

### Fermentation of different sugars by Bifidobacteria

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#### Abstract

The genus of *Bifidobacteria* stands out for being one of the most used probiotic bacteria for food applications. Identification of bifidobacterial species remain elusive, biochemical tests for the identification of strains of *Bifidobacteria* are now superseded by use of genus-specific PCR primers. The aim of this study is to identify of some *Bifidobacteria* strains by chemical tests non the method of PCR, in this study it's found the ability of four strains of *Bifidobacteria* (*Bifidobacterium longum* ATCC 15707, *Bifidobacterium bifidum* LMGD 10645, *Bifidobacterium animalis and Bifidobacterium angulotum*). To fermented by glucose, galactose, fructose, starch, lactose, sucrose, ribose and mannitol. Carbohydrate fermentation test was performed in Basal Liquid Medium (BLM). The development of a yellow color after incubation was considered a positive result. All strains in this search are fermented all sugars, and we found that *B. bifidum* and *B. longum* can ferment ribose, galactose and mannitol or can't.

Keywords: *Bifidobacteria*; Fermented; MIRCEN; Purchased.

#### 1. Introduction

Probiotics are the microorganisms (including bacteria, mould and yeasts) that have various health benefits to the host, when it consumed in sufficient amounts. Functional foods are food or food products that containing probiotics, have several therapeutic benefits and health-promoting effect (Nadia *et al.*, 2022).

The genus of *Bifidobacteria* stands out for being one of the most used probiotic bacteria for the food applications. The probiotics are live microorganisms that when continuously administered in ample amounts, inundates several benefits to consumer health (Verruck and Prudencio, 2019).

Bifidobacteria play an important and beneficial role in the proper balanced of hindgut microflora (Rada *et al.*, 2002). The most common

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Bifidobacterium species found in the gut of neonates and breast - fed infants are B. bifidum, B. infantis and B. breve, while in the intestine of adults are B. longum and B. adolescentis. (Zohreh, 2016 and Eva Vlkova et al., 2004). Some of Bifidobacterium strains are considered to be important probiotic that used in the food industry and are widely used as freeze - dried additives in the food industry for the production of beverages, cheese products, cultured milk and milk powder, the employment of strains belonging to B. animalis, B. longum, B. bifidum and B. infantis as probiotic starter cultures is due to their important role played in gut. They suppress putrefactive bacteria by production of lactic and acetic acids in large intestine that control pH (Eva Vlkova et al., 2002), also, the mechanism of antibacterial activity for bifidobacteria against pathogens bacteria waylays in inhibit the pathogen adhesion to surfaces and, producing of iron-siderophore (Verruck and

Prudencio, 2019). From other health benefits which have been attributed to Bifidobacterium species include the alleviation of lactose malabsorption (intolerance), antitumoral activity, reduction of cholesterol levels and immune system activation effect (Eva Vlkova *et al.*, 2004).

Biochemical tests for the identification of strains of *Bifidobacteria* are now superseded by use of genus-specific PCR primers described by (Kok *et al.*, 1996). Identification of bifidobacterial species remain elusive. Considerable developmental work is required in this area since DNA-DNA reassociation is currently the only reliable method of bifidobacterial species identification, (Ballongue 1993 and Yaeshima *et al.*, 1996). So, the aim of this study is using chemical tests (not all of them are shown here) for identification of *Bifidobacteria* non-PCR.

# 2. Material and methods

Bifidobacterium longum strain ATCC 15707, B. bifidum LMGD 10645, B. animalis and B. from angulatum were purchased Cairo Microbiological Resource Center (MIRCEN), Faculty of Agriculture, Ain Shams University. Measurement of fermentation rate or carbohydrate fermentation test was performed in 5 ml of (BLM) basal liquid medium, Bifidobacteria were inoculate in basal medium with (1gL) for saccharide source. The following saccharides were used: glucose, galactose, mannitol, lactose, sucrose, fructose, ribose and starch. The development of a yellow color after incubation at 37°c for 3 to 7 days was considered a positive result. This result agrees with (Migual *et al.*, 2004; Rada *et al.*, 2002).

# 3. Results and discussion

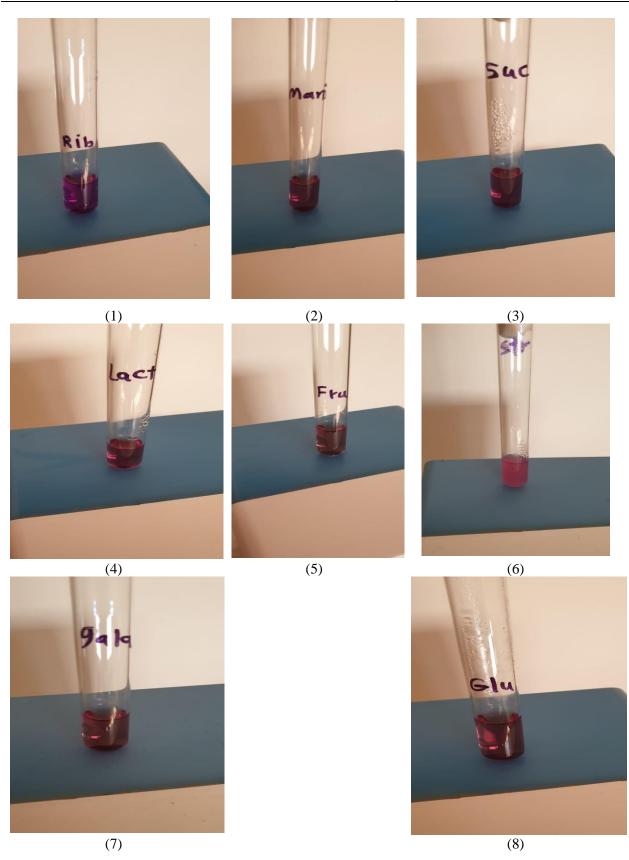
As illustrated in the table (1); Fermentation profiles of carbohydrates displaying variability among commercial strains of Bifidobacterium included in this study, all strains fermented all of sugars, this result agrees with (Rada *et al.*, 2002; Migual *et al.*, 2004; Masco *et al.*, 2004; Mitsuoka, 1969; Scardovi and Trovatelli, 1974; Scardovi and Crociani, 1974; Orla. Jensen, 1924; Tissier, 1900; Mattarelli *et al.*, 2008; Reuter, 1963; Wytske and Stouthamer, 1968; Sakata *et al.*, 2002; Paola *et al.*, 2018).

All strains can ferment mannitol, this result agrees with (Wytske and Stouthamer, 1968) and this result incompatible with (Migual *et al.*, 2004; Rada *et al.*, 2002; Paola *et al.*, 2018).

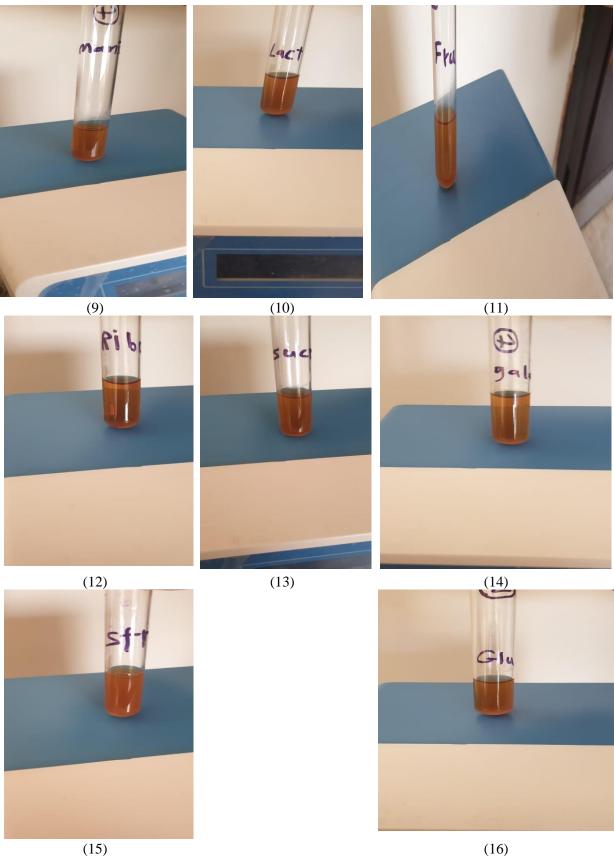
Upland *B. bifidum* might ferment fructose and galactose, this result agrees with Paola *et al.* (2018), and this result incompatible with Migual *et al.* (2004). And it can ferment ribose, this result agrees with Migual *et al.* (2004) and this result incompatible with Paola *et al.* (2018). Also, it can ferment lactose this result agrees with Paola *et al.* (2018), and this result agrees with Paola *et al.* (2018), and this result agrees with Paola *et al.* (2018), and this result agrees with Paola *et al.* (2018), and this result agrees with Paola *et al.* (2018), and this result incompatible with Paola *et al.* (2004).

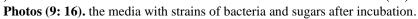
Regarding to *B. longum* can ferment sucrose this result agrees with Rada *et al.* (2002) and Paola *et al.* (2018), and this result incompatible with Migual *et al.* (2004). Also, it can ferment ribose and galactose this result agrees with (Paola *et al.*, 2018; Mattarelli *et al.*, 2008; Reuter, 1963; Sakata *et al.*, 2002) and this result incompatible with Migual *et al.* (2004).

strain	Glucos	e Lactose	Sucrose	Galactose	Starch	Ribose	Fructose	Mannitol
B. angulatum	+	+	+	+	+	+	+	±
B. animalis	+	+	+	+	+	+	+	±
B. bifidum LMGD10645	+	±	+	±	+	±	±	±
B. longum ATCC15707	+	+	±	$\pm$	+	±	+	<u>+</u>



Photos (1: 8). the media with sugars before adding bacteria and incubation.





As illustrated from the photos; the strains that ferment the sugar are convert the color of media from purple to yellow; While the development of a yellow color after incubation at 37°c for 3 to 7 days was considered a positive result. This result agrees with (Migual *et al.*, 2004; Rada *et al.*, 2002).

## 4. Conclusion

This study was performed to use another method to detect of the *Bifidobacteria* and distinguish among their strains non – PCR Method.

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There is no funding for this research. Institutional Review Board Statement All Institutional Review Board Statements are confirmed and approved. Data Availability Statement Data presented in this study are available on fair request from the respective author. Ethics Approval and Consent to Participate Not applicable Consent for Publication Not applicable. Conflicts of Interest

The author disclosed no conflict of interest starting from the conduct of the study, data analysis, and writing until the publication of this research work.

## 5. References

- Ballongue, J., (1993). 'Bifidobacteria and probiotic action'. P.357-428.In S. Salminen and A. von Wright(ed), Lactic acid bacteria. Marcel Dekker Inc., New York.
- Eva Vlkova, Jana Medkova and Vojtech Rada., (2002) 'Comparison of four methods for identification of Bifidobacteria to the genus level'. *Czech J. Food Sci. Vol.20, No.5:171-*174.
- Eva Vlkova, Vojtech Rada and Iva Trojanova., (2004). 'Enumeration, isolation, and identification of Bifidobacteria from dairy products'. *Acta agriculturae slovenica*,84(2004)1,31-36.
- Kok, R.G., A. de Waal, F. Schut, G. W. Welling,G. Weenk, and K. J. Hellingwerf., (1996).'Specific detection and analysis of a probiotic

Bifidobacterium strain in infant faeces'. *Appl. Environ.Microbiol.* 62:3668-3672.

- Masco, L., Ventura, M., Zink.R., Huys.G, Swings, J., (2004) 'Polyphasic taxonomic analysis of Bifidobacterium animalis and Bifidobacterium lactis reveals relatedness at the subspecies level: Reclassification of Bifidobacterium animalis as Bifidobacterium animalis subspecies. Animalis subspecies'.nov and Bifidobacterium lactis.Int.J.Syst. Evol. Microbiol.54,1137-1143.
- Mattarelli, P., Bonaparte, C., Pot, B., Biavati, B., (2008). 'Proposal to reclassify the three biotypes of Bifidobacterium longum as three subspecies: Bifidobacterium longum subspecies. longum subsp'. nov., Bifidobacterium longum subsp. Infantis comb, nov., *Bifidobacterium* longum subsp.suiscomb.nov., Syst.Evol. Int.J. Microbiol.58,767-772.
- Miguel Gueimonde, Susana Delgado, Baltasar Mayo, Patricia Ruas- Madiedo, Abelardo Margolles, Clara G. de los Reyes-Gavilan., (2004). 'Viability and diversity of probiotic Bifidobacterium Lactobacillus and populations included in commercial fermented milks'. Food Research International 37(2004) 839-850.
- Mittsuoka, T., (1969).'Comparative studies on bifidobacteria isolated from the alimentary tract of man and animals (including descriptions of Bifidobacterium thermophilum nov, spec., and Bifidobacterium pseudolongum nov, spec)'. Zentralbl.Bakteriol.Orig.210,52-64.
- Nadia, S, Alkalbani, Tareq, M, Osaili, Anas, A, Al-Nabulis, Amin, N, Olaimat, Shao-Quan Liu, Nagendra, P, Shah, Vasso Apostolopoulos and Mutamed, M, Ayyash., (2022) 'Assessment of yeasts as potential probiotics: a review of gastrointestinal tract conditions and investigation methods. J Fungi (Basel). 2022 Apr 2;8(4): 365.doi:10.3390\jof8040365.

- Orla. Jensen, M.L.D., (1924). 'La classification des bacteries lactiques'. *Lait 4,468-474*.
- Paola Mattarelli, Bruno Biavati, Wilhelm H. Holzapeel and Brian J.B. Wood., (2018).
  'The Bifidobacteria and Related Organisms Biology, Taxonomy, Applications'. *Elsevier Inc. ISBN:978-0-12-805060-6*.
- Rada, V., Bartonova, J., and Vlkova, E., (2002).
  'Specific growth rate of Bifidobacteria cultured on different sugars'. *Folia Micobiol.* 47(5), 477-480(2002).
- Reuter, G., (1963). 'Vergleichende Untersuchunge uber die Bifidus-Flora im Sauglins-und Erwachsenenstuhi'. Zentralbl Bcteriol Orig A191,486-507.
- Sakata, S., Kitahara, M., Sakamoto, M., Hayashi,
  H., Fukuyama, M., Benno, Y., (2002).
  'Unification of Bifidobacterium infantis and
  Bifidobacterium suis as Bifidobacterium
  longum'. *Int.J. Syst, Evol.Microbiol.52,1945-1951.*
- Scardovi, V., and Crociani, F., (1974). 'Bifidobacterium catenulatum, Bifidobacterium dentium, and Bifidobacterium angulatum. Three new species and their deoxyribonucleic acid homology relationships'. *Int.J.Syst. Bacteriol. 24, 6-20.*
- Scardovi, V., and Trovatelli, L.D., (1974). 'Bifidobacterium animalis (Mitsuoka)

comb.nov. and the "minimum" and "subtile" groups of new bifidobacteria found in sewage'. *Int.J. Syst.Bacteriol.24,21-28.* 

- Tissier, M.H., (1900). 'Recherches sur la flore intestinale normale et pathologique du nourisson (thesis)'. University of Paris, Paris, France.
- Verruck, S. and Prudencio, E.S., (2019). 'Survival of Bifidobacterium ssp. During gastrointestinal passage and their mechanism of action for pathogenic bacteria inhibition in the gut: A concise review'. *Food Biology* 2019, 8:1-6. Doi: 10-25081\fb. 2019.v8.3856.
- Wytske De Vries and A.H. Stouthamer., (1968). 'Fermentation of glucose, lactose, galactose, mannitol and xylose by Bifidobacteria'. *Bacteriology.p.* 472-478.vol, 96, No. 2.
- Yaeshima, T., S. Takashashi, N. Ishibashi, and S. Shimamura., (1996). 'Identification of bifidobacteria from dairy products and evaluation of a microplate hybridization method'. *Int. J. Food Microbiol.* 30:303-313.
- Zohreh Mashak., (2016). 'Identification of Bifidobacterium strains isolated from Kashke zard: A Traditional Iranian fermented cereal- dairy based food'. Avicenna J Clin Microb Infec. (2016) November; 3 (4) ce39240.doi:10.17795\ajcmi-39240.