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RESEARCH ARTICLE

Physiological and biochemical characteristics of some strains of *Bifidobacteria*

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Abstract

Identification of *Bifidobacterial* species still remain difficult and the chemical tests for identification of species of *Bifidobacteria* are now superseded by use of PCR. This study aimed to identify and examine some *Bifidobacteria* strains by chemical tests and not the method of PCR, identification of the bacterial strains was performed where the strains were initially tested for Gram stain, catalase test, production of Co2 (gas) from glucose, determined in Man, Rogosa, and Sharp (MRS) broth, as well as the ability to grow at different temperatures (5, 15, 25, 37° C) for 5 days, were considered to identify the strains. The strains were activated in sterile de Man, Rogosa, and Sharp broth inoculation and then incubated at 37° C for 7 days. The MRS broth was supplemented with various salt concentrations (4 % & 6.5%) of NaCl, to determine the ability of strains to grow at different concentrations of salt (NaCl). The results showed that *Bifidobacterium* is a genus of Gram positive, and the bacterial colonies on MRS agar are rod shapes, catalase negative, and it can't produce gas from glucose. Strains of *Bifidobacterium* can grow in the temperature range from 5°C - 37°C, and *Bifidobacterium* species can't tolerate the salt concentration.

Keywords: Catalase, Gram stain, Probiotic, *Bifidobacteria*

1. Introduction

As consumers grow more health conscious, there is a growing market for functional foods and beverages that contain *Bifidobacterium* due to its potential applications in both food and therapeutic applications. Additionally, more consumers are interested in purchasing probiotic food and beverage products because they may strengthen their immune systems. (Abdul Kalam *et al*., 2023).

Bifidobacteria was isolated from different origin and it isolated for the first time in 1899 by Tissier from the stools of breast-fed infants and commonly found in the gut of infants and the uterine region of pregnant mothers. it's a Gram-positive and anaerobic microorganism (obligate anaerobes) and not producing gas, nonspore-forming irregular anaerobic rods. were given the Latin word "bifidum" for their bifurcate morphology.

L. bifidus, or *Bacillus bifidus*. A number of *Bifidobacteria* have a long history of safe use as dietary adjuncts; *Bifidobacterium breve, bifidum, Lactis, animalis,* and *adolescentis* have GRAS (generally regarded as safe). *Bifidobacterium* is a non-spore-forming, Gram-positive, anaerobic probiotic. Like all probiotics, it confers health benefits on the host when administered in adequate amounts. The starter cultures are generally designed to assure food safety, economic feasibility, shelf-life and technological criteria. Apart from these traditional properties, that new starter cultures should take into account the risks posed by the

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formation of the biogenic amines in the food, the development and spreading of bacterial resistance to antibiotics, protection against harmful bacteria either by acidification or by the production of antimicrobials (bacteriocins). The ability of starter cultures to compete with the natural microbiota of raw materials, as well as technological performances, relies upon the ability to survive in the conditions encountered in the food (salt, temperature, pH, preservatives) (Antonio *et al*., 2012; *Chen et al.*, 2021).

One of the most prevalent microbes in the human gut, *Bifidobacterium* can make up as much as 3% of the gut microbiota of adults. However, it is more prevalent in the stomach of infants, where it can make up as much as 91% of the gut microbiota of breast fed infants. While *Bifidobacterium breve* and *Bifidobacterium infantis* are the most prevalent species in the digestive tract of human newborns, *Bifidobacterium adolescentis* and *Bifidobacterium longum* are the principal species in the adult intestine.

In addition, *B. catenulatum, B. bifidum, B. pseudocatenulatum, B. angulatum, B. dentium*, and *B. gallicum* have also been reported to be human intestinal bifidobacteria. *Bifidobacterium* constitutes a significant proportion of probiotic cultures that are used in the food industry. 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 the gut **(**Zohreh, 2016).

As one of the most widely used probiotic bacteria for food applications, Bifidobacteria is regarded as a probiotic starter because of its role in maintaining and promoting gastrointestinal health, as well as the possibility that it plays a significant role in preserving overall health (Verruck and Prudencio, 2019).

According to Eva Vlkova *et al*. (2004) *Bifidobacteria* are helpful and crucial for maintaining the right balance of the gut microbiota. By regulating the pH of the large intestine through the generation of lactic and acetic acids in a molar ratio of 2:3, it inhibits dangerous microorganisms. *Bifidobacteria* have also been linked to additional health benefits, such as vitamin synthesis, the reduction of lactose intolerance or malabsorption, antitumoral activity, anticholesterolemic (lower cholesterol), and immune system activation. To distinguish these bacteria from other bacteria, it is crucial to identify *Bifidobacteria* species down to the genus level, something that is still difficult to perform. Since the detection of PCR or F6PPK-tests is the conventional method for identifying *Bifidobacterium*, and DNA-DNA reassociation is currently the only reliable method of *Bifidobacterium* species identification, significant development work is needed in this area. Nowadays, PCR primers, as described are used to identify *Bifidobacterium* strains instead of chemical tests. Therefore, the goal of this study is to identify *Bifidobacteria* using a different chemical test (not all of them are displayed here) that is not the PCR method or F6PPK.test. These tests include the Gram stain, Catalase test, the ability to produce CO_2 or gas from glucose, the ability to grow at different temperatures (5, 15, 25, and 37° C) for five days, and the ability of strains to grow at different concentrations of salt (NaCl).

2. Materials and Methods

2.1. Strains

Bifidobacterium. bifidum LMGD 10645, *B. longum* strain *ATCC* 15707, *B angulotum.* and *B. animalis* were purchased from Cairo Microbiological Resource Center (MIRCEN), Faculty of Agriculture, Ain Shams University.

2.2. Physiological and biochemical characteristics of Bifidobacteria

2.2.1. Identification Tests

The strains were studied under a microscope to check for morphological traits, cell shape, and Gram stain reactivity **(**Choksi and Desai, 2012; Alrekaby and Alwendawi, 2014; Zohreh, 2016).

2.2.2. Catalase Test

A sterile microscopic slide was coated with a drop of 3% hydrogen peroxide. Using a nicrome wire loop, remove cells from the middle of a test strain's well colony and place them in the hydrogen peroxide drop. After mixing the two, watch for the formation of gas bubbles. Strains that exhibit catalase negativity and do not produce gas bubbles are classified as *Bifidobacterium* strains. According to (Choksi and Desai, 2012 , Imene et al, 2013, Alrekaby and Alwendawi, 2014 and Mashak, 2016).

2.2 .3. Growth at Different Temperatures

One ml of overnight cultures was transferred into the tubes which contain 5 ml temperature test media of MRS broth, then incubated, they were incubated for 5 days at 5, 15,25 and 37° C. changes in turbidity of MRS media after incubation for 24, 48 and 72 h, was the positive result these results agreement with **(**Azhari, 2011).

2.2.4. Growth at Different NaCl Concentrations

One ml of overnight cultures was transferred into the tubes which contain 5 ml NaCl test media (media of MRS added with 4 % or 6.5 % NaCl, and containing bromocresol purple as a pH indicator with final pH 6.2 to 6.6 (Harrigan and McCance, 1976). Then the strains were tested for growth at 4 % and 6.5 % NaCl concentrations, they were incubated for 7 days. The changing from purple to yellow is interpreted as a favorable outcome or taken as a positive result this accordance with (Choksi and Desai, 2012).

2.2.5. Gas from Glucose

According to Choksi and Desai. (2012), the addition of Ca (Oh)2 to the glucose-fermented tube the presence of Co2 resulted in strong turbidity of CaCo3 on the fermented tube.

3. Results and Discussion

Table 1. Identity characteristics of *Bifidobacterium* strains

No. of strains	1	2	3	4
Morphology	Rod	Rod	Rod	Rod
Gram reaction	+	+	+	+
Catalase reaction	-	-	-	-
Growth at 5° C temperature	-	+	-	-
Growth at 15° C temperature	±	-	-	±
Growth at 25° C temperature	-	-	-	+
Growth at 37° C temperature	+	+	+	+
Tolerance of 4% salt	-	-	-	-
Tolerance of 6.5% salt	-	-	-	-
Co2 from glucose	-	-	-	-

Where 1,2,3 and 4 are (*B. angulotum, B. animalis, B. bifidum LMGD*10645 and *B. longum ATCC*15707) *respectively.*

As illustrated in the table (1), the rod-shaped bacterial colonies on MRS agar belong to the Gram-positive species *Bifidobacterium*. These findings concur with those of (Zohreh,2016). The bacterial colonies on MRS agar are rod-shaped, catalase negative, and incapable of generating gas from glucose, these findings concur with (Alrekaby & Alwendawi, 2014; Zohreh, 2016).

3.1. Temperature-dependent growth.

The commercial strains of *Bifidobacterium* used in this study exhibit variability, as shown in table (1) and photographs 1, 2, 3, and 4. These strains can grow at temperatures ranging from 5 to 37 °C. This result agrees with (Verruck and Prudencio, 2019).

The investigation's findings indicated that 37°C was the ideal temperature to boost *Bifidobacterium* performance. Additionally, the stability of different strains of *Bifidobacteria* varied, which may be related to the fact that *Bifidobacteria* thrive in anaerobic environments, particularly when 0.05% L-cysteine is present (Dave and Shah, 1997).

Bifidobacterium species can't tolerate the salt concentration, as illustrated in table (1) and photos (5,6,7 & 8) (Zhang *et al*., 2022).

Reduction of viable count of bacteria was observed on exposure to salt concentration, it's displayed a low tolerance to stress, possibly owing to the injury caused by salt to the integrity of the bacterial membrane. Although some *Bifidobacterium* strains are able to survive under more extreme conditions.so; the physiology of these bacteria is far from understood (Gandhi and Shah, 2016).

Photo (1) media before incubation as general

photo (2) positive at 15 °C

Photo (3) negative & positive at 5

Photo (4) positive at 5° C

Photos (5 & 6) Media with NaCl before inoculated with bacteria

photo (7) negative at 6.5% NaCl

photo (8) negative at 4% NaCl

4. Conclusion

This research was performed to use another method to detect of the *Bifidobacterium* species and distinguish among their strains non – PCR Method or F6PPK test, that by using biochemical tests.

Declarations

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Data presented in this study are available on fair request from the respective author
Ethics Approval and Consent to Participate
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Consent for Publication
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The authors disclosed no conflict of interest

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