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Bifidobacterium from infant stool: the diversity and potential screening

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Abstract. *Kusharyati DF, Pramono H, Ryandini D, Manshur TA, Dewi MA, Khatimah K, Rovik A. 2020. Bifidobacterium from infant stool: the diversity and potential screening. Biodiversitas 21: 2506-2513.* Bifidobacteria spp. are a group of Lactic Acid Bacteria commonly found in the gastrointestinal tract of adults and infants. LAB are known as probiotics and have many health benefits. This research aimed to isolate Bifidobacteria from infant stool, identify, explore their diversity, and screen their potential as probiotics. Stool samples were collected from 3 healthy infants in Banyumas Regency. The potential screening included lysozyme resistance, antimicrobial activity, and exopolysaccharide production. A total of 7 Bifidobacterium species were isolated from infant stool: *B. catenulatum, B. minimum, B. indicum, B. dentium, B. asteroides, B. galicum,* and *B. coerinum. B. indicum* isolates (Bb3F and Bb1B) had the greatest inhibition activity against *Escherichia coli* and *Candida albicans* with 10.80 and 9.70 mm, respectively. Bifidobacteria isolates were resistant to lysozyme from egg whites up to 200 µg.mL^{-1}. *B. catenulatum* Bb1A isolate had the highest yield of exopolysaccharide production with 74 mg.L^{-1}. Among them, three Bifidobacterium strains (Bb1B, Bb2A, and Bb2E) were considered potentially as probiotics.

Keywords: Bifidobacterium, diversity, exopolysaccharide, infant stool, microbes

INTRODUCTION

The human digestive tract contains many niches of microbial ecosystems. Microbes can grow and colonize certain parts of the human digestive system. These microbial groups are estimated to reach as many as 400-500 species which vary according to the location of the intestinal tract (Ramakrishna, 2013). Lactic acid bacteria (LAB) and Bifidobacteria naturally form dynamic ecosystems in the human digestive tract. The main LAB found in the human intestines consists of Lactobacillus and Leuconostoc. Meanwhile, Bifidobacterium is dominant among the first invaders of newborns, a month after birth (Sirilun et al., 2015).

Bifidobacterium is commonly found in the vagina and the human digestive tract (Attri et al., 2018) as well as newborn digestive tracts (Penders et al., 2006). Bacterial colonization in the digestive tract begins during birth due to the infant contact to the vaginal tract and fecal microbiota (Palmer et al., 2007). LAB and Bifidobacteria are acquired from the environment of which ingested food is presumably the main source. The group of bacteria that dominates the intestines in adult individuals is LAB, including Lactobacillus, Bifidobacterium, and Bacteroides (Attri et al., 2018).

Bifidobacterium is a LAB that can be potentially used as a probiotic. Probiotics are a group of living bacteria that provide beneficial health effects when consumed in sufficient quantities (Bermudez-Brito et al., 2012). Bifidobacterium may help to establish the mucosal barrier, produce beneficial metabolites, develop the immune system, and prevent the pathogenic colonization (Liu et al., 2016; Yan et al., 2017). Bifidobacterium produces bacteriocin that expresses an inhibition to some bacteria from different genera, such as *Arcobacter butzleri, Brochothrix thermosphacta, C. difficile, H. pylori, L. monocytogenes, S. aureus,* and *S. typhimurium* (Martinz et al., 2013).

Information about human microbes can be obtained by analyzing the microbial content in stool samples. Subijanto and Reza (2006) stated that the titer of Bifidobacterium and Enterococcus from breast-milk-feed infants are 10^{10}-10^{11} CFU.g^{-1} and 10^{8} CFU.g^{-1}, respectively, as well as other anaerobic bacteria. Palmer et al. (2007) stated that the infant digestive tract contains a diverse and dynamic pattern of microbial composition. Therefore, infant stool is an appropriate source to explore the diversity of Bifidobacteria. The exploration of Bifidobacterium species from feces samples was considered important. Recently, research has focused on identifying new strains of Bifidobacterium and their safety confirmation (Hadadji et al., 2005; Hendrati et al., 2017; Rada et al., 2010; Zidene & Faid, 2007). This research aimed to isolate Bifidobacteria from infant stool, identify, explore the diversity, and screen their potential use as probiotics.

MATERIALS AND METHODS

Isolation

The stool samples were collected from 3 healthy infants (less than one month-aged) in Banyumas Regency: Banyumas, Sokaraja, and Purbalingga District. Stool samples were collected in a sterile container and directly transported to the laboratory, then stored at -20ºC. Upon experiment, a gram of stool samples was diluted up to 10^{-5} using sterile-distilled water. The last two dilutions were surface-plate cultured on deMann Rogosa and Sharpe Agar (MRSA) medium (Oxoid) and incubated at 37ºC for 4 days under anaerobic condition. Colonies that appeared as smooth, white, and round colonies were considered as Bifidobacteria. A total of 22 colonies from the highest dilution of 3 different samples were picked up, randomly. Selected colonies were then re-cultured on the MRSA medium to obtain pure cultures.

Colony characterization

The colony morphology of purified culture on the MRSA medium was studied after 24 hours of incubation, such as their size, elevation, shape, and color.

Cellular characterization

Gram's staining. Gram's staining can differentiate the bacteria by the physical and chemical properties of the cell wall. A bacterial smear was colored by adding Crystal violet dye. After 1 min, it was washed and air-dried, Next, Lugol's Iodine was added for 1 min, then washed and air-dried, followed by the 96% Ethanol addition for a few seconds. As the second dye solution, Safranin was added for 1 min, then washed and air-dried. The Gram-positive bacteria were characterized as violet cells under the microscopy observation.

Motility. The bacterial colony was grown by stab inoculation on Sulfide Indole Motility Agar for 2x24 hours at 37ºC. The growth of motile bacteria was indicated with pellicle formation.

Biochemical tests

Catalase test. A catalase-positive bacteria produced oxygen bubbles after the addition of 2 drops H_2O_2 reagent on the bacterial smear.

Oxidase test. A single colony was smeared (then was covered with a paper) on a glass slide. Next, 3 drops of reagent (tetramethyl-D-phenylenediamine dihydrochloride) were added on the bacterial smear. The color changes into maroon blue were indicated a positive result.

Indole test. The bacterial colony was cultured in Tryptone Broth medium at 37ºC for 2x24 hours. The formation of red-colored compound on the medium after the addition of Kovac's reagent was indicated a positive result.

Methyl Red-Voges Proskauer test. The Bifidobacteria isolates were cultured in Methyl Red & Voges Proskauer medium at 37ºC for 48 hours. For Methyl Red: A total of 5 drops of Methyl-Red reagent were added. For Voges Proskauer: A total of 5 drops of 40% KOH and next α-naphthol reagents were added. After 5 minutes, a pink-color development was indicated a positive result.

The pH ranges test

The bacterial colony was cultured in the MRSB medium (pH 4, 7, 9). A positive result was indicated by the medium turbidity after 2x24 hours of incubation at 37ºC, anaerobically.

Carbon source test

The bacterial colony was cultured in mineral medium containing some sugar types: arabinose, fructose, galactose, glucose, glyculose, maltose, mannose, lactose, raffinose, ribulose, and sucrose, then incubated at 37ºC for 2x24 hours, anaerobically. A positive result was indicated by the turbidity of the growth medium.

Resistance test against lysozyme

The MRSA medium was supplemented with egg whites as a source of lysozyme (200 µg.mL^{-1}). One milliliter of Bifidobacteria isolate was surface-plate grown on MRSA medium containing lysozyme, then incubated at 37ºC for 2x24 hours, anaerobically. The growing colonies expressed their resistance to lysozyme (200 µg.mL^{-1}).

Antimicrobial activity against pathogenic microorganisms

A 3 mL of *E. coli* and *C. albicans* culture were added into 100 mL of Nutrient Broth (Merck) medium, then was incubated with shaking (incubator S1-600) at 150 rpm for 8 hours. Bifidobacteria isolate was grown on MRSB medium and incubated at 37ºC for 18 hours anaerobically, then, were centrifuged (Thermo Scientific) for 10 min at 13,000 rpm. The inhibition activity of supernatant was further tested against *E. coli* and *C. albicans*, referred to as the previous study by Hendrati et al. (2017).

Exopolysaccharide production

A 0.75 mL of Bifidobacteria isolate was inoculated in 25 mL of MRSB medium, then was incubated at 37ºC for 2x24 hours, anaerobically. Bacterial cell breakdown was done by cold centrifugation. A 10 mL of bacterial culture was transferred into micro-centrifuge tubes, then was centrifuged for 10 min at 10,000 rpm, 4ºC. A 96% ethanol was added (200%, v/v) to the supernatant, then was stored overnight at 4ºC. Next, the supernatant was centrifuged for 25 min at 4,000 rpm, 4ºC. The pellets were dried at 100ºC. The dry weight of the pellets was weighed as the exopolysaccharide weight in mg.L^{-1}.

RESULTS AND DISCUSSION

Isolation, Characterization, and Identification

Twenty-two isolates were randomly picked up from the highest dilution-plates (six different plates) that appeared as white and round colonies on MRSA medium were considered as Bifidobacteria (Figure 1). The morphology of the bacterial colonies included the following characteristics: pinpoints, small and medium-size, round-shaped, milky-white, shiny surface, raised elevation, and

flat edges (Table 1). Bifidobacterial colonies have morphological characteristics of milky white or near creamy colonies, in the form of round colonies, and wool edges with a diameter of 0.1-0.5 mm (Hadadji et al. 2005).

The Bifidobacterium-type isolates were further analyzed for their characterization and identification. Conventional identification and classification of Bifidobacterium species have been based on phenotypic and biochemical characteristics, such as cell morphology and sugar fermentation patterns. Manual characterization referred to Bergey's Determinative Bacteriology and Cowan & Steel's Manual for the Identification of Medical Bacteria. Bifidobacteria have several key characteristics that can be used as references in determining the genus or species, such as Gram-positive, palisade shaped cell, non-motile, and catalase-negative (Garrity et al., 2005; Okamoto et al., 2008; Zinedine & Faid, 2007). All Bifidobacterium-type isolates were confirmed as Gram-positive, rod-shaped, and non-motile (Table 1). They did not undergo decolorization during the Gram's staining process; therefore, they stay colored crystal violet. They did not perform a spreading pellicle formation; therefore, it was categorized as non-motile bacteria. These characterizations of Bifidobacterium-type isolates were also described by Hendrati et al. (2017).

The previous study of Hadadji et al. (2005) found that Bifidobacteria are categorized as catalase-negative. The catalase enzymes produced by the bacteria mediate the breakdown of H_2O_2 into O_2 bubbles and water. The tested Bifidobacteria showed a positive result on the oxidase test, expressed by the maroon blue color formation. This positive result was also found by Lindawati and Suardana (2016). The Bifidobacteria did not form red-colored compounds on the surface of the Trypthon Broth medium. The color of the MR-VP broth medium was changed into pink or dark red. Then, it confirmed that the Bifidobacteria showed a positive result in Methyl Red, while negative in Indole and Voges-Proskauer tests. Since, Bifidobacteria do not grow in fermented mixed acid or butanediol fermentation (Venkatesn et al., 2012; Zinedine & Faid, 2007). The Bifidobacteria were able to grow better at pH 7 and 4 than pH 9 medium (data not shown), which showed their tolerance to acidic conditions. Lindawati and Suardana (2016) found that Bifidobacteria are acid-tolerant bacteria.

The patterns of sugar-type fermentation provide a guideline for microbiological identification of Bifidobacteria which have been demonstrated to be strain-specific, rather than species-specific (Roy & Ward, 1990). Limitations in using certain types of sugars can be used as a key to identify specific bacterial types. The sugar conversion is influenced by the enzymatic activity in bacterial cells. Not all bacteria can use all types of sugars as carbon sources, due to limited enzyme activities (Lawson et al., 2020; Okamoto et al., 2008). Bifidobacteria are capable of utilizing glucose, galactose, lactose, maltose, fructose, and some other sugar type as carbon sources (Munoz-Quezada et al., 2013; Okamoto et al., 2008). Bifidobacteria are positive to most of the carbon sources test: arabinose, fructose, galactose, glucose, glyculose, maltose, mannose, lactose, raffinose, ribulose, and sucrose (Table 2, Figure 2). The patterns of sugar-type fermentation identified the isolates as *B. catenulatum* (27.27%), *B. minimum* (27.27%), *B. indicum* (27.27%), *B. dentium* (4.54%), *B. asteroides* (4.54%), *B. galicum* (4.54%), and *B. coerinum* (4.54%) (Table 2).

Table 1. Characterization of Bifidobacterium-type Isolates

Isolates	Colony characters							Other characters		
	Size	Shape	Color	Surface	Elevation	Edge	Motility	Gram	Catalase	Oxidase
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	-	+	-	+
Bb2A	Medium	Round	White	Shiny	Raised	Flat	-	+	-	+
Bb2B	Medium	Round	Milky-white	Shiny	Raised	Flat	-	+	-	+
Bb2C	Small	Round	Milky-white	Shiny	Raised	Flat	-	+	-	+
Bb2D	Medium	Round	Milky-white	Shiny	Raised	Flat	-	+	-	+
Bb2E	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	-	+	-	+

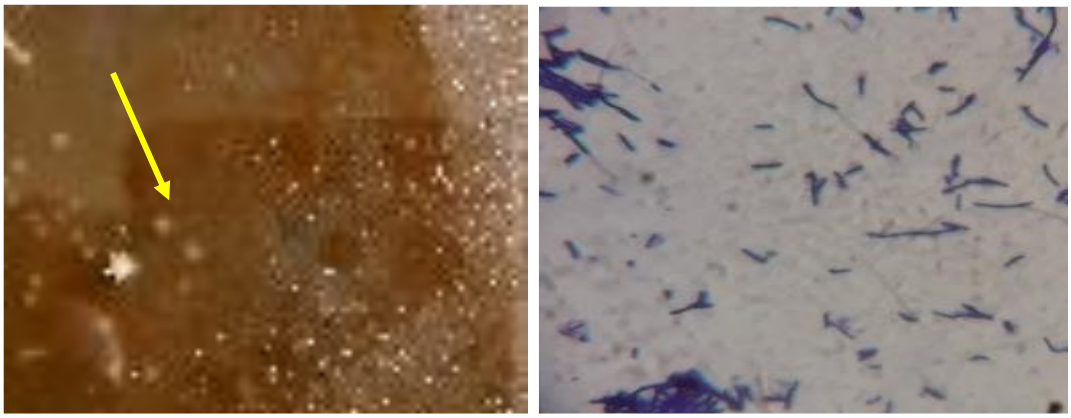

Figure 1. Colony of Bifidobacterium on MRSA (*left*) and microscopic cell morphology (*right*; 1000x magnification)

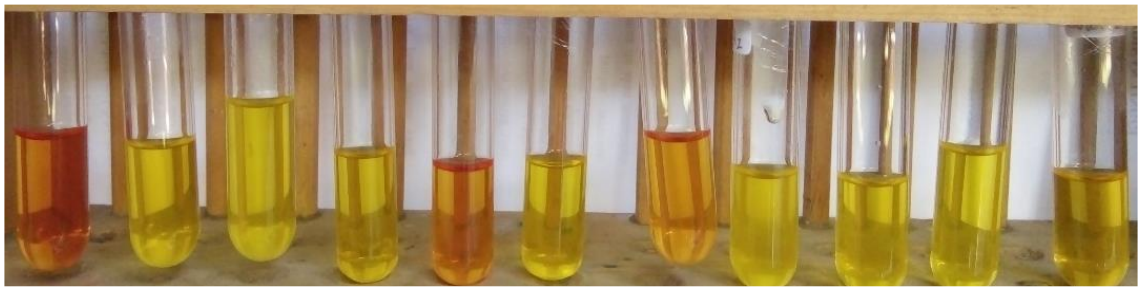

Figure 2. Carbon Source Test of Bifidobacterium Isolates

Table 2. Biochemical Test of the Isolated Bifidobacteria

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*
Bb2A	-	+	-	+	-	-	-	-	-	-	-	-	+	-	+	*B. minimum*
Bb2B	-	+	+	+	-	+	-	+	+	+	+	-	+	-	+	*B. indicum*
Bb2C	+	+	-	+	-	-	-	-	-	-	-	-	+	-	+	*B. galicum*
Bb2D	+	+	+	+	+	+	+	+	+	+	-	-	+	-	+	*B. minimum*
Bb2E	+	+	+	+	+	+	+	+	-	-	-	-	+	-	+	*B. dentium*
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; Gal: Galactose; Fru: Fructose; Glu: Glucose; Gli: Glyculose; Mal: Maltose; Man: Mannose; Lac: Lactose; Raf: Raffinose; Rib: Ribulose; Suc: Sucrose; Ind: Indole; MR: Methyl Red; VP: Voges Proskauer, SC: Simmon's Citrate.

Some studies have been identified around 32 species of Bifidobacterium, including 11 species from infant stool, such as *B. breve, B. longum* subsp. *longum, B. bifidum,* and *B. longum* subsp. *infantis* (Mariat et al., 2019; Narayanan & Subramonian, 2015; Nomoto et al., 2017) as well as adult-associated species i.e. *B. catenulatum* and *B. adolescentis* (Duranti et al., 2017). The Bifidobacteria colonization is thought to begin during birth (Hendrati et al., 2017; Turroni et al., 2012). The microbial colonization of the infant digestive tract becomes more complex by the exposure of environmental microorganisms, breast-milk, and food consumption (Nomoto et al., 2017; Palmer et al., 2007; Turroni et al., 2012; Vaishampayan et al., 2010).

Resistance to lysozyme

Bifidobacteria as probiotic agents are recommended to be resistant to lysozyme. In some sources, lysozyme acts as an anti-microorganism since its enzymatic activity can disrupt the stability of the bacterial cell walls (Rockova et al., 2013; Sakurai et al., 2017). In this study, all Bifidobacteria isolates were qualified as probiotic which may be applied in the foods industry, because they were able to grow on lysozyme-supplemented MRSA medium (data not shown). This result indicated that the Bifidobacteria were resistant to 200 $\mu g.mL^{-1}$ lysozyme from egg whites. It might because the Bifidobacteria modify their peptidoglycan (Sakurai et al., 2017). Some studies found that Bifidobacteria are more resistant to 300 $\mu g.mL^{-1}$ lysozyme than Gram-negative bacteria (Rada et al., 2010). In some conditions, Bifidobacteria may tolerate up to 500 $\mu g.mL^{-1}$ lysozyme, although some cells have imperfect growth and die with varying percentages (Zinedine & Faid, 2007). Probiotics are recommended to be resistant against lysozyme at 25-35 $mg.L^{-1}$ and 50 $mg.L^{-1}$ for the dairy industry and cheese products, respectively (Cardarelli et al., 2007; Guglielmonti et al., 2007).

Antimicrobial Activity against Pathogenic Microorganisms

Bifidobacteria play an important role in human immunity (Biasucci et al., 2008). As a probiotic agent, Bifidobacteria fights against bacterial pathogens through some mechanisms, such as decline the pH, compete for adhesion sites and nutrients, and produce antimicrobial agent e.g. hydrogen peroxide, organic acids and bacteriocins (Barzegari et al., 2020). Bacteriocins are secondary metabolites produced by LAB which act the same way to antibiotics (Pandey & Malik, 2019). Some research studied the antimicrobial activity of bacteriocins against both Gram-negative and positive bacteria (Duranti et al., 2017; Martinz et al. 2013), and Candida infections (Kohler et al., 2012).

Figure 3 showed the different inhibition capacity of Bifidobacterium isolates against pathogenic *E. coli* and *C. albicans*. Some inhibition zones showed different appearances, ranging from clear to cloudy edged (Figure 4). A clear zone with cloudy edge indicates that some indicator bacteria remain alive. While, the clear edge indicates that the isolate has the ability of bactericidal metabolites which means it can kill the indicator bacterial cells (Pan et al., 2009). The largest antimicrobial activity against *E. coli* was shown by the Bb3F isolate, with an inhibition zone of 10.80 mm, while the Bb1G isolate showed the smallest inhibition of 6.0 mm. According to Pan et al. (2009), the more than 6 mm clear zone express a strong inhibition activity, a 3-6 mm clear zone as moderate, while a 0-3 mm clear zone as weak inhibitory.

Candida spp. are common microbiota living in the human gastrointestinal tract and other mucosal surfaces (Vicariotto et al., 2012). For example, *C. albicans* is transmitted from mothers to infants during birth (Ward et al., 2017). Figure 3 showed that the largest inhibition activity of Bifidobacterium isolates against *C. albicans* was shown by Bb1B and Bb3F isolates with an inhibition zone of 9.70 mm, while Bb2C isolate showed the smallest inhibition zone of 7.0 mm. To inhibit the *C. albicans* growth, Bifidobacteria disrupt the physical structure of cells membrane, reduce the development of cell filamentation (Cizeikiene et al., 2013; Kohler et al., 2012) and eradicate the Candida-biofilm formation (Barzegari et al., 2020; Ujaoney et al., 2014; Vicariotto et al., 2012).

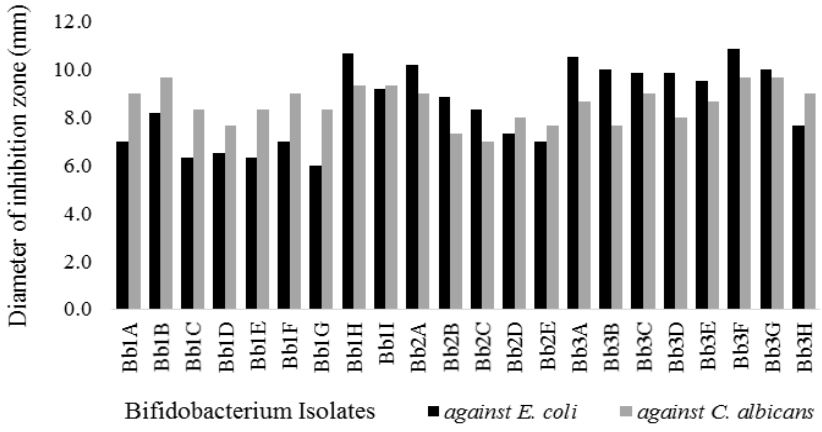

Figure 3. Inhibition Zone of Bifidobacteria Isolates against *C. albicans* and *E. coli*

Figure 4. Inhibition zone of Bb2E isolate against *E. coli* (*left*) and Bb1B isolate against *C. albicans* (*right*)

Exopolysaccharide (EPS) production

Exopolysaccharides (EPS) are carbohydrate polymers produced by bacteria in two forms, i.e. capsular and slime polysaccharide (Wu et al., 2010; Yan et al., 2017). Bacteria use the EPS to protect them from phagocytosis, stimulate immunomodulation activities, tolerate acidic conditions, and reduce pathogenic colonization in the gastrointestinal tract (Alp & Aslim, 2010; Salazar et al., 2009). The bacteria group that produces EPS, e.g. Bifidobacteria spp. have the potential to be a probiotic agent. Figure 5 showed that EPS production by Bifidobacteria ranged from 5-74 $mg.L^{-1}$. The highest yield of EPS was performed by Bb1A isolates, with 74 $mg.L^{-1}$, while the lowest were Bb1F and Bb2C isolates i.e. 5 $mg.L^{-1}$.

The medium composition e.g. carbon sources, temperature, pH, and incubation time directly affects the EPS production (Behare et al., 2009; Prathima et al., 2014). Gayathiri et al. (2017) found the maximal EPS production at 24th hour of incubation which decrease during the next growth stage. In the present study, MRSB medium containing glucose type sugar is used as the growth medium for EPS production. Meanwhile, Zubaidah et al. (2012) found that a good medium for EPS production is a medium containing lactose type sugar. In carbohydrate metabolism, lactose will be converted into galactose and glucose. The galactose acts as the precursor which increases the activity of related enzymes. It may also be correlated to Bifidobacterium ability in using lactose as the carbon source, since lactose is one of the most abundant components of human milk, besides human milk oligosaccharides, lipid, and protein (Pacheco et al., 2015). In breastfeed-infants gut, lactose and human milk oligosaccharides encourage the growth of Bifidobacterium (Lawson et al., 2020).

In conclusion, a total of 7 *Bifidobacterium* spp. were isolated from infant stool: *B. catenulatum*, *B. minimum*, *B. indicum*, *B. dentium*, *B. asteroids*, *B. galicum*, and *B. coerinum*. The *B. indicum* isolates (Bb3F and Bb1B) had the greatest inhibition activity against *C. albicans* and *E. coli* with 9.70 and 10.80 mm, respectively. Bifidobacterium isolates were resistant to lysozyme from egg whites up to 200 $\mu g.mL^{-1}$. The *B. catenulatum* (Bb1A isolate) had the highest yield of exopolysaccharide production with 74 $mg.L^{-1}$. Three Bifidobacterium strains (Bb1B, Bb2A, and Bb2E) were considered potentially as probiotics.

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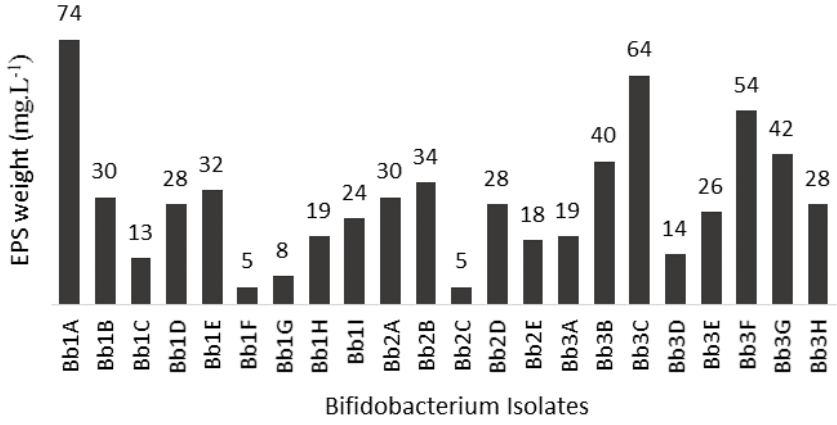

Figure 5. Exopolysaccharide Weight Produced by Bifidobacterium Isolates

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