 days. About 1g of freshly voided infant faecal samples collected in sterile sample vials containing Yoshioka broth were plated on Yoshioka agar and incubated at 37°C for 48 hours under anaerobic condition using Anaero gas pack (Hi media cat.no. LE002F).Presumptive individual colonies were selected for Gram's staining, and biochemical tests.

Phenotypic Identification:

Phenotypic identification of bifidobacterial species was done by Grams staining and viewed using Nikon Model YS100 Binocular Microscope. ANAERO23 Test kit of LA CHE MA MIKRO-LA-TEST (Ref: 10003366 PLIVA LACHEMA Diagnostics, Czechoslovakia) was used for the biochemical characterization of bifidobacterials pecies

Electron microscopy:

Electron Microscopic studies were done to establish the morphological features of various strains. Phosphotungstic acid 1 per cent was used for staining bifidobacterial isolates. Carbon coated copper grids were used in Tecnai 10 Philips model of electron microscope for morphological identification of the isolated Bifidobacterium .

.RESULTS

The study of identification of Bifidobacterium in infant faeces by conventional methods is shown in Table 1. Out of forty six infant faecal samples collected thirty five isolates showed growth in anaerobic conditions on Yoshioka agar. Eighteen out of the thirty five samples when inoculated in selective media developed Gram positive colonies**.** Out of the eighteen Gram positive isolates, six showed biochemical characteristics typical of bifidobacterial species using Anaero – 23 test kit

Gram positive bifid rods on Gram's staining are shown in Plate 1. The Plate reveals pleomorphic cell morphology (as rods of various shapes characteristically club shaped in single form or chain or 'v' groupings).

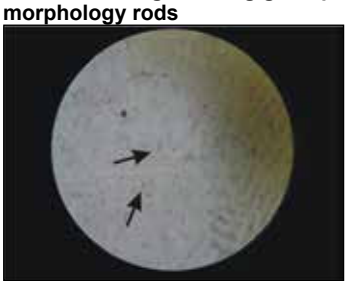
TABLE – 1
Identification of bifidobacterial species from infant faeces

Sl. No.	Infant identity	Age group in days	Anaerobic growth	Gram staining	Biochemical reaction	Species
1.	IB_1	3 days	Yes	Positive	+	B.breve
2.	IB_2	11 days	Yes	Positive	NCR	
3.	IB_3	88 days	No	-	-	-
4.	IB_4	90 days	No	-	-	-
5.	IB_5	60 days	Yes	Negative	-	-
6.	IB_6	48 days	Yes	Positive	NCR	-
7.	IB_7	81days	Yes	Negative	-	-

8.	IB_{8}	88days	Yes	Negative	-	-
9.	IB_{9}	79days	Yes	Negative	-	-
10.	IB_{10}	20 days	Yes	Positive	+	B.longum
11.	IB_{11}	88days	Yes	Negative	-	-
12.	IB_{12}	18 days	Yes	Positive	+	B.longum
13.	IB_{13}	68days	Yes	Negative	-	-
14.	IB_{14}	44days	Yes	Positive	NCR	-
15.	IB_{15}	47days	Yes	Positive	NCR	-
16.	IB_{16}	39days	Yes	Positive	NCR	-
17.	IB_{17}	63days	Yes	Negative	-	-
18.	IB_{18}	35days	Yes	Positive	+	B. bifidum
19.	IB_{19}	29days	Yes	Positive	NCR	-
20.	IB_{20}	33days	Yes	Negative	-	-
21.	IB_{21}	65days	Yes	Negative	-	-
22.	IB_{22}	48days	Yes	Positive	NCR	-
23.	IB_{23}	90days	Yes	Negative	-	-
24.	IB_{24}	88days	Yes	Negative	-	
25.	IB_{25}	68days	No	-	-	-
26.	IB_{26}	90days	Yes	Negative	-	-
27.	IB_{27}	31days	No	Positive	NCR	-
28.	IB_{28}	33days	Yes	Negative	-	-
29.	IB_{29}	40days	Yes	Positive	NCR	-
30.	IB_{30}	48days	No	-	-	-
31.	IB_{31}	90days	No	-	-	-
32.	IB_{32}	26days	Yes	Positive	NCR	-
33.	IB_{33}	90days	Yes	Negative	-	-
34.	IB_{34}	38days	Yes	Positive	NCR	-
35.	IB_{35}	88days	No	-	-	-
36.	IB_{36}	73days	No	-	-	-
37.	IB_{37}	41days	Yes	Positive	NCR	-
38.	IB_{38}	67days	No	-	-	-
39.	IB_{39}	23 days	Yes	Positive	+	B.breve
40.	IB_{40}	21days	No	-	-	-
41.	IB_{41}	44days	No	-	-	-
42.	IB_{42}	14 days	Yes	Positive	+	B. bifidum
43.	IB_{43}	58days	Yes	Negative	-	--
44.	IB_{44}	60days	Yes	Negative	-	-
45.	IB_{45}	65days	Yes	Negative	-	--
46.	IB_{46}	90days	Yes	Negative	-	-

Positive : Gram positive reaction
Negative : Gram negative reaction
NCR : Non Characteristic Reaction

Plate - 1
Phenotypic Characterisation
Gram's staining showing gram positive pleomorphic cell morphology rods



The ability of microorganisms to metabolise different types of carbohydrates has been used for identification purposes and Table 2 illustrates the fermentative characteristics distinguishing the bifidobacterial species using Anaero 23 – test kit (Plate 2).

Table – 2
Biochemical characterization for *Bifidobacterium*using ANAERO – 23 KIT

Sl. No.	Isolate Code	Species	H	G	F	E	D	C	B	A	H	G	F	E	D	C	B	G	H	G	F	E	D	C	B
			INDOLE	GLUCOSE	MALTOSE	FRUCTOSE	GALACTOSE	LACTOSE	MELIZITOSE	UREASE	NITRATE	SUCROSE	SALICIN	TREHALOSE	MANNITOL	RHAMNOSE	NAG	BGL	ESCULIN	MANNOSE	RAFFINOSE	CELLIBIOSE	XYLOSE	ARABINOSE	SORBITOL
1	IB_{1}	B.breve	-	+	+	+	+	+	-	-	-	+	+	d	d	-	d	+	+	d	+	d	d	-	d
2	IB_{39}	. B.breve	-	+	+	+	+	+	-	-	-	+	+	d	d	-	d	+	+	d	+	d	d	-	d
3	IB_{18}	B.bifidum	-	+	-	+	+	+	-	-	-	d	-	-	-	d	d	+	d	-	-	-	-	-	-
4	IB_{42}	B.bifidum	-	+	-	+	+	+	-	-	-	d	-	-	-	d	d	+	d	-	-	-	-	-	-
5	IB_{10}	B.longum	-	+	+	+	+	+	+	-	-	+	-	d	-	-	d	+	d	d	+	-	d	+	-
6	IB_{12}	B.longum	-	+	+	+	+	+	+	-	-	+	-	d	-	-	d	+	d	d	+	-	d	+	-

Plate - 2
Bifidobacterial species identified by fermentative characteristics using Anaero – 23 kit

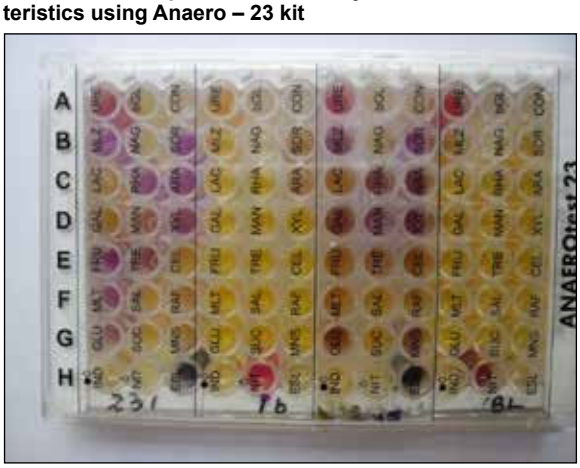
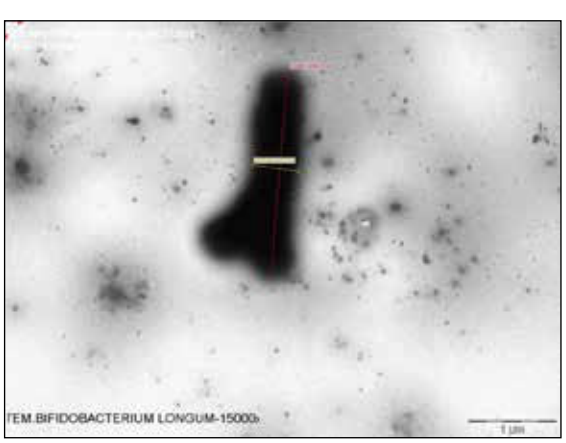


PLATE – 3
Transmission electron microscopic photograph showing typical bifid nature of *Bifidobacteria*



The results obtained were compared with those in standard tests for identification (Scardovi, 1986) and the isolates were assigned to appropriate species**.** The fermentative characters of IB_1, IB_{39}, IB_{18}, IB_{42}, IB_{10}, IB_{12}, corresponded to the fermentative patterns of B.breve, B.breve ;B. bifidum ,B. bifidum; and B.longum, B.longum respectively. Thus six isolates of bifidobacterial species were identified by their ability to ferment carbohydrates.

Discussion

Identification of bifidobacterial species in infant faeces

The incidence and isolation of Bifidobacteria from breast fed infant faeces in the present study corroborates with Roberts et al. (1985), Tamime et al. (1995), Silvi et al. (1996) and Martin et al. (2009) who reported that human milk is favourable for the growth and sustenance of Bifidobacteria in the large intestine of infants. The opaque, white and concave colonies observed in the present study after anaerobic incubation was similar to the findings of Dubey and Mistry (1996). Wasilewska and Bielecka (2003) also isolated and identified fourteen bifidobacterial strains from faeces harbouring the gut of 3-month old breast fed infant. Vlkova et al. (2005) reported the presence of Bifidobacteria from twenty nine infant faeces out of the ninety five faecal samples collected. Hence the breast fed infant faecal sample seemed to be an ideal source of Bifidobacteria.

The results and illustrations in Plate 2 showed Gram positive pleomorphic patterns. The cell morphology in the present study depicts various cell morphology patterns and is concurrent with the findings of Scardovi (1986) who described the morphology of Bifidobacteria as club shaped or with spatulated extremities, star like arrangement or disposed in “V” or palisade arrangement. Gerald (1999) and Matsuki et al. (2003) also affirmed the pleomorphic gram positive nature of Bifidobacteria uniform to branched, bifurcated ‘Y’ and ‘V’ forms, spatulate or club shape as observed in the present study. Microscopic examination also revealed non bifid Gram negative bacilli and bacteriodes which were not considered for further studies. Comparison of cell morphology of bifidobacterial isolates grown on Yoshioka medium was supplemented with the results of biochemical test to differentiate the species by Anaero Test Kit. The fermentative characters of the isolates IB_{10} and IB_{12} categorize these isolates under the species of B. longum. Isolates IB_{10} and IB_{12}fermented glucose, maltose, fructose, galactose, lactose, melizitose, sucrose, raffinose and arabinose and was negative for nitrate reductase. They showed a negative reaction with indole, salicin, mannitol, rhamnose, cellobiose and sorbitol. This corresponds to the findings of Otto Kandler and Norbert Weiss (1986) who w

Isolates of B.breve (IB_1 and IB_{39}) in this study fermented glucose, maltose, fructose, galactose, lactose, sucrose, salicin, esculin and raffinose. But they did not react with melezitose, nitrate, rhamnose and arabinose which corresponds to the findings of Otto Kandler and Norbert Weiss (1986).

B.bifidum isolates (IB_{18} and IB_{42}) fermented lactose, fructose, galactose but showed inability to ferment arabinose, cellobiose, maltose, mannose, mannitol, melezitose, raffinose, salicin, sorbitol, trehalose, and xylose. This corresponds to the findings of Otto Kandler and Norbert Weiss (1986) who mentioned that B.bifidum fermented lactose, fructose and galactose and could not ferment maltose, trehalose, mannitol, mannose, salicin, raffinose and xylose.

From the present study it is evident that B.longum can utilise a wide range of carbohydrates followed by B.breve and B.bifidum.Mitsuoka (1984) reported that any strain belonging to the Bifidobacterium genus must be nitrate reductase negative, does not form indol, does not have urease activity and does not liquefy gelatin. Selected isolates of Bifidobacteria in this study correlated with these conditions. The Gram staining observation of Bifidobacteria paralleled biochemical findings thus affirming the findings of Resnick and Levin (1981). Plate 4 showed the typical bifid nature of the bifidobacterial isolates as viewed through Transmission Electron microscopy and is in consonance with the observation of Jaya Prasad et al. (1999)who also confirmed the morphological feature of Bifidobacteria using a scanning microscope.

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