



IRAQI
Academic Scientific Journals



العراقية
المجلات الأكاديمية العلمية

TJAS

Tikrit Journal for
Agricultural
Sciences

ISSN:1813-1646 (Print); 2664-0597 (Online)

Tikrit Journal for Agricultural Sciences

Journal Homepage: <http://tujas.tu.edu.iq>

E-mail: tjas@tu.edu.iq

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KEY WORDS:

Detection Bacteria, *Bacillus spp*,
childrens milk powder

ARTICLE HISTORY:

Received: 21/09/2021

Accepted: 17/12/2021

Available online: 31/12/2021

Tikrit Journal for Agricultural Sciences (TJAS)

Detection for *Bacillus spp* In Milk Powder Infant Formula

ABSTRACT

This study was included isolation and identification *Bacillus spp* bacteria from childrens milk powder under 2 years of age .45 milk sample were collected with 3 replicates per label. Dialak , Novolac AD, Similac 1, Nactalia 1, Celia2, Sunny baby1, Nursoy, Primalac1, Alpha1, Biomil plus, Dovelac1, Liptomil plus2, DoveGER, S-26 LF, S-26 AR ,of the various pharmacies from the location of Baghdad for the period from 1/11/2018 to 30/4/2019. Isolates were examined by microscopic, agricultural and chemical test, as it was possible to obtain 8 isolats, all of which belonged to the various strains of wax and included 3 isolates of *Bacillus cereus* of milk samples contamination of 1.35% and two isolates of bacteria in Dialak 1, *B.stearotherophilus* from Novolac AD milk sample with 0.9 contamination and isolation of one *B.stearotherophilus* from each of the Nursoy,sunny baby1,Celia 2 (0.45%).

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INTRODUCTION

Milk and its derivatives are a rich source of nutrients for people in most countries of the world (Park, 2009). International trade has set laws to ensure the safety and suitability of food such as milk, protect consumers 'health and facilitate trade within the provisions of food hygiene in the Codex Alimentarius Procedures Manual under the title“ Relations between Commodity Committees and General Committees ”to set various standards for products Dairy Where these laws take into account the principles of health production in the manufacture of milk and dairy products and directives on their application to the fullest in various methods and treatments and this law takes into account the specifications of milk from different animals by the producing countries and it focuses on acceptable results for food safety in accordance with the principles of the food system and these laws apply to the production, processing and handling milk (O'Mahony ,1987) . It becomes similar to breast milk and is offered to infants who do not breastfeed their mothers' milk whatever the reasons, so the method of manufacturing it takes the most important aspect in terms of preparation, sterilization, discharge of air and pressure with inert nitrogen gas to keep milk from any contamination (Hawra and Naji, 2005) especially *bacillus*, because it is the main micro biome of the products as it is widespread in the environment, especially soil, which is from bacteria that make up the spore forming bacteria is able to grow in different degrees of temperature and storage

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conditions where some strains work to degradation the protein material and lead to the spoilage of milk and some of these are a product of endo and exotoxin which causes some diseases, especially for a very sensitive ages such as children (United States Food and Drug Administration, 2008). There are a large number of bacilli in soil and various water and in different families, including a thermophilic, acidity, alkali and mesophilic, some of which are responsible for food spoilage and some play a role in the production of antibiotics (Esbelin, 2009). The genus bacilli contains about 148 species of them with an important effect on the fertility of soil (Belma *et al.*, 2002) and there are different types of sorority sores in food, including *B. cereus*., *B. megaterium*, *B. mycoide*, *B. subtilis*, *B. lentus*, *B. pumilus*. *B. Stearothermophilus*, one of its characteristics is a bacillus bacteria ranging in size from 0.9-1.2 micrometers and a length of 2-4 micrometers of aerobic and anaerobic optional (Shaheen, 2009), positive for a pigment of gram and driven by the surrounding cilia appearing pair or chains (Holt *et al.* , 1994) formed for spore forming and internal spore forming, terminal or central necessary for its growth ranges from 4-55 degrees Celsius and the optimum temperature from 30-40 degrees Celsius, while the optimum temperature for the growth of its psychrotrophic strains is 4-5 degrees Celsius (Roberts *et al.*,1996). It can also grow in a 7.5% salt content (Forsythe, 2000), contains the vegetative cells of bacillus in some of its strains on a surface protein layer that forms the outer layer of the cell wall and is fully covered (S-Layer secures the existence of this layer Its adherence to human skin cells and its ability to stick to the surface of manufacturing devices causing problems in the production of milk and dairy products (Kotiranta *et al.*, 2000), and forms the types of bacilli internal spor forming which is a solid composition used by bacteria to stay in difficult conditions as the bacilli is covered with non-existent in other types of bacilli (Shaheen, 2009). Some bacilli isolated from milk belong mainly to the bacillus group (*Bacillus cereus*) in that they form a single inner spore with an oval or elongated shape (Health Protection on Agency, 2007) Therefore, implementation of proper health control along the food chain is necessary to ensure the safety and suitability of this .Food to be used and to prevent unhealthy practices and conditions in the production, processing and handling of milk and dairy products, as is the case in many milk and milk producing countries which constitute a large part of the diet of consumers, especially infants. The aim of the study is to detect the bacilli in the medical milk of infants and their growth at different temperatures with morphological and biochemical tests performed on them.

MATERIALS AND METHODS

Infant formula milk powder sample marks were taken from different pharmacies from Baghdad for the period from 1/11/2018 to 4/30/2019, with a total number of 45 samples, were taken randomly and at different time periods, and bacteriologically analyzed by three replications of each mark, and biochemical tests were performed on them.

Cultivation circles:

- 1 Buffered peptone
- 2- Bacillus cereus agar
- 3- Blood Agar Base

Microbial test

the sample was prepared with a weight of 10 g of it and put it in 90 ml of buffer saline and left it for 30 minutes to activate. Then it was transferred to a water bath at 85 °C for 30 minutes to get rid of the vegetative cells. Then a series of dilution was done where 0.1 ml of the third, fourth and fifth dilution was transporting on the culture medium Nutrient Agar was incubated at different temperatures ranging from (30 – 80 °C) to find out the optimum temperature for growth.

Count the bacteria that make up the spore forming:

This is to detect the bacteria by transport the bacterial in a water bath of 85 degrees Celsius for a period of 30 minutes to kill the vegetative cells and then incubating on the nutrient medium at 37 degrees.

A test for *B. sterothermophilus* Incubated at different temperatures (30, 37, 40, 45, 50, 55, 60, 65, 70) C for 15 minutes. Microscopic examination to view the internal spore using a gram dye.

Biochemical tests for *B. cereus*

1-Glucose fermentation: Suspicious colonies were taken and inoculated in tubes containing glucose medium and incubated at 37 ° C for 24 hours.

2-Voges-Proskauer reaction: The reaction was performed to detect acetyl methyl carbinol

3-Catalase testing of the bacteria's ability to produce the catalase enzyme by adding H₂O₂ to colonies that are 24 hours old.

4-The starch degradation test is a test for the ability of bacteria to degrade the amylase enzyme.

5-The hemolysis test to observe the hemolysis and on the center of the hematological mantle, and incubation at 37 degrees Celsius to observe the hemolysis of the beta type that distinguishes it.

Test for growth at different temperatures for isolated bacteria

Tubes containing the liquid nutrient medium were used, according to (Claus *et al.*, 1986). The test was all conducted at temperatures (20, 25, 30, 37, 45, 55) °C in a water bath with a cold and hot heating circuit. The tubes were inoculated with the bacterial strains and submerged in the water bath. The growth of colonies was observed every day for a period of three days.

RESULTS AND DISCUSSION**Table (1) Results of *B. sterothermophilus* isolation test at different temperatures for three days**

Date of production	Sample	Number	°C Temperatures								
			30	37	40	45	50	55	60	65	70
2/2019	Novolac AD	1	+	+	+	+	+	+	+	+	—
7/2018	Novolac AD	1	+	+	+	+	+	+	+	+	—
3/2018	Celia 2	1	+	+	+	+	+	+	+	+	—
4/2018	Sunny baby	1	+	+	+	+	+	+	+	+	—
2/2018	Nursoy	1	+	+	+	+	+	+	+	+	—

Table (2) Results of testing *B. cereus* isolates at different temperatures for a period of three days

Date of production	Sample	Number	°C Temperatures					
			20	25	30	37	45	55
2/2018	Dialak	1	+	+	+	+	—	—
4/2018	Dialak	1	+	+	+	+	—	—
10/2018	Dialak	1	+	+	+	+	+	+

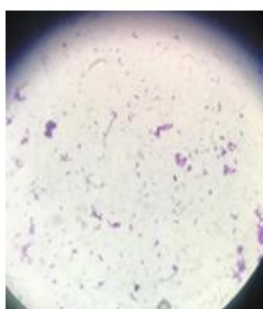
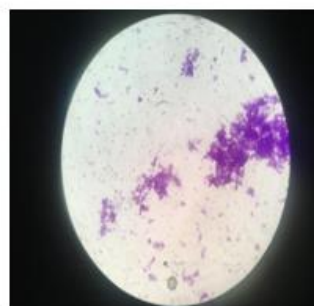
*B. sterothermophilus**B. sterothermophilus*

figure (1, 2)



figure (3,4)

figure (5) *Bacillus Cereus* Beta hemolysis

The results showed the presence of *Bacillus cereus* bacteria in some infant milk powder samples and at different rates *B. stearothermophilus* from Novolac AD milk samples with 0.9% contamination and one *B. stearothermophilus* isolate from each of Nursoy, Sunny baby1, Celia2 milk samples with 0.45% contamination. This indicates that these samples were not exposed to the better thermal treatment, while it was found that the samples that were exposed to the heat treatment were intact and were not exposed to any microbial contamination. The results of the *B.sterothermophilic* isolation test at different temperatures (30, 37, 40, 45, 50, 55, 60, 65, 70) C showed three days carrying the enzymes of these bacteria to resist high temperatures down to a temperature of 70, where it did not appear any growth in it and the growth varied according to the temperature shown in the study, where the best temperature for growth was 40 and 45 degrees Celsius, as the results showed that *B. cereus* isolates tested at different temperatures for a period of three days (20,25,30,45,55) . In Dialak milk, some strains are resistant to a temperature of 45, 55, and this may be due to the stability of the enzymes of these bacterial strains and the resistance of the internal spore to them. From the above we see that some isolates are positive for the catalase test, the VP test, and fermented for glucose and a analyzer for starch, and these tests give an indication of the possibility of these isolates to excrete Enterotoxin, as a study conducted by (Valero *et al.*,

2002) showed that the bacterial strains belonging to the wax bacillus are capable of analyzer the starch.

As for the motility test, some strains in the study showed their spread on the surface of the hemolysis, and with regard to hemolysis, studies have shown that the characteristic of bacteria is *B.cereus* according to a previous study (Chorin *et al.* ,1997). In a study of the world (Shadlia-Matug *et al.*, 2008) it was confirmed that 95% of medical infant formula and food, 60% of medicinal infant formula with fruits, and 65% of infant formula fortified with vegetables were contaminated with bacillary bacteria. In a study of the two worlds (El-Gendi and Wahba, 2013) 42.9% of infant formula fortified with banana fruit and 67% of medicinal infant formula with vegetables were contaminated with bacillary springs. In another study of the world (Sadiq *et al.*, 2016) samples of medicinal infant formula fortified with grains contaminated with bacillus spores were observed. The researcher Yacoub *et al.*(2017) recently recorded indicators of contamination of medical infant formula, contamination with bacillus bacteria, where spores were observed that ranged between 2.85 - 10 units forming a cell per gram., The isolation of *B. cereus*, which is an important bacterium due to its water activity inside the food and its ability to secrete toxins and grow even in cold temperatures (Darwesh *et al.*, 2015), in a study of the factor (Logan,1988) four types of bacilli were isolated. *B. cereus*, *B. licheniformis*, *B. pumilus* and *B. coagulans* Of medical infant formula, which has a relationship with food poisoning The results most common bacteria isolated from medicinal infant formula were *B. subtilis* and *B. licheniformis* .As mentioned (Al-Timimi, 2007) *B. cereus*, *B. circulans* and *B. pumilus* strains from medicinal infant formula were confirmed . Also confirmed (Abdelhameed *et al.* , 2018 ; Emam *et al.*, 2018) contamination of medical milk for children with *B. cereus* bacteria, where the total number of bacterial cells was from 4.0×10^1 to 2.1×10^2 1.45 x 10²) (Reduces bacterial contamination)

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تشخيص بكتريا *Bacillus spp* في حليب الاطفالمحمد موسى جعفر¹ حسين عوده كريدي²

1-دائرة البحوث الزراعية /وزارة العلوم والتكنولوجيا

2- كلية الهادي الجامعة / وزارة التعليم العالي والبحث العلمي

الخلاصة

اجريت هذه الدراسة لعزل وتشخيص بكتريا *Bacillus spp* من حليب الاطفال الرضع دون عمر السنتين. اذ جمعت 45 عينة حليب بواقع 3 مكررات للعلامة الواحدة وهي Dialak , Novolac AD, Similac 1, Nactalia 1, Celia2, Sunny baby1, Nursoy, Primalac1, Alpha1, Biomil plus, Dovelac1, Liptomil plus2, DoveGER, S-26 LF, S-26 AR, من صيدليات محليه مختلفة من مناطق بغداد للفترة من 2018/11/1 ولغاية 2019/4/30 وشخصت العزلات بالفحوصات المجهرية والمزرعية والكيموحيوية اذ امكن الحصول على 8 عزلات كانت جميعها تعود لمختلف السلالات العصوية والعصوية الشمعية وتضمنت 3 عزلات من *B.Cereus* من عينات حليب Dialak1 بنسبة تلوث 3.5 % وعزلتين من بكتريا *B. stearothermophis* من عينات حليب Novolac AD بنسبة تلوث 0.9% وعزله واحد بكتريا *B. stearothermophilus* من كل من عينات حليب Nursoy, Sunny baby1, Celia2 بنسبة تلوث 0.45.

الكلمات المفتاحية:

التحري , بكتريا , *Bacillus spp* , حليب الاطفال الطبي