American Journal of Biology and Natural Sciences
https://biojournals.us/index.php/AJBNS
ISSN: 2997-7185

Isolation, Identification, and Extraction of Probiotics from Bifidobacterium Bifidum

Wassan Rodha Hassan, Yasemin Khudiar Alghanimi, Heyam Abdul Ridha Al-Awade
Department of Biology, College of Education for Pure, University of Kerbala, Kerbala, Iraq

Received: 2024, 15, Nov
Accepted: 2024, 21, Nov
Published: 2024, 13, Dec

Annotation: The current study aims to Isolation and identification of the Bifidobacterium bifidum isolates from different sources, including fresh cow's milk, breastfeeding mothers' milk, and imported and locally produced dairy products, and their comparison with the standard isolate. The Diffusion of Oils test and the Emulsification Index (E24) test were conducted to investigate the ability of Bifidobacterium bifidum to produce biosurfactants (BS). The biosurfactant was then extracted and freeze-dried using a lyophilizer. Which can later be used in many applications due to its unique properties and be widely used in the food and pharmaceutical industry as probiotics.

1. INTRODUCTION,

Bifidobacterium bifidum has been identified within the last century *Bifidobacterium* spp. bacteria constitute 99% of the content of the large intestine, It is mainly found in the lining of the intestines and vaginal canal and helps digest fiber and prevent infection [1]. They produce important compounds such as vitamin B and healthy fatty acids, It is one of the beneficial bacteria known as probiotics [2]. The *Bifidobacterium* genus includes about 118 different species, which were isolated from various sources such as humans, animals, birds, insects, milk and its derivatives, and honey , All species of the genus *Bifidobacterium* are nonpathogenic except one species, *B. dentiuum* which is isolated from dental caries [3].

Biosurfactants are biological substances produced by many microorganisms, including *B. bifidum*,

that reduce surface tension and are antimicrobial and anti-adhesive substances [4]. Biosurfactants are a diverse group of surface-active molecules that have unique chemical properties and are of microbial origin and range in size from 500 to 1500 daltons , They were first discovered in the The sixties of the 17th century, and have since been widely accepted as an effective alternative to their traditional synthetic counterparts[5]. Biosurfactants (BS) and bioemulsifiers (BE) are Product from many microorganisms such as bacteria, fungi and yeasts , (BS) contains in its biological structure one of the following: glycolipid, mycolic, lipoprotein, lipid composite-polysaccharide, lipopeptide, or phospholipide [5].

2. MATERIALS AND METHODS

Sample collection

Many samples were collected from which *Bifidobacterium bifidum* bacteria could be obtained from different sources such as fresh cow's milk, milk of nursing mothers, and dairy products Imported and local such as yogurt and whey , Five isolates of *Bifidobacterium bifidum* were obtained two from cow's milk , two from dairy products, and one from human breast milk.

Sample cultur

They were grown directly on Petri dishes containing MRS-L-Cytiene culture medium.

Sample diagnosis

After growth of individual colonies from positive culture cases *B. bifidum* was diagnosed based on the diagnostic methods used in Bergey's Manual of Systematic Bacteriology [6]. Their identity was confirmed after conducting microscopic and laboratory examinations , examination of the systemVITEK-2 Compact , polymerase chain reaction (PCR).

Molecular diagnostics (PCR)

PCR was used to detect *Bifidobacterium bifidum* from bacterial cell extracts based on the 16S rRNA gene, with an amplicon size of 379 bp, using specific primers specifically designed for this study to target the 16S rRNA gene. Five isolates of B. bifidum were identified The primers used in the PCR reaction are detailed in the table (2-1).

Table (2-1) Primers used in the PCR reaction

Gene name	Primers Sequence 5′→3′		Primer length	Amplific-ation size (bp)	Annealing Temperatu-re
16S rRNA	F	GAAGAACCTTACCTGGGCTTG	**21bp**	**379**	**58**
	R	**ATTACTAGCGACTCCGCCTTC**	**21bp**		

Testing the ability of *B. bifidum* to produce Biosurfactant

To verify the ability of bacteria to produce biosurfactants, the following two tests were performed:

1. Diffusion of oils

The test result is read by observing the displacement of the oils. And spread it in the water [7].

2. Emulsification index E 24

The test results are read by observing the appearance of a foam-like emulsion layer over the solution, which indicates that the bacteria have the ability to emulsify fats. The greater the foam, the stronger the emulsifying capacity[8].

Extraction of biosurfactant produced from Bifidobacterium bifidum

The Biosurfactants can be extracted by physical methods such as filtration or centrifugation, chemical methods using solvents such as ethanol or methanol, or biological methods using specific enzymes, which are effective for extracting biosurfactants from cells [9]. Then use Lyophilizer to dry the biosurfactant and filtrate to obtain a white to pale yellow powder and store it until use.

3. Results and discussion

Bifidobacterium is a genus of Gram-positive, non-motile, often branching anaerobic bacteria. They are found almost everywhere in the digestive system , *Bifidobacterium bifidum* is an important species in the field of probiotics, playing a vital role in promoting digestive health and immune support, This research aims to study how to isolate, diagnose and extract probiotics from these bacteria.[10].

The bacterial isolation process involves the use of special selective nutrient media such as MRS-L-Cysteine -HCL, It is effective in isolating *B. bifidum* from different sources , Appropriate growth conditions are chosen that promote the growth of *B. bifidum* , and prevent the growth of harmful bacteria [11].

The isolates were diagnosed based on the phenotypic characteristics of the growing colonies, which appeared in the form of circular colonies resembling dew drops, as in the picture (3-1).

The bacteria were identified through microscopic characteristics and biochemical tests, and were diagnosed based on the fermentation of sugars, as in Tables (3-1), (3-2), and (3-3).

Picture (3-1) B. bifidum bacteria colonies growing on solid MRS-L-Cystiene-HCL medium

Table (3-1) Phenotypic tests for diagnosing *B. bifidum* isolated from different sources

Seq	test	test result
1.	Gram stain	+
2.	Growth Aerobic	-
3.	Growth Anerobic	+
4.	Morphology rods	+
5.	Spore forming	-

Table (3-2) Biochemical tests to diagnose *B. bifidum* isolated from different sources

Seq	test	test result
1.	KOH	-
2.	Oxidase	-
3.	Catalase	-
4.	Indole	-
5.	Citrate Utilization	-
6.	Urease	-
7.	Gelatinase	-
8.	Starch hydrolysis	-
9.	Ammonia from arginine	-
10.	Nitrate Reduction	-
11.	Gasein hydrolysis	-
12.	Lecethinase	-
13.	Lipase	-
14.	Motility test	-
15.	Growth temperature (35-45) °C	+
16.	Growth temperature (5-15) °C	-
17.	Gas production	-

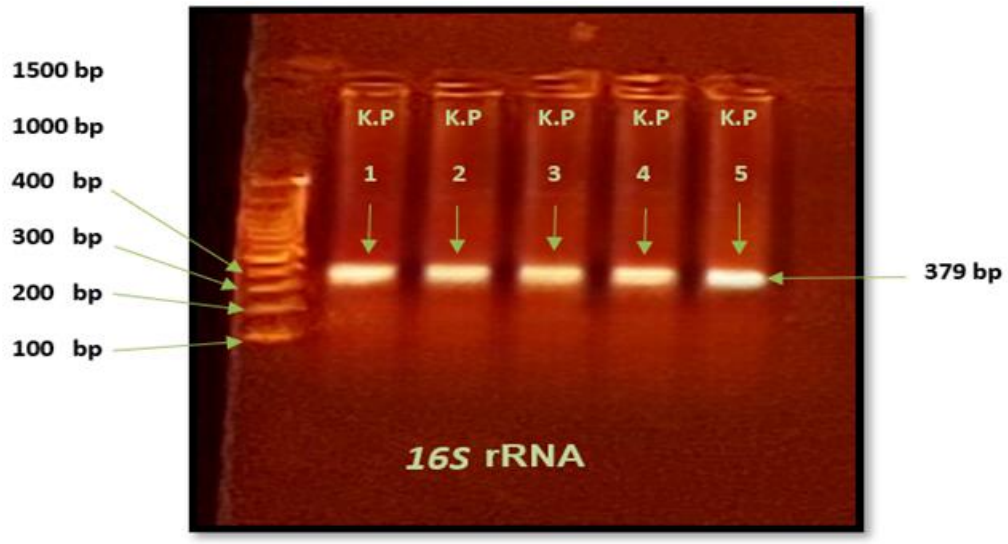
Table (3-3) Fermentation of sugars to identify *B. bifidum* bacteria isolated from different sources

Seq	Sugar name	test result
1.	Glucose	+
2.	Fructose	+
3.	Calactose	+
4.	Sucrose	+
5.	Lactose	+
6.	raffinose	+
7.	Manthol	+
8.	mannose	-
9.	Arabinose	-
10.	Xylol	-
11.	Manthol	-

+: positive test - : negative test

Molecular diagnosis of *B. bifidum* using (PCR)

Molecular techniques: Techniques such as Polymerase Chain Reaction (PCR) are used to confirm the identity of *Bifidobacterium bifidum*. These methods provide high accuracy in diagnosis and help in identifying different species of *Bifidobacterium* bacteria [12]. In the current study, the 16S rRNA gene was used to identify *B. bifidum* with a strength of 379, employing specific primers designed for this gene. The 16S rRNA gene is highly stable and does not undergo changes over time, making it a reliable standard for identification Additionally, the 16S rRNA gene contains highly variable regions among bacterial species, This will provide a specific sequence for each bacterial species, aiding in species identification [13]. as shown in picture (3-2). Isolating and diagnosing *B. bifidum* is vital for understanding its role in gut health and its clinical applications. These processes require the use of precise techniques to ensure correct species identification, contributing to the enhancement of scientific understanding of this beneficial bacterium.



Picture (3-2) Electrophoresis of the PCR product of B. bifidum using the principles of the 16S rRNA gene (379 bp), at a gel concentration of 1.5 % and a voltage of 70 V, for 50 minutes

Tests for the ability of *B. bifidum* to produce biosurfactants

Although research on the production of biosurfactants by this species is limited, there is growing interest in exploring its potential in this field.Other bacterial species, such as *Pseudomonas* and *Bacillus* , are known for their high capacity to produce biosurfactants, suggesting the possibility of similar properties in *B. bifidum* [14].

In another study, it was shown that *B.bifidum* is considered an important species in research related to biosurfactants, which are compounds that play a vital role in reducing surface tension between liquids , The oil spreading technique has been employed as an effective tool to evaluate the ability of this bacterium to produce biosurfactants [15]. The results in the current study yielded amazing results for all bacterial isolates in displacing oils, and this is evidence of the possibility of bacteria producing biosurfactants. The results of this study were comparable to those of a study conducted in India on the ability of *Pseudomonas spp.* , isolated from oil-contaminated soil, to produce biosurfactants [16].

Another study [17]. demonstrated that the biosurfactant extracted from *Bifidobacterium* spp. inhibited the biofilm of pathogens isolated from cardiac catheterization patients and influenced the phagocytosis process. Research by [18]. showed that probiotic bacteria *Lactobacillus* produce a biosurfactant of the type Glycosyldiglycerides which exhibits antimicrobial activity and inhibits the adhesion of pathogens.

Emulsification test: Measures the ability of bacteria to emulsify oils in waterThe ability of *B. bifidum* isolates to emulsify kerosene was tested as an indicator of glycolipid production. The results demonstrated that all bacterial isolates exhibited emulsification capability , as evidenced by the formation of an emulsified, foam-like layer on the surface of the solution , This indicates that the bacteria possess the ability to emulsify lipids , Studies indicate that *B. bifidum* can contribute to enhanced emulsifying properties, [19]. Despite the lack of studies Related to *B. bifidum*, which produces surfactants in modern sources available, however, these few studies have proven the importance of these materials in This makes them valuable in many applications [7].

References

1. Henry, T. (2023). Recherches sur la flore intestinale des nourrissons:(état normal et pathologique).

2. Sakanaka, M. ; Gotoh, A. and Yoshida, K.(2019). Varied pathways of infant gut-associated bifidobacterium to assimilate human milk oligosaccharides: prevalence of the gene set and its correlation with bifidobacteria-rich microbiota formation. Nutrients.

3. Rampelli, S. ; Soverini, M. and D'Amico, F.(2020). Shotgun metagenomics of gut microbiota in humans with up to extreme longevity and the increasing role of xenobiotic degradation. mSystems., 5:e 00124-20.

4. Chen, J. ; Xinyi, C. and Loong Ho, C.(2021).Recent Development of Probiotic *Bifidobacteria* for Treating Human Diseases Department of Biomedical Engineering, Southern University of Science and Technology (SUSTech)., Synthetic Biology Volume 9.

5. Marcelino, P.R.F. ; Gonçalves, F. ; Jimenez, I.M. ; Carneiro, B.C. ; Santos, B.B. and da Silva, S.S.(2020). Sustainable production of biosurfactants and their applications, Lignocellulosic Bioref. Technol.,159–183.

6. gbavwe, C. (2017). Identification and characterisation of novel glycan binding bacterial adhesins encoded by the human gut microbial metagenome. PhD thesis, National University of Ireland, Galway.

7. Cristina Diez, M. ; Llafquen, C. ; Paola,F. ; Claudio, L.; Briceño, G. ; Schalchli, H.(2022).Biosurfactant Production by Bacillus amyloliquefaciens C11 and Streptomyces lavendulae C27 Isolated from a Biosurification System for Environmental Applications.,23;10(10):1892.

8. Nayarisseri , A. ; Singh , P. and Singh , S. K.(2018).Screening, isolation and characterization of biosurfactant producing Bacillus subtilis strain ANSKLAB03 30;14(6):304–314.

9. Weng ,Y. ; Jiang , D. ; Liang , J. ; Ye , S. ; Tan , W. ; Yu , C. and Zhou,Y. (2021).Effects of Pretreatment with Bifidobacterium bifidum Using 16S Ribosomal RNA Gene Sequencing in a Mouse Model of Acute Colitis Induced by Dextran Sulfate Sodium., 9;27:e928478-1–e928478-12.

10. Mayo, B. and van Sinderen D. (2010). *Bifidobacteria: Genomics and Molecular Aspects*. Caister Academic Press. ISBN 978-1-904455-68-4.

11. Zavaglia, A. ; Kociubinski , G. ; Pérez, P. Pérez, ; De Antoni, G.(1998).Isolation and characterization of Bifidobacterium strains for probiotic formulation.,61(7):865-73.

12. Kim, H. ; Kim , E. ; Yang , S. ; Lee , S. ; 1, Kim , M. ; Kim , H. (2020).Development of Real-Time PCR Assay to Specifically Detect 22 Bifidobacterium Species and Subspecies Using Comparative Genomics., 28;11:2087.

13. Shehata , H. R. ; Hassane , B. ; Newmaster , S. G. (2024).Real-time PCR methods for identification and stability monitoring of Bifidobacterium longum subsp. longum UABl-14 during shelf life ., Systems MicrobiologyVolume 15.

14. Jungersen , M. ; Wind , A. ; Johansen , E. ; Christensen , J. E. ; Stuer-Lauridsen , B. and Eskesen , D.(2014). The Science behind the Probiotic Strain Bifidobacterium animalis subsp. lactis BB-12, Microorganisms.,2(2):92–110.

15. Ku , S. ; Park, M. S. ; Ji , G. E. ; You , H. J. (2016). Review on Bifidobacterium bifidum BGN4: Functionality and Nutraceutical Applications as a Probiotic Microorganism., 14;17(9):1544.

16. Anandaraj, B. and Thivakaran, P. (2010). Isolation and production of biosurfactant producing organism from oil spilled soil . J.Biosci Tech.,1(3) :120-126.

17. Abed AL-Majlawi, B.(2015). Study of the Effectiveness of Biosurfactant Extracted from Bacteria Bifidobacterium spp. In the Inhibition of Biofilm of Pathogens Isolated from Cardiac Catheterization Patients and Its Effect in Phagocytosis. University of Karbala College of Education for Pure Sciences Department of Biology.

18. Gudin, E. J. ; Rocha, V. ; Texeira, J. A. and Rodrigues, L. R. (2010). Antimicrobial and antiadhesive properties of a biosurfactant isolated from Lactobacillus paracasei ssp. paracasei A20. Lett. Appl. Microbiol. 50: 419– 424.

19. Devale , A. ; Rupali Sawant , R. ; Pardesi , K. ; Perveen , K. ; Khanam , M. N. ; Shouche , Y. ; Mujumdar , S. (2023). Production and characterization of bioemulsifier by *Parapedobacter indicus*. Front Microbiol.,16;14:1111135.