



PROBIOTIC BACTERIA ISOLATED FROM FERMENTED MILK

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PMDC #: 87796-P.

Article Received on 20/03/2018

Article Revised on 10/04/2018

Article Accepted on 30/04/2018

ABSTRACT

Studies have shown that probiotic bacteria or so-called human-friendly bacteria are a group of organisms which have certain requirements and specifications to stay alive during their journey in the gut of man until they reach the last part of the small intestine, and studies have shown that this group has a significant impact on improving human and public health as well as help the body to resist many diseases and some of the most important strains of these bacteria are Lactobacillus and Bifid bacterium which were used to produce many dairy products raised on Worldwide for their important benefits. So the study focused on probiotic bacteria. The study was divided into two main parts. First: the definition of probiotic bacteria isolated from fermented milk products. Second: the preparation of some probiotic extracts from certain herbs and nutrients from the local environment to improve and increase the productivity of probiotic bacteria and comparison between these sources to identify the best materials and which have the capacity to improve the dynamic growth of these friendly bacteria through study. There was first: Isolation and identification of each of the bacteria Streptococcus. saccharolyticus, Bifidobacterium.sp, and Lactobacillus acidophilus. As well as the yeast Saccaromycess .sp was isolated by definition tests such as dye test gram – movement of bacteria tests –oxidize catalase test or the analytical profile index (API). Second: water extracts were prepared from sources which are Green tea – turmeric – naekhh – cloves – sage-Mint-Ginger-carobs (spectrophotometer) and it was found that the three best extracts in improving growth of bacteria probiotics were cloves then green tea followed by carob compared to the control sample grown on the liquid environment of the growth of probiotic bacteria MRS.

KEYWORDS: so-called human-friendly, Saccaromycess, saccharolyticus, Bifidobacterium

INTRODUCTION

Bacteria probiotics or so-called human-friendly bacteria organisms have certain requirements and specifications so that they can survive the arduous journey in the gut until they reach the last part of the small intestine or colon.^[1] The Latin word "probiotic" is composed of two "Pro" means "for" and the second "biotic" means "life" and include the probiotics group of bacteria, the most important. And these types of bacteria live in the intestines of human for ecological balance with other bacteria, and help prevent the type of pathological bacteria, some of these bacteria also produce vitamin (b), (c). And assist in digestion, improve absorption of nutrients, and dramatically reduce cancer-causing substances, and strengthen the body's immune function.^[2]

The Probiotics also plays an important role in easing digestive pain, and help sensitive bowel, or: ulcerative colitis, and helps in the prevention of allergies. Preliminary research also said that probiotics are

effective in preventing the risk of eczema in children, studies conducted on samples from animals, showed that probiotics effective in preventing tumors, so it does have some antioxidants, which may help fight infections.^[3]

Probiotics bacteria that human get from nutrition have different benefits for microbial balance within the intestine and prevents the body many diseases as eating probiotics and especially after the cases requiring antibiotics as antibiotics eliminates all organisms whether useful or sick so you must eat foods rich probiotics after antibiotic medication to restore natural balance of flora within the body.^[4]

Role of Probiotics in Human Health

- Keep balance of natural microbes in the gut and respiratory system.^[5]
- Improve resistance to settle for microbes in the urogenital.^[6]
- Reduce the level of cholesterol.^[7]

- Suppress mutations in components of the gut and intestinal tumors.^[8]
- Decrease allergies to lactose.^[9]
- Improve the digestion of protein.^[10]
- Helps in synthesis of vitamins and mineral salts by metabolic activity.^[11]
- Prevent or reduce vulnerability to digestive diseases and prevent digestive disorders, constipation, liver disease, and inflammation of the skin.^[12]
- Reduce the raised blood pressure.^[13]

In a study of scientists indicated that hot extracts from natural sources are used as sources for the development of useful bacteria in the body.^[14] In another study it was shown that the use of certain formulations containing water extracts of carob contributes positively in the treatment of recurrent and chronic diarrhea in children with diarrhea to high mortality among children. The following are the most important species said that was used as a source of probiotics to recognize their importance and worth of the human person.^[15]

Sage is aromatic herbaceous Muammar known scientifically *Salvia officinalis* and studies have shown that vegetation Sage strengthens weak memory and back in a short time.^[16] Another study had confirmed the quote where Sage was found to inhibit the enzyme responsible for breaking down the acetylcholine brain which causes Alzheimer's.^[17]

Green tea has many significant benefits, although most people don't realize its benefits but they didn't even try drinking it but they only drink black tea which is less useful for the benefits of green tea. Tea has more than 3000 species,^[18] and is named each type according to cultivation area, and tea tree bushes grow about 7.5-9 cm depending on the viability, But when planted, they are clipped up to 1.5 cm only the leaves are harvested every 6-14 days, and each bush produces about 125 grams of tea leaves every year and continue to be productive of 25-50 years or more than 100 years, especially if grown with organic materials, and after harvesting, the tea leaves extracts from more versatile types from those extracts of black tea. There is also a third type called (Oolong) which is partly fermentation, tea is also referenced as leavened in part by drying bringing properties of black tea and green together.^[19] Some of the most important components of green tea and its health benefit are.

First: The bitter component in green tea, or what is called (catechin) this component works to reduce the likelihood of human infection by the cancer and protects against tumors, and also works to reduce the level of cholesterol in the blood and reduces the risk of turning into cancerous tumors, polyps and also works to prevent high blood pressure, it is also noteworthy that fights influenza viruses and prevents bad breath.^[20] Secondly: Caffeine that helps alertness also frees from fatigue and drowsiness. And also it works as diuretic.

Carnations Clove is an evergreen tree with leaves and clove oils extracted from leaves and flowers, and is considered one of the basic shapes used in medical purposes using the mature flower buds dried in herbal formulations also in traditional Chinese medicine. Clove oil has anti-inflammatory properties and microbes it also analgesic and antifungal, and grants the plant has special privileges in the treatment of some medical conditions including facial nerve paralysis, chronic stress, which helps reduce food poisoning kills clove oil some bacteria including *Pseudomonas aeruginosa* all types of *Shigella* as well as *Streptococcus pneumoniae*, which can cause food poisoning all clove oil reduces the feeling of gas pressure felt by patients with stomach ulcers usually so frustrates alioginol found in clove oil transmission Neural signals that a person feels bloated and gas pool although not working directly to prevent grass production also protect the body from cancer of the stomach, periodontal and dental pain: used flowers and clove oil worldwide to generations of tooth pain relief.^[22]

Alnankhah leaves (Ajowan) is a herbal plant gives a bunch of fruits on white umbrella body turn after maturity to brown. These fruits contain volatile oils like Thymol. It increase urine exertion, relief urinary colic, dysuria, and nausea.

Mint is green plant with aromatic smell especially Peppermint tree does not prevail on the Earth more than 30 centimeters remains. When eaten fresh, it appetizes and relieves heart burn, suppresses nausea, stomach aches and cramps. Also it is helpful in gout itching and hemorrhoids, poor vision, headaches, chest phlegm, soothes toothache, and is antispasmodic effect and contains acceptable amount of dietary fibers.^[24]

Turmeric is rhizomes as seed near the surface of the earth blocks herbal everlasting but his yearbook, containing turmeric on oils by 4.2-14% oil, Studies have proven its benefits are used in folk medicine and has a fantastic force especially for digestive function of liver and bile. It is useful for treating many diseases like diabetes.^[25]

Ginger contains volatile oils, resins, and is stimulates beneficial bacteria in the body. Used as a condiment, manufactured in drink powder. It is useful for colic and diuretic, strengths teeth and gums and treats diarrhea and dysentery. It is helpful for diabetics.^[27, 28]

Carob is an evergreen tree up to 10 meters in height and has a large violet horns the fruit structure and grind these in some countries of the world. It's fruit contains about 50-70% sugar and fibers which helps in activating the body's beneficial bacteria that treats irritable bowel syndrome (IBS) and also it contains starch, fat and protein and vitamins and acid, tannins.^[27, 28]

Therefore, the study focused on isolating and defining some types of probiotics in some products in the

domestic market and also prepare some extracts of some sources of local environment probiotics and determine the best of these sources to improve the development of the probiotics bacteria.

AIM OF STUDY

To isolate and define some types of probiotics found in natural and dairy products.

OBJECTIVES

- To isolate the probiotics bacteria sources of fermented structure in specialized environments.
- To identify bacteria by standard microbiological tests as well as by bacterial identification system by API.
- To prepare watery extracts of some food sources for probiotics and development of bacterial isolates and compare these sources to improve the growth of probiotics by measuring the density of bacterial growth by spectrophotometer.

MATERIALS AND METHODS

A Fermented milk was used to isolate the probiotic bacteria. MRS environment (de Man, Rogosa and Sharpe) specialized to isolate and develop bacteria anaerobic probiotic in dairy products. MRS broth liquid environment used for the isolation of Lactobacilli bacteria.

M17 liquid environment to isolate Streptococcus. By using both H₂O₂ and oxidase test reagent in both catalase and oxidase respectively. The nomination paper and credit no. 42. Natural materials from the local environment for testing green tea, cinnamon, turmeric, ginger, carob, mint, Nankh, and sage.

The API test used from (Biomeri company France eux), and Centrifuge type Td4 table-Typelow Speed Centrifuge Spectrophotometer model/ GENESYS-10 uv-modison/ WI 53711 from the USA.

Measurement of microbial growth by spectrophotometer

Quantification of bacterial growth is very important in most of the applied studies and essential in assessing the impact of various physical or chemical transactions such as temperature and pH on the growth and proliferation of bacteria. There are many ways to calculate it, and one of those ways is to assess the degree of turbidity.

Assessing the degree of turbidity using spectrophotometry

The basis of this method is that when the growth of bacterial cells in the center of the liquid diet increases all increase significantly resulting in disturbance in the Middle, the perfuming degree depends on type and number of bacterial cells in each case taking into account the selection of wavelength optical wave length appropriate for bacteria and is the wavelength where the

degree of absorption cells or solution or commenter used the largest possible.

Method of work

Media was prepared for 18-24 hours of bacterial species on various water extracts in addition to nutritious liquid. Nutrient broth environment about 50 ml per 250 ml flask capacity. Bacterial growth was checked the (from different extracts prepared) by centrifugation at 3000 cycle per minute for 15 minutes. 10 ml of sterile water was added in the tube, that cells are distributed regularly, then transfer 1 ml sterile pipette through the test tub tube containing 9 ml sterile water to get ease 1:10 10-Referred the commenter diluted with tube and then transfer 1 ml sterile pipette through another tube containing 9 ml sterile water to get ease 1:100 10-2.

Absorption was measured by spectrophotometer for the light cascading using the device at 570 nm wavelength. Then bacterial growth was Checked again in various extracts prepared for comparability in extended dynamic growth of probiotics isolated bacteria.

RESULTS AND DISCUSSION

The study focused on beneficial bacteria probiotic its broad health effects already mentioned To split the results to two main parts.

1. Isolation and identification of bacteria from probiotic basic dairy products raised in local markets.
2. Prepare extracts of probiotics from domestic markets products and to improve the growth of bacteria, probiotic and compared to identify the best sources probiotic.

Isolation and identification of probiotic bacteria: Producing bacteria were isolated two types of dairy products raised are fermented milk, as well as the case of curdled and by isolating bacteria from both producers on specialized environments for the development of lactic acid bacteria is MRS as well as M17 environment which is specialized in the development of globular lactic acid bacteria through the total count of bacteria developing on both environments found that the numbers of bacteria isolated from sour to sample the environment MRS 40 x 10 and the numbers of bacteria on the M17 environment is 25 x 10 while preparing bacteria isolated from a sample fermented milk the environment MRS 60 x 10 while setting an M17 52 x 10 comparing these numbers with effective daily dose of bacteria probiotics note that these products covering the needs of the person of the bacteria are beneficial to human health.

(1) The growth of the bacteria probiotic the environment MRS – it is clear from the shape of the initial qualities characteristic of lactic acid bacteria that grow inside the environment composed of a pinhead-sized colonies shape.

Following the total bacterial count on the development of specialized environments, isolation of bacteria strain Identified through several preliminary tests to identify developing strains and these tests as follows.

Colonial gram description of movement cells oxidase catalyst. The table shows the differences between the strains isolated from the sample of fermented milk where

some isolated strains in each test g movement but differ in both sensitivity to catalase and oxidase in the Description cell. Isolated from the sample of fermented milk where some isolated strains in each test g movement but differ in both sensitivity to catalase and oxidase in the description cells.

Table 1: Types of strains isolated from the sample

Strain	Gram	Shape	Movement	Catalase	Oxidase
1	+	Spherical in clusters	Dynamic	-	-
2	+	Bacillus	Dynamic	-	+
3	+	Bacillus	Dynamic	-	-

Table 2: Types of strains isolated from fermented milk sample

Strain	Gram	Movement	Catalase	Oxidase
1	+	Dynamic	-	+
2	+	Dynamic	-	-
3	+	Dynamic	+	+

It is clear from the table (2) difference between the strains isolated from the sample case of curdled and had noted that the major isolates in deferential cells where the second and third isolates were stick figures while the first isolation spherical.

Followed by initial definition tests is affirmative and testing that identifies the type of isolated strains is the API test.

Sample	IND	MR	GLU	MAN	LAC	SAB	MAL	SAL	TRV	ARA	GEI	ESC	GHY	CEI	MVE	MILZ	RAF	SOR	RHA	TRE	CAT	SPOR	Green	Coc
Y (1)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
MRS																								
Y (2)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
M17																								
R (1)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
MRS																								
R (2)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
M17																								

And through the use of tables to test API20A tests to While strains isolated from a sample case of curdled identify developing strains were found to be strains were. on sample fermented milk.

- 1 - Streptococcus saccharolyticus 1_Lactobacillus acidophilus
- 2 - Lactobacillus acidophilus 2- Bifidobacterium sp
- 3 - Bifidobacterium sp.



Table 3: API test results for the samples and selection bacterial strains.



Figure (6-2): shows the tubes inoculated with bacteria containing different extracts and liquid environment.

After completion of the vaccination with strains isolated over water extracts prepared nurseries – bacterial growth was measured by increased turbidity for liquids.

(3) The API tapes

And types of strains that were isolated and identified it all from lactic acid bacteria group and it is one of the most important types of beneficial bacteria (probiotic) of man where bacteria *Lactobacillus acidophilus* and *Bifidobacterium*, *Streptococcus saccharolyticus*, All of the microorganisms most commonly used resistance to harmful bacteria is already sick and living in the intestine and the vagina, and protects the body against bacteria and all species are useful to the body.

There are several theories about the role of microbes in digestive symptoms of ageing, namely health status and degree of human activity. Where intensified studies in this field, choose strain of treatment isn't easy, it must be of a type that is not the disease occurs and produces no toxins, and can be transported in the body without causing disease or harm him, and most commonly used treatment is a bacteria *Lactobacillus allaktobasilas* and *albevidobaktrame* bacteria *Bifidobacterium*, are present in some types of milk and yogurt and have a large interest in digestive health and significantly useful microbial treatment to maintain a good balance of beneficial microbes in the colon, The problem with eating food containing useful microbial bio, she confronts the digestive chemical and natural obstacles such as Succulents intestinal and bile salts, as a result, few can access and settlement area of the small intestine and the colon, to overcome this problem add probiotic Probiotics are foodstuffs help friendly bacteria breed probiotic Probiotics encourage the growth of beneficial intestinal microbes resistant to intestinal enzymes did not only benefit the friendly microbes. The most important of these nutrients are sugars of type alaligoskri.

II. Preparing water extracts of various sources to improve the growth of bacteria isolated probiotic

Following the isolation of bacteria useful probiotic and recognized during the first part of the study the collection and transfer of probiotic bacteria cells into specialized liquid environment (MRS Broth). For the development of water extracts prepared from natural sources in the environment and learn about the best any of these sources to improve the dynamic growth probiotic.



(4) Development of probiotic bacteria to the environment compared to liquid control.

It was at the beginning of this stage the transfer of bacteria isolated from environment to environment specialized MRS solid liquid to liquid environment and the results were as follows.

Development in preparation for transfer to water extracts prepared under study.



(5) **Different bacteria genera isolated** The study of preparation of water extracts of many food sources used in the local environment (green tea – turmeric – *trachyspermum Ammi* – pink – Sage – Mint – Ginger – carob).

Races were moved to 5 ml bacteria isolated from liquid (4 ml liquid environment MRS + 1 ml of the aqueous extract of different specimens under study) compared with sample Officer (control) and the transfer of the same genera of bacteria but to 5 ml of environment MRS broth only without any additives.

After the transfer of bacteria best growth in two isolated from each (case of curdled – fermented milk (yogurt) probiotic on various water extracts are being stocked and measure growth after incubation period using photometric device spectrophotometer for determining the best homemade extracts improve the growth of bacteria by increasing turbidity in liquid environment.

The following figure placed pipes that were the development of bacteria at all prepared extracts.



Figure (6-1): shows the tubes containing the liquid environment only without inoculation with bacteria.



Figure (6-2): shows the tubes inoculated with bacteria containing different extracts and liquid environment.

After completion of the vaccination with strains isolated over water extracts prepared nurseries – bacterial growth was measured by increased turbidity for liquid environment and the results were as follows.

Table 4: Results of reading different breeds of water extracts prepared.

Sample	Fermented Milk		Fermented Milk	
	1	2	1	2
Green Tea	2.34	2.11	2.41	2.15
turmeric	2.05	1.47	1.43	1.44
Nakha	1.47	1.81	1.59	1.80
Clove	3.47	3.82	2.84	3.82
Sage	1.89	1.67	1.77	1.74
Mint	1.47	0.75	1.37	1.88
Zingber	1.90	0.85	1.82	1.48
Carobs	1.78	2.02	2.39	1.16
Control = 1.428				

Comparing different samples with the sample control and through the table note that all samples were higher than reading the sample control, this indicates that water extracts have improved dynamic growth of bacteria. On the other hand, comparing both extracts find the best water extracts in improving growth of bacteria was aqueous extract of cloves, followed by green tea and this trend is consistent with the 1997 Vanderhoof stated that some natural extracts used probiotic improve probiotic while growth was lower than in extracts improve growth is apparent from these results Mint how important such different elements to improve the growth of friendly bacteria to humans, these results agreed with numerous studies and scientific research in the world.^[29]

In a study of Custodio *et al.*, 2011 stated that carob of important nutrients which have been used in the medical aspects, the most important of which is to improve the growth of beneficial bacteria in the human body. These results are consistent with the Su *et al.* 2008 stating that green tea has been instrumental in improving the dynamic growth of bacteria useful body.^[31]

Wasson *et al.*, 2008 stated that green tea has many benefits using probiotic as well as other health benefits contains phenol polyurethane compounds that are potent antioxidant and contains many articles which are counter to the occurrence of tumors.^[32]

1. In quantities that meet human needs we mentioned during the study about the importance of these types of foods to improve the overall health of the human and natural flora natural rebalancing of the body as well as the protection of many human diseases, which spread widely especially in domestic environments, has identified through this research on the importance of these foods in reducing risks and prevention of diseases by consuming such types of safe foods that contain many useful bacterial species such as the, as well as some important races such as the these food sources are completely secure and free from any chemicals or vital human harm but, on the contrary, they return it to benefit and improve public health and, indeed, through the study were isolated and identified many of the useful bacteria and most bacteria are isolated.

2. The importance of the use of certain nutrients that improve growth and preparation of the bacteria are beneficial to the body or the Probiotic for balance of beneficial bacteria to the body, especially in the wake of diseases requiring medication some > and antibiotics, which kill bacteria causing disease but also kill beneficial bacteria to the body and thus notable decline of beneficial bacteria in the body, and studies have shown that there are many nutrients that help improve the growth of friendly bacteria And restore balance within the body of this study dealt with several articles of the test environment and identify the extent to which these substances improve the dynamic growth of these friendly bacteria of Group of nutrients that study aqueous extract of cloves were followed by aqueous extract of green tea – then aqueous extract of carob, as the study proved that most prepared extracts led the improvement of dynamic growth of beneficial bacteria, so if compared to the growth of beneficial bacteria to the traditional environment for the development of probiotics.

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