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INVESTIGATION OF FERMENTED FOODS FOR ISOLATION OF BENEFICIAL PROBIOTIC MICROORGANISMS

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Abstract
A "potential probiotic" refers to a microorganism that has the potential to confer health benefits when consumed. Probiotics are beneficial bacteria, or they can be known as friendly to human health, especially for the digestive system, because they are very beneficial to the intestines. In the current study, we highlight products that contain therapeutic alternatives for human health found in home-fermented foods, as well as those available in local markets, where tests were positive for the presence of probiotics in these foods.
Out of 50 samples, there were 60 isolates, of which only 40 belonged to Lactic acid bacteria LAB, and the appearance on MRS agar media is shown white, large, smooth, little convex, little mucoid, and round colonies with entire margin, under the microscope, The cells exhibited a positive reaction to Gram staining, presenting as purple rods or cocci with rounded ends, occurring individually, in pairs, or in short chains, and non-spore-forming, and the biochemical characteristics of the catalase and oxidase tests were negative, there were two genera, Lactobacillus and Lactococcus as a potential probiotic found in foods.
The twenty isolates were excluded because they were growing with difficulty on MRS medium and had different shapes and colours of colonies, also the cells under the microscope had a negative Gram stain, while oxidase and catalase tests were positive.

Keywords: Bacteria, Lactic acid bacteria, Lactobacillus, Lactococcus, probiotics, Gram-positive, Gram-negative.

Introduction

Probiotics are living microorganisms that, when consumed, can positively impact health by enhancing the natural intestinal microbiota. Certain types of lactic acid bacteria (LAB) are considered probiotics, while others may have potential as probiotics or are used as fermentation cultures in the food industry. LAB encompass a group of microorganisms characterised by being gram-positive, tolerant to oxygen, resistant to acidic conditions, typically not forming spores, and not requiring oxygen for respiration. Many LAB strains play vital roles in fermenting foods and are recognised as safe for consumption (GRAS status). Numerous laboratory studies have demonstrated that probiotic LAB can inhibit the

growth of harmful foodborne pathogens. Additionally, LAB can break down prebiotics in the host, exhibit immune system-modulating properties, and reduce cholesterol levels [1].

Scientific evidence supports the therapeutic benefits of probiotics in preventing and treating various health issues. These benefits include relieving lactose intolerance, safeguarding against gastrointestinal infections, boosting the immune system, reducing serum cholesterol levels, exhibiting anti-allergic properties, and preventing urogenital diseases. LAB can be sourced from a variety of foods, including fruits, vegetables, juices, grain products, and fermented foods. Recent research in Nigeria has identified LAB strains from fermented foods that demonstrate desirable characteristics for probiotic use[2].

Probiotics are actual microorganisms used as food additions to enhance health by equilibrating the microbial milieu in the gastrointestinal system. Lactic acid bacteria (LAB), frequently used in protective isolates, are among the most prevalent probiotic bacteria and are regarded as safe vs pathogenic populations. LAB is naturally found in the environment and can inhibit the increase of harmful microorganisms by producing specific substances. Scientific studies have reported various beneficial effects of probiotics, including anti-allergic and anti-cancer properties; promoting fat loss; enhancing the host's immune response; and alleviating symptoms of irritable bowel, enteric inflammation, and antibiotic-induced diarrhoea [3].

In recent times, probiotics are not only recognised for their growth-promoting effects but also for their role in stimulating the immune system and preventing numerous diseases. The nutritional value and therapeutic effects of food probiotic products have gained significant attention in the health sector. One of the most important types of bacteria that are beneficial to the stomach is a good probiotic. Lactobacilli are considered one of these beneficial bacteria. They are gram-positive and catalase-negative bacteria, as they are natural bacteria found in the human mouth, female reproductive system, and intestines, where they act as a preservative and work to limit the effect of microorganisms that are harmful to health. [4].

Lactobacilli play a significant role in regulating the undesirable microflora in the gut, effectively preventing the proliferation of pathogenic bacteria through the production of antimicrobial metabolites. They serve as natural biological preservatives and are naturally present in foods. When screening bacteria for their probiotic potential, three important criteria include antimicrobial activity, tolerance to bile salts and acids, which are essential for their medicinal use. During lactic fermentation, lactic acid bacteria (LAB) generate a range of chemicals, including organic acids such as peroxide of hydrogen peroxide and diacetyl, bacteriocins, and antifungal agents such as fatty acids. Bacteriocins are protein molecules that can suppress the growth of susceptible pathogenic bacteria and have a unique breakdown mechanism in the digestive tract, differing from that of antibiotics [5]. When using these bacteria, LAB works to resist lysozyme, stomach acidity, and other stomach secretions, as well as the bile gland. It also works to resist many harmful bacteria and reduces the effectiveness of some germs due to the chemicals they produce, as these substances have direct effects on pathogenic bacteria and reduce their toxic secretions. Lactic acid bacteria in dairy products have preventive properties against pathogenic bacteria, serving as a natural defensive mechanism. A multitude of studies have examined the antibacterial properties of

probiotics versus both Gram-positive and Gram-negative bacteria, including E. coli, Pseudomonas aeruginosa, as well as Staphylococcus aureus [6].

The main objective of this study was to isolate, identify, and characterise potential probiotics.

MATERIALS AND METHODS

Chemicals Materials

Chemical materials used in the study are listed in Table 1.

Table 1: Chemicals and Materials used in this study.

Chemical	Company	Origin
Absolute Ethanol	ROMIL pure chemistry	UK
Absolute Methanol	Sigma	U.S.A
Agar – Agar	Himedia	India
Calcium Carbonate ($CaCO_3$)	Sigma –Aldrich	Germany
Catalase solution	Oxoid	Germany
Methanol	BDH	England
Nystatin	Local market	Iraq
Catalase reagent 3%	Oxoid	Germany
Oxidase reagent	Oxoid	Germany

Culture Media

All media mentioned in Table 2 were prepared and sterilised according to the manufacturer's instructions by sterilising them for 15 minutes at 121°C.

Table 2 lists the cultural media and their sources used in the current work.

Culture media	Company	Origin
Man, Rogosa Sharpe MRS-Agar	Oxoid	England
Man, Rogosa Sharpe MRS-Broth	Oxoid	England

Stains and Reagents

All reagents are ready to use. It is recommended to filter all stains before use. These reagents are imported from the Al Hnoof factory, Jordan

Table 3 shows the Gram stain composition

reagent composition	Percentage
Gram's Crystal Violet (Methyl Violet)	0.5%
Gram's Iodine (Lugol's iodine) -Potassium Iodide -Resublimed iodine	 2.0% 1.0%
Gram's Decolouriser -Methanol -Acetone	 80% 20%
Gram's Safranin (Counterstain)	1.0%

Methods

The collection and processing of samples for the isolation of possible probiotic Lactobacillus and Lactococcus species from various sources is being undertaken.

We selected 50 fermented foods from various sources, such as bread, fermented vegetables, cheese, yoghurt, and milk. It was used to isolate food samples. From each sample, 1 g or 1

ml of solid and liquid, respectively, was taken and placed in a test tube. 9 ml of MRS medium was added and mixed well by continuous stirring to obtain a homogeneous consistency.

Fermented Vegetables

Vegetables (cabbage, cucumber, carrot, cauliflower, lettuce, eggplant, turnip and olives) were collected from local markets, gently washed and cut into small pieces, then placed in a clean, suitable container. 3% NaCl solution was added to them, covered and incubated at 35°C for 3-7 days [7].

Isolation of LAB: Lactobacillus and Lactococcus spp.

One millilitre of the sample was carefully added to each of the nine millilitres of MRS broth in each tube. For 48 hours, all of the tubes that were inoculated were kept in an anaerobic incubator at 37°C. Following that, dilutions ranging from 10^{-1} to 10^{-6} were made serially. Proceeding to add the bacteria to MRS agar plates, 100 microliters of the third dilution was then put on top. A candle jar was used to incubate the plates anaerobically with 3% CO_2 and 1% calcium carbonate. After 48 hours at 37°C, cultures were treated with 0.1% antifungal (Nystatin) to stop fungal growth. Following incubation, big white colonies of more purified MRS agar were streaked, and colonies were transferred onto it. Before being tested for identification, they were cultured under the identical conditions described earlier. [8]

Identification of Lactococcus and Lactobacillus spp.

Morphological identification

Morphological properties of isolates were examined, involving diameter, colour and shape of colonies as well as the microscopic morphology of cell shape and Gram staining.

Gram staining

A full loop of water was placed on a circular area on a slide, and small amounts of bacteria were added and mixed into this water drop. After spread and drying of smear, the slide was rapidly passed through the flames of the burner three times to fix the colonies on the slide. Then, stain heat-fixed smears with Gram's crystal violet for 1 minute and wash off with tap water and flood the slide with Gram's iodine for 2 minutes. Then wash off with tap water and decolourise smear with Gram's decolouriser for a few seconds, and wash well in tap water and counterstain with safranin for 2 minutes. Only then, wash with tap water and allow drying. After this step, examine under a microscope using an oil immersion objective.[9] The results will be if it occurs in black or blue, the result will be gram-positive bacteria if the bacteria under the microscope are black, purple or blue, but if it is red, it will be gram-negative bacteria

Biochemical identification

Biochemical tests were used for the characterisation and identification of Lactobacillus and Lactococcus isolates as follows:

Oxidase test

This test was performed by soaking a piece of filter paper in 1% solution of the oxidase test reagent, and then a small amount of culture was placed using a loop. After 5 to 10 seconds, the positive reaction was noted as the appearance of a deep-purple colour [10].

Catalase test

After an overnight of bacterial culture, a single colony was smeared with a sterile wooden stick on a clean microscope slide. Then a drop of 3% H2O2 was added to the slide. After the test is done, the positive result is indicated by the formation of gaseous bubbles, which means production of catalase [11].

Bacterial Isolates' Preservation and Maintenance

Bacterial isolates' maintenance was carried out as follows:

Short-Term Preservation

For a few weeks, bacterial isolates were grown on MRS agar slants before being firmly covered using parafilm, then kept at four degrees Celsius.

Sterilisation methods

Three methods of sterilisation were used first and second methods in the current study for the sterilisation of media, solution and glasses.

Moist heat sterilisation (autoclaving)

for 15 minutes at 121ºC and pressure (15 lb/in²) all Media and solutions were sterilised by autoclaving.

Dry heat sterilisation

An electric oven was used to sterilise glassware at 160 ºC for 3 hrs. or 180 ºC for 2 hrs**.**

Membrane sterilisation (filtration)

Millipore filtering was used to sterilise heat-sensitive solutions by a Millipore filter paper (0.22 µm in diameter).

RESULTS AND DISCUSSION

Collection, Isolation, and Identification of probiotics

Isolation and identification of Lactic acid bacteria

All these samples were collected during the period from the beginning of November 2023 until the beginning of January 2025 and were prepared according to the aforementioned protocol in the presence of CO_2 gas at a concentration of 3-5% generated from a candle jar. As a result, the results showed the presence of 40 isolates distributed equally between Lactobacillus bacteria and Lactococcus bacteria, with 20 isolates for each. The data were collected, as shown in Table 4, using the selective enrichment culture technique, that is, culturing the cells on MRS agar, which allows the selective growth of (Lactobacillus and Lactococci) and prevents other species. To verify it accurately, it was subjected to several tests, including biochemical, microscopic and morphological.

Table 4: Food and clinical samples used to isolate probiotics

Type of samples	No. of samples
Fermented vegetables	20
yoghurt, cheese, cream and liquid milk	25
Sourdough	5

Morphology and Microscopy of probiotics

MRS agar is a very suitable medium for the growth and isolation of Lactobacillus and Lactococcus bacteria. It contains calcium carbonate, so it was used after identifying these two types of bacteria. It contains all the nutrients necessary for their growth. As a result, they were described and their condition was shown. They appeared in different sizes and within circular colonies surrounded by clear areas. They were white in colour and had a smooth, convex texture. [12].

Microscopically, the bacteria appeared under an oil immersion lens (100x) with Gram stains as the gram-positive purple sphericals (cocci), rods (bacilli) that existed singly, in pairs, or in short chains, as shown in the following figures (1,2). [13]

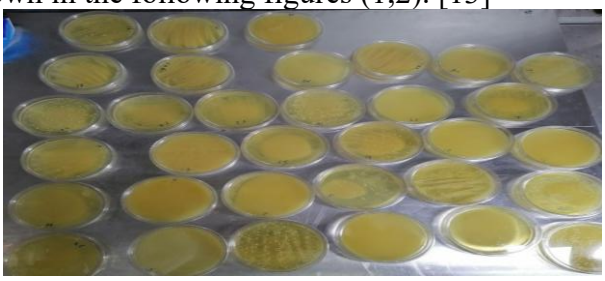

Figure 1: morphology examination of the lactic acid bacteria grown on MRS agar containing calcium carbonate

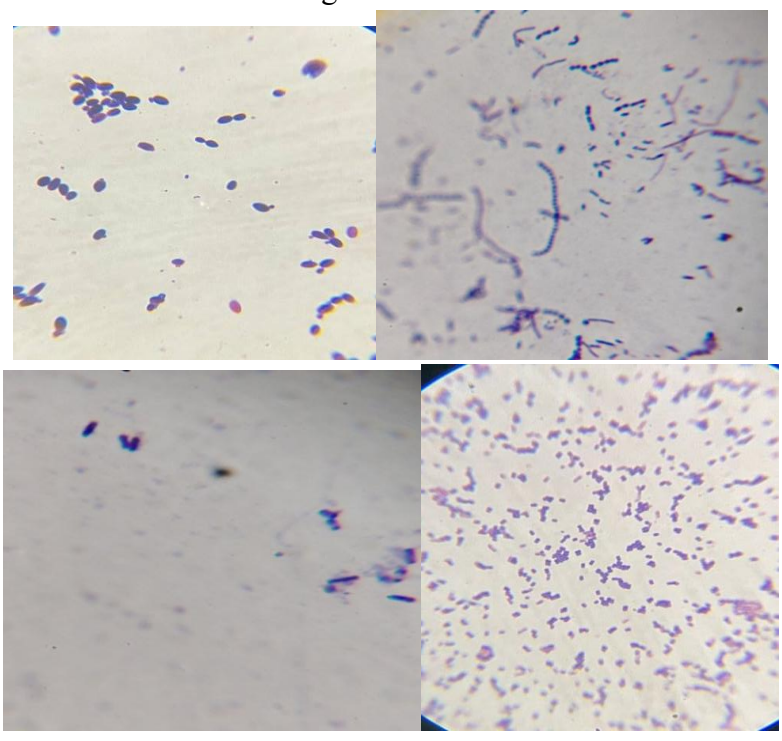

Figure (2): morphology examination of the lactic acid bacteria under a microscope (100x).

Biochemical identification of lactic acid bacteria

Forty isolates that confirmed belong to the genus Lactobacillus and Lactococci were negative for catalase and oxidase, while twenty isolates were positive and did not belong to lactic acid bacteria, as shown in the following figures (3)

Moreover, catalase and oxidase tests were valuable tests to identify the genus of Lactobacillus, and therefore, the above tests were performed for all isolates. [14]

Figure (3): catalase test, a: positive, and b: negative.

Discussion

Alternative medicine or natural medical alternatives is a new global trend that many countries have begun to rely on instead of synthetic pharmaceutical compounds to treat medical conditions and diseases. Among these is the use of certain types of beneficial bacteria, including probiotic bacteria, as they are already present in the human intestine as natural beneficial bacteria that work to combat many microorganisms that are harmful to public health by reducing the incidence of intestinal infections and cholesterol and strengthening the body's immunity, as these bacteria work to form certain acids, including lactic acid. The high acidity value in the intestine can eliminate many numbers and types of bacteria. They also produce substances that work as preservatives, such as bacteriocin [15]**.**

In this study, we found that fermented foods, such as dairy, dough, and vegetables, can be a medium for microorganisms that are beneficial to human health, thus obtaining healthy food that contains therapeutic alternatives for most of the diseases faced by humans, and also putting an end to drug resistance caused by excessive use of medication.

The results showed, based on the growth of colonies on MRS culture medium in non-aerobic conditions, that they are lactic acid bacteria, and also by the lysis of calcium carbonate, evidence that the bacteria secrete lactic acid, and based on the Gram-negative stain and their appearance under the microscope, most of them belong to the genera Lactococcus and Lactobacillus.

It has long been known that dairy products are beneficial for people's health. Recently, many scientists from all over the world have separated and isolated lactic acid bacteria, also known as LAB, and the lactobacilli from traditional foods and looked at how well they fight off different diseases. Microorganisms like lactobacilli, as well as other bacteria, can kill infections in several ways, such as by competitive exclusion, which helps keep food safe [16].

CONCLUSION and Recommendations

Conclusion

In conclusion, the findings of this study highlight the significant role of the antagonistic effects of bacterial by-products against a broad spectrum of microorganisms in both food preservation and human health. These bacteria hold potential for use in the production of various food and pharmaceutical items and can contribute to the development of novel functional foods. Therefore, it is advisable to increase the consumption of dairy products enriched with probiotics and to focus on identifying and producing foods containing the most potent and effective lactobacilli for inclusion in daily dietary habits.

Recommendations

Conduct further research to explore the specific mechanisms underlying the antagonistic effects of the isolated bacterial by-products. This could involve molecular studies to identify the active compounds responsible for inhibiting the growth of harmful microorganisms**.**

Investigate methods to optimise the production processes for incorporating probiotic bacteria into dairy products and other food items. This could involve studying factors such as fermentation conditions, strain selection, and storage conditions to maximise the viability and efficacy of probiotics in the final products**.**

Increase public awareness and education regarding the health benefits of consuming probiotic-enriched foods. This could involve educational campaigns to inform consumers about the importance of probiotics in maintaining gut health and overall well-being**.**

Encourage the development and production of a wider range of functional foods containing probiotics. This could include exploring new food matrices and formulations to appeal to different consumer preferences and dietary needs.

References

1. Anitha, P. M. D., Periyar Selvam, S., & Kulkarni, S. A. (2018). Isolation and characterization of potential probiotics from fermented ragi (Eleusine coracana). International Journal of Pharmacy and Pharmaceutical Sciences, 10, 145-151.
2. Kunchala R, Banerjee R, Mazumdar SD, Durgalla P, Srinivas V, Gopalakrishnan S. Characterization of potential probiotic bacteria isolated from sorghum and pearl millet of the semiarid tropics. Afr J Biotechnol 2016;15: 613-21.
3. Hawaz E. Isolation and identification of probiotic lactic acid bacteria from curd and in vitro evaluation of its growth inhibition activities against pathogenic bacteria. Afr J Microbiol Res 2014; 8:1919-425.
4. Nsofor CA, Sarah U, Chinyere U. Isolation and characterization of lactic acid bacteria from ogi sold in Elele Nigeria. J Biol Food Sci Res 2014; 3:19-22.
5. Salehi M. Antagonistic effect of lactobacilli isolated from native food. J Food Sci Technol Innov 2012; 5:1.
6. Sharafi H, Maleki H, Ahmadian G, Shahbani Zahiri H, Sajedinejad N, Houshmand B, et al. Antibacterial activity and probiotic potential of Lactobacillus plantarum HKN01: A

new insight into the morphological changes of antibacterial compound-treated Escherichia coli by electron microscopy. J Microbiol Biotechnol 2013;23:225-36.

7. Fleming, W. G. K. (1992). A new method for signle pile settlement prediction and analysis. Geotechnique, 42(3), 411-425.

8. Mannan, A. A., Liu, D., Zhang, F., & Oyarzún, D. A. (2017). Fundamental design principles for transcription-factor-based metabolite biosensors. ACS synthetic biology, 6(10), 1851-1859.

9. Erum, S., Naeemullah, M., Masood, S., Qayyum, A., & Rabbani, M. A. (2012). Genetic divergence in amaranthus collected from Pakistan.

10. Benson, A. D., Johnson, S. D., & Kuchinke, K. P. (2002). The use of technology in the digital workplace: A framework for human resource development. Advances in Developing Human Resources, 4(4), 392-404.

11. Kok, Jan. Genetics and Molecular Biology of Streptococci, Lactococci, and Enterococci. eds Dunny, Gary M. Cleary, P. Patrick. McKay, Larry L. Special Purpose Vectors for Lactococci. American Society for Microbiology, 1991. pg. 97

12. De Man J. C.; Rogosa M.; and Sharpe M. E. (1960). A medium for the cultivation of Lactobacilli. J. Appl. Bacteriol. 23: 130 – 135.

13. Kandler, O. and Weiss, N. (1986) Genus Lactobacillus. In: Bergey's Manual of Systematic Bacteriology, Vol. 2. (Edited by Sneath, P.H. A.; Mair, N.S. and Hold, J.G.), William and Wilkins Co., Baltimore, USA.

14. Thakur, M.; Deshpande, H. W. and Bhate, M. A. (2017). Isolation and identification of lactic acid bacteria and their exploration in non-dairy probiotic drink. Int. J. Curr. Microbiol. App. Sci., 6(4): 1023-1030.

15. Sewell RD, Rafieian-Kopaei M. The history and ups and downs of herbal medicine usage. J Herbmed Pharmacol 2014; 3:1-3.

16. Karami, S., Roayaei, M., Hamzavi, H., Bahmani, M., Hassanzad-Azar, H., Leila, M., & Rafieian-Kopaei, M. (2017). Isolation and identification of probiotic Lactobacillus from local dairy and evaluating their antagonistic effect on pathogens. International journal of pharmaceutical investigation, 7(3), 137.