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Isolation of *Bifidobacterium* from Infant's Feces and Its Antimicrobial Activity

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Abstract

Bifidobacterium is a group of Lactic Acid Bacteria (LAB) that commonly found in the gastrointestinal tract and vagina. LAB has many health benefits, such as produce an antimicrobial substance against a pathogen. This research aims to isolate *Bifidobacterium* from an infant's feces and know its antimicrobial activity against *Escherichia coli* and *Candida albicans.* A total of 5 isolates *Bifidobacterium* spp. were isolated from the sample. The largest inhibitory activity against *E. coli* was shown by isolate Bb3F, with the inhibitory zone of 10.80 mm. While the largest inhibition activity against *C. albicans* was shown by isolate Bb1B and Bb3F with the inhibitory zone of 9.70 mm.

Keywords

antimicrobial substances, infant's feces, *bifidobacterium*, *candida albicans, escherichia coli*

1 Introduction

Bifidobacterium is a Gram-positive bacterium, anaerobic, hetero-fermentative, non-spores-forming, non-motile and included in Lactic Acid Bacteria [1]. Many *Bifidobacterium* is also recognized as probiotics where probiotics are living bacteria that give some health benefits to the host when administrated in a sufficient amount and have wider applications in food, feed, dairy and fermentation industry as non-pharmacological approaches for health management [2,3].

Bifidobacterium commonly found in the human digestive tract and vagina [4]. These bacteria can also be found in the digestive tract of newborns [5]. Bifidobacterium is the dominant bacterium that lives in the digestive organs of newborns and the number decreases with age. Its colonization in infancy when the baby gets this bacterium from the mother's fecal and vaginal microbiota [6].

Bifidobacterium can help the intestine colonization. The competition of microorganism attachment to intestinal epithelial cells is affected by exopolysaccharide production. Exopolysaccharide is a candy polymer that is fully attached to the cell surface [7]. *Bifidobacterium* produces some antimicrobial substances, such as hydrogen peroxide, organic acids, and bacteriocin. Bacteriocin is able to inhibit bacterial growth from the same species (narrow spectrum) or different genera (broad-spectrum), e.g. *Listeria monocytogenes*, *Escherichia coli* and *Salmonella* sp. [8].

2 Methods

2.1 Bacterial Isolation

The samples were collected from 2 areas at Banyumas i.e. Sokaraja and Purbalingga sub-district. Samples were collected from less than 1 month-aged babies by using a sterile jar. They were diluted until 10^{-5} dilutions. The last two dilutions were surface plate cultured on de Mann Rogosa Sharpe (MRS) agar (Merck) and incubated at 37^{0}C for 4 days under anaerobic condition. The representative morphological types of *Bifidobacterium*-like colonies (milky white or near creamy colonies, in the form of round colonies, wool edges with a diameter of 0.1-0.5 mm) were isolated. Selected colonies were then cultured on MRS agar in order to obtain pure cultures.

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2.2 Bacterial Characterization and Identification

Further diagnostic and evaluation tests including Gram-staining for microscopic and morphologic inspection, oxidase test, catalase activity, motility test, indole production, carbohydrates fermentation patterns were performed to confirm the presence of the genus *Bifidobacterium* in the samples. Isolate identification at the species level was conducted through biochemical testing. Carbohydrate fermentation profiles including fructose, glucose, arabinose, maltose, mannose, galactose, lactose, raffinose, ribulose, and sucrose were compared against a standard table.

2.3 Antimicrobial Activity against *Escherichia coli* and *Candida albicans*

A total of 3 mL of *E. coli* and *C. albicans* were inoculated into 100 mL Nutrient Broth medium, then incubated in a shaker incubator at 150 rpm for 8 hours. *Bifidobacterium* was inoculated on MRS broth and incubated for 18 hours at 37°C. Then, centrifuged for 10 minutes at 13.000 rpm to obtain *Bifidobacterium* cell-free supernatant. The antimicrobial activity of cell-free supernatant against *E. coli* and *C. albicans* was conducted by using Kirby's Bauer assay.

3 Discussions

3.1 Characterization of *Bifidobacterium*-like colonies

Seventeen isolates that appeared as round and white colonies on MRS agar were considered to be *Bifidobacterium*. The morphology of the bacterial colonies has the following characters, pinpoint, small and medium-size, round in shape, cream-white, shiny surface, raised elevation, and flat edge (**Table 1**). *Bifidobacterium* has morphological characteristics of milky white or near creamy colonies, in the form of round colonies, wool edges with a diameter of 0.1-0.5 mm [9].

Bifidobacterium is Gram-positive bacteria with rods or branches shape and non-motile [10,11]. Characterization data showed that *Bifidobacterium*-like isolates were Gram-positive, rods in shape, and non-motile. Biochemical test also showed that isolated bacteria were Indole negative, Methyl Red positive, Voges Proskauer negative and Simmon's Citrate positive (**Table 2**).

Bifidobacterium has several key characters that can be used as references in determining the genus or species, such as Gram-positive, V-shaped or palisade-shape cells, non-spore-forming, non-motile, anaerobic, positive to most of the carbon sources test i.e. monosaccharide, disaccharide, and polysaccharide [12]. The biochemical test showed that *Bifidobacterium*-like isolates were identified as *B. catenulatum* (6 isolates), *B. minimum* (4 isolates), *B. indicum* (5 isolates), *B. asteroids* (1 isolate), and *B. coerinum* (1 isolate). While, Mariat et al. [13] found that most commonly *Bifidobacterium* species found in infant's feces are *B. longum, B. bifidum, B. breve,* and *B. infantis.* Identification by Duranti et al. [14] shows the populations of these bacteria is not only found in infants, such as *B. bifidum, B. breve,* and *B. pseudocatenulatum,* but also in adult humans, like *B. catenulatum* and *B. adolescentis.*

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Table 1 Characterization of Bifidobacterium-like Colonie

Isolates	**Morphological characters**						**Cell character**	
	Size	**Shape**	**Color**	**Surface**	**Elevation**	**Edge**	**Motility**	**Gram**
Bb1A	Pin point	Round	Milky-white	Shiny	Raised	Flat	-	+
Bb1B	Pin point	Round	Milky-white	Shiny	Raised	Flat	-	+
Bb1C	Pin point	Round	Milky-white	Shiny	Raised	Flat	-	+
Bb1D	Medium	Round	Milky-white	Shiny	Raised	Flat	-	+
Bb1E	Medium	Round	Milky-white	Shiny	Raised	Flat	-	+
Bb1F	Medium	Round	Milky-white	Shiny	Raised	Flat	-	+
Bb1G	Pin point	Round	Milky-white	Shiny	Raised	Flat	-	+
Bb1H	Medium	Round	Milky-white	Shiny	Raised	Flat	-	+
Bb1I	Medium	Round	Milky-white	Shiny	Raised	Flat	-	+
Bb3A	Medium	Round	White	Shiny	Raised	Flat	-	+
Bb3B	Medium	Round	White	Shiny	Raised	Flat	-	+
Bb3C	Small	Round	White	Shiny	Raised	Flat	-	+
Bb3D	Small	Round	White	Shiny	Raised	Flat	-	+
Bb3E	Medium	Round	White	Shiny	Raised	Flat	-	+
Bb3F	Small	Round	White	Shiny	Raised	Flat	-	+
Bb3G	Medium	Round	White	Shiny	Raised	Flat	-	+
Bb3H	Small	Round	White	Shiny	Raised	Flat	-	+

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Table 2 Biochemical Test of Bifidobacterium-like Isolates

Isolates	Ara	Fru	Gal	Glu	Gli	Mal	Man	Lac	Raf	Rib	Suc	Ind	MR	VP	SC	Species
Bb1A	+	+	+	+	+	+	+	+	+	+	+	-	+	-	+	*B. catenulatum*
Bb1B	-	+	+	+	-	+	-	+	+	+	+	-	+	-	+	*B. indicum*
Bb1C	+	+	+	+	+	+	+	+	+	+	+	-	+	-	+	*B. catenulatum*
Bb1D	+	+	+	+	+	+	+	+	+	+	+	-	+	-	+	*B. catenulatum*
Bb1E	-	+	-	+	-	+	-	-	-	+	-	-	+	-	+	*B. indicum*
Bb1F	+	+	+	+	+	+	+	+	+	+	+	-	+	-	+	*B. catenulatum*
Bb1G	-	-	-	+	-	+	-	-	-	-	-	-	+	-	+	*B. minimum*
Bb1H	-	+	-	+	-	+	-	-	-	-	-	-	+	-	+	*B. minimum*
Bb1I	+	+	+	+	+	+	+	+	+	+	+	-	+	-	+	*B. catenulatum*
Bb3A	-	+	-	+	-	-	-	-	+	+	-	-	+	-	+	*B. indicum*
Bb3B	-	+	-	+	-	+	-	-	-	-	-	-	+	-	+	*B. minimum*
Bb3C	-	+	-	+	-	+	+	-	-	-	-	-	+	-	+	*B. minimum*
Bb3D	-	-	-	+	-	-	-	-	-	-	-	-	+	-	+	*B. asteroides*
Bb3E	-	+	-	+	-	+	-	+	-	-	-	-	+	-	+	*B. indicum*
Bb3F	-	+	-	+	-	+	-	-	-	-	-	-	+	-	+	*B. indicum*
Bb3G	+	+	+	+	+	+	+	+	+	+	+	-	+	-	+	*B. catenulatum*
Bb3H	-	+	-	-	-	+	-	-	-	-	-	-	+	-	+	*B. coerinum*

Note: Ara: Arabinose; Fru: Fructose; Gal: Galactose; Glu: Glucose; Gli: Gliculose; Mal: Maltose; Man: Mannose; Lac: Lactose; Raf: Raffinose; Rib: Ribulose; Suc: Sucrose; Ind: Indole; MR: Methyl Red; VP: Voges Proskauer, SC: Simmon's Citrate.

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3.2 Antimicrobial Activity against *Escherichia coli* and *Candida albicans*

Bifidobacterium showed different inhibition against *E. coli* and *C. albicans* (**Table 3**), expressed by the zone of inhibition. The largest inhibitory activity against *E. coli* was shown by isolate Bb3F, with the inhibitory zone of 10.80 mm, while the smallest inhibition was shown by isolate Bb1G i.e. 6.0 mm. The largest inhibition activity against *C. albicans* was shown by isolate Bb1B and Bb3F with the inhibitory zone of 9.70 mm, while the smallest inhibition was shown by isolate Bb3H i.e. 7.3 mm. *Bifidobacterium* from breast-milk can protect the infant from several infections and diseases [14]. *Bifidobacterium* can inhibit the growth of pathogens, such as *E. coli, Salmonella* sp., *Candida* sp., *Listeria monocytogenes* and others [15,8,16].

Table 3 Antimicrobial Activity of Isolated Bifidobacterium against E. coli and C. albicans

Isolates	**Against *E. coli***				**Against *C. albicans***			
	The diameter of the inhibitory zone (mm)				The diameter of the inhibitory zone (mm)			
	U1	**U2**	**U2**	**Average**	**U1**	**U2**	**U2**	**Average**
Bb1A	6	7.5	7.5	7	11	9	7	9
Bb1B	9.5	9	6	8.2	8	11	10	9.7
Bb1C	6	6.5	6.5	6.3	8	9	8	8.3
Bb1D	6	7.5	6	6.5	7	8	8	7.7
Bb1E	6.5	6.5	6	6.3	8	9	8	8.3
Bb1F	7	7	7	7	8	9	10	9
Bb1G	6	6	6	6	9	8	8	8.3
Bb1H	11	10.5	10.5	10.7	10	9	9	9.3
Bb1I	10	9	8.5	9.2	9	10	9	9.3
Bb3A	9.5	10.5	11.5	10.5	9	9	8	8.7
Bb3B	10	10	10	10	7	8	8	7.7
Bb3C	10	10.5	9	9.8	9	9	9	9
Bb3D	8.5	9	12	9.8	8	8	8	8
Bb3E	10	9.5	9	9.5	9	9	8	8.7
Bb3F	10	11	11.5	10.8	10	10	9	9.7
Bb3G	9.5	10.5	10	10	8	7	9	8
Bb3H	8	7	8	7.7	7	7	8	7.3

Fig. 1 Antimicrobial activity of isolate Bb3E against E. coli (left) and isolate Bb1B against C. albicans (right)

Some isolates produce an inhibitory zone with the false-clear and clear zone (**Fig. 1**). The false-clear zone shows that the active metabolites contained in the supernatant can inhibit the pathogen's growth but do not kill the pathogen. Clear inhibitory zone indicates that bacterial metabolites have a bactericidal effect that can kill pathogenic cells [17].

Bifidobacterium from infant feces showed inhibition against *E.coli* [18]. The antagonistic activity of several species of Bifidobacteria against Gram-positive and Gram-negative pathogens was related to the ability of the tested strain to excrete broad-spectrum antimicrobial substances [19]. Lactic Acid Bacteria exert a biostatic effect on the growth of yeasts including *C. albicans* [20]. Lactic Acid Bacteria may compete for receptors on the surfaces of digestive tract cells and nutrients with the pathogenic microorganisms, the production of biosurfactants that interfere with microbial desorption dan adhesion, the production of hydrogen peroxide (H_2O_2) and bacteriocins, the release of exometabolites such as capric, acetic, and lactic acid are the possible mechanisms for probiotic activity [21].

4 Conclusions

Seventeen isolates of Bifidobacterium spp. were isolated from the infant's feces. The largest inhibitory activity against *E. coli* was shown by isolate Bb3F, with the inhibitory zone of 10.80 mm. While the largest inhibition activity against *C. albicans* was shown by isolate Bb1B and Bb3F with the inhibitory zone of 9.70 mm.

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