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(REVIEW ARTICLE)

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Formulation of probiotics from fermented food products: A review

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Abstract

Probiotics are living microorganism which may that they provide health benefits when consumed. The most common fermented foods are, yogurt, sauerkraut, soyabean, Honey, Apple cider vinegar etc…, Yogurt: Probiotic fermented milks and yoghurts are acidified and fermented by viable bacteria, usually L. bulgaricus and S. thermophilus, resulting in a thicker product with a longer shelf life. They are a nutrition-dense food, providing a good source of calcium, phosphorus, potassium, vitamin A, vitamin B2, and vitamin B12. Sauerkraut: Probiotic properties of Lactobacillus plantarum. During the fermentation process, the antioxidant content (vitamins, minerals, phenolics) of fruits and vegetables is increased due to the involvement of LAB in the decomposition and metabolism, enhancing the antioxidant capacity is enhanced. Soyabean: Bacillus subtilis is the most broadly used strain for soybean fermentation, which can increase antioxidant activity, anti-allergic activity and fibrinolytic function of the soybean. Honey: Evaluation of L. casei antimicrobial activity; test was conducted to determine the ability of L. casei to inhibit the growth of pathogenic bacteria. Probiotics have been used to treat bowel problems (such as diarrhoea, irritable bowel), eczema, vaginal yeast infections, lactose intolerance, and urinary tract infections.

Keywords: Lactobacillus; Probiotics; Vitamin; Fermentation; Food; Microbes

1. Introduction

Probiotics, in the form of supplements or food products, have emerged as the most prominent ingredient in the era of functional foods. Probiotics have always been a vital component and commercial target for providing potential health benefits. The term “probiotic” was first presented by Werner Kollath in 1953, which is known to be a derivative of the Latin word pro meaning “for life.” Kollath defined probiotics as active bodies with essential functions for promoting various health aspects. Food and Agriculture Organization (FAO) and World Health Organization (WHO) described them as “live microbes when administered in adequate quantities, confer health benefits on host organisms”. Several bacteria belonging to the genera Pediococcus, Lactococcus, Enterococcus, Streptococcus, Propionibacterium, and Bacillus are considered potential microbes for probiotic status. The frequently used strains belong to the divergent group of Bifidobacterium and Lactobacillus that significantly affect health with various actions. Probiotics can be consumed either by incorporating them into foods or drinks in the form of dairy or non-dairy foodstuffs or as supplements an ecological consideration of the gut flora is necessary to understand their relevance in human health, as well as the probiotic food concept. Each individual has a unique signature of more than 100–1000 microbial species in gastrointestinal tract (GIT) Probiotics are also known to demonstrate promising results like improved gut barrier function; adding to their unique ability to compete with pathogenic microbiota for adhesion to the gut and improve their colonization. Probiotics are used to treat various diseases that are mentioned in the flow chart below.

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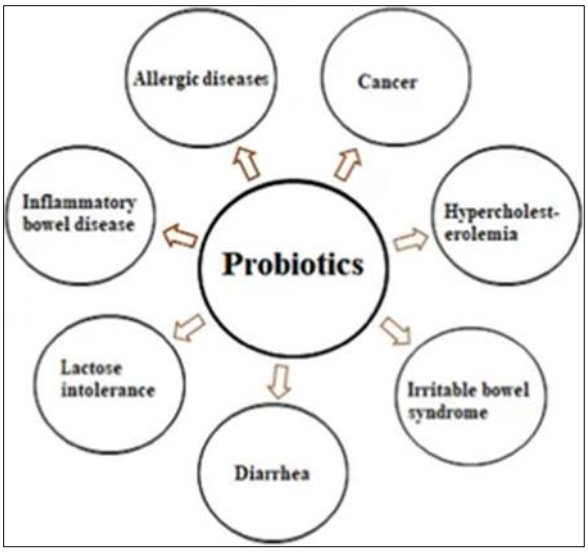

Figure 1 Benefits of probiotics

1.1. Mechanism of action of probiotics

- Probiotics perform their function by competing with pathogens for nutrients and receptors for binding thereby making their survival and adherence to gut mucosa difficult.
- Probiotics produce anti-microbial substances which inhibit pathogens growth.
- Probiotics promote epithelial barrier function by enhancing mucus production and increasing the expression of tight junction proteins which prevents the translocation of pathogens from intestine into the blood.
- Probiotics regulate immunity of the host by modulating maturation and function of dendritic cells subsequently increasing the activity of T cells which play important role in immune homeostasis.
- Probiotics also regulate the production of neurotransmitters including serotonin, dopamine and gamma aminobutyric acid (GABA).

1.2. Health benefits of probiotics

1.2.1. Improved digestion

They can alleviate digestive issues like bloating, gas, constipation, reduced diarrhoea and enhanced nutrient absorption.

1.2.2. Boosted Immunity

Probiotics support a strong immune system by strengthening gut barrier function and influencing immune cell activity and reduced risk of infection.

1.2.3. Mental well-being

There is growing evidence suggestion a connection between gut health and mental health with probiotics potentially playing a role in mood regulation and cognitive function it reduces anxiety, depression and improve cognitive function.

1.2.4. Allergies

Probiotics can enhance the body immune response thus playing a vital role in managing allergies. Heart: Probiotics are a natural safe option to add into your daily regime to maintain healthy cholesterol levels and contributed to heart health.

1.2.5. Weight loss

Prebiotics whether consumed on their own or combined with probiotics can be on important part of a comprehensive weight loss strategy. Good bacteria: Help our body's digest food and absorb nutrient and they produce several vitamins in the intestinal tract.

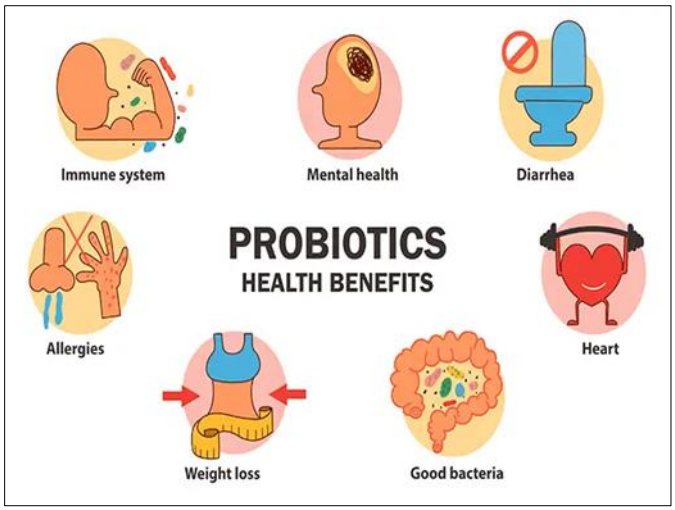

Figure 2 Health Benefits of probiotics

1.3. Fermentation of food products

1.3.1. Honey

Honey, sweet, viscous liquid food, dark golden in colour, produced in the honey sacs of various bees from the nectar of flowers. Flavour and colour are determined by the flowers from which the nectar is gathered. Some of the most commercially desirable honeys are produced from clover by the domestic honeybee. Evaluation of L. casei antimicrobial activity. This test was conducted to determine the ability of L. casei to inhibit the growth of pathogenic bacteria. The testing process followed a modified method by Muche et al. (2020). Single colonies of L. casei were cultured in 5 mL of liquid MRS media without test sugar and phenol red; they were then incubated at 37 °C for 18 h. Subsequently, 1 mL of the L. casei culture was centrifuged at 4 °C for 10 min at4000 rpm to separate the supernatant and the L. casei cell pellet [1]. The cell suspension was then made using 1x PBS. Pathogenic bacteria were cultured in 5 mL of liquid NB media and incubated at 37 °C with shaking at 150 rpm for 18 h using a shaker incubator. A standardized 0.1 mL of the pathogen culture, adjusted to 0.5 McFarland, was inoculated onto nutrient agar media and spread onto Petri dishes until dry. Subsequently, test discs soaked in L. casei isolate suspension were placed on labelled Petri dishes and incubated at 37 °C for 18 h. Observations were made by examining the clear zones formed around the test discussed.

Acid resistance test 50 µL of bacterial suspension, corresponding to an optical density of 0.5 McFarland, was introduced into 5 ml MRS broth with pH values of 2.5 and 4.0. After 3–4 hours of incubation at 37°C, a solution loop was streaked onto MRS agar plates. MRS agar plates were then incubated for 24–48 hours, and the number of colonies counted should not be less than 106 cfu/ml, indicating the resistance of the bacteria to acid.[2]

Tolerance to gastric intestinal conditions was carried out according to [43,44]. Strains were grown in modified MRS overnight at 37 °C, then centrifuged (5000× g for 15 min at 4 °C), and bacterial pellets were washed twice with phosphate-buffered solution (PBS). Afterwards, bacterial cells were re-suspended in PBS at a final concentration of 108 CFU/mL. A volume of 0.2 mL of the bacterial suspension was added to 1.8 mL of simulated gastric solution at pH 2 and 3 (NaCl 6.2 g/L, KCl 2.2 g/L, CaCl2 0.22 g/L, NaHCO3 1.2 g/L, 0.3% (w/v) pepsin) and another aliquot of 0.2 mL of bacterial suspension was added to 1.8 mL of simulated intestinal solution at pH 7.5 (NaCl 6.2 g/L, KCl 2.2 g/L, CaCl2 0.22 g/L, NaHCO3 1.2 g/L, 0.1% (w/v) pancreatin, 0.3% (w/v) bile salts). Cells in 0.1 M PBS pH 6.5 were used as control. After 2 and 4 h of incubation in gastric and intestinal juices, respectively, viability testing was performed via plate counts on modified MRS agar after 48 h at 37 °C under anaerobic conditions. [3]

Figure 3 Process of fermentation of Honey

1.4. Isolation and identification of lactobacillus from honey

Phylogenetic analysis of Lactobacillus bacteria in honey bees collected from different regions of Iran (marked by bold face type). Phylogenetic tree based on a distance matrix analysis of 1275 positions in the 16S rDNA gene. The phylogenetic tree was constructed by Crustal using the neighbour-joining method within the MEGA (4) package. Closely related type and reference strains are shown in parentheses together with accession numbers from GenBank. Bootstrap values based on 1000 resampling display the significance of the interior nodes and are shown at branch points. Cluster I L. kunkeei group, cluster II L. plantarum group, cluster III L. Apis group, and cluster IV as out group**.** [4]

1.4.1. Yogurt

Following the antimicrobial assay, a total of 50 potential lactobacilli isolates were obtained. The number of isolates was reduced to 20 by evaluating the results obtained in the antimicrobial activity test applied to these isolates. While evaluating, among the isolates showing inhibition on both indicator strains, priority was given to those with high inhibition rate on E. coli. Determination of antibiotic resistance of the isolates in the study, 6 antibiotic discs were used to determine the antibiotic resistance of lactobacilli strains. These antibiotic discs (Oxoid, Hampshire, England) were as follows: ampicillin (10 µg), vancomycin (30 µg), oxacillin (1 µg), cephalothin (30 µg), cefpodoxime (30 µg) and tobramycin (10 µg). Activated cultures were grown on slopes and the bacterial cells were removed from the surface with saline [5]. Cell suspensions (0.5 on the McFarland scale) were inoculated to Mueller-Hinton agar plates (Oxoid)containing horse blood and glucose. Standard yogurt is typically manufactured from the conventional starter culture strains, Lactobacillus delbrueckii subsp. bulgaricus and Streptococcus thermophilus. Meanwhile bio-yogurt or probiotic yogurt is supplemented with probiotic strains such as Bifidobacterium and Lactobacillus acidophilus that are claimed to have numerous health benefits and should remain live at adequate numbers. [6]

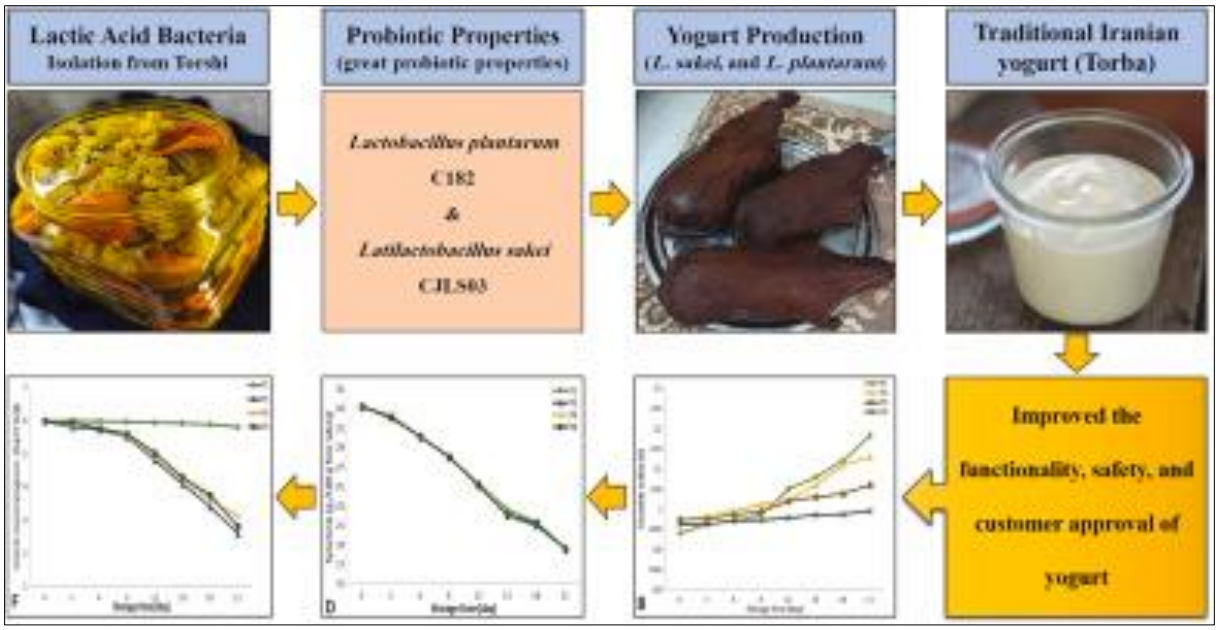

Figure 4 Process of fermentation of yogurt

1.5. Isolation and identification of lactobacillus from fermented yogurt

Two grams of each sample was transferred in a flask containing MRS Broth as enrichment media and added distilled water to 100 ml and incubated. After 24 100 µl enriched samples was spread on MRS agar and incubated at 37°C and anaerobic condition for 48 h. Bacterial colonies were purified by subsequent sub cultures. Final identification was done using classic microbiology tests including Gram-staining for detecting morphology, catalase and oxidase tests, motility, indole producing, growth at 15°C, and carbohydrates fermentation (arabinose, fructose, galactose, lactose, mannitol, salicin, sucrose, and trehalose) test. All Gram-positive and catalase-negative bacilli were selected for the assessment of antimicrobial ability [7]. Antimicrobial effect of isolates was evaluated by disc diffusion test on MHA medium plated with three pathogens. For this purpose, fresh culture of isolates was centrifuged (8000 rpm, 15 min) and supernatants were removed. Blank discs were inoculated with 40 µl supernatant of each isolate and were placed on separate MHA medium inoculated with S. aureus (ATCC-6538), B. subtilis (ATCC-12711), and P. aeruginosa (ATCC-27853) strains. Growth inhibition zones of pathogens and isolated lactobacilli inhibitory ability were assessed after incubation of all agar media at 37°C for 24 h. The antimicrobial assay, a total of 50 potential lactobacilli isolates were obtained. The number of isolates was reduced to 20 by evaluating the results obtained in the antimicrobial activity test applied to these isolates. While evaluating, among the isolates showing inhibition on both indicator strains, priority was given to those with high inhibition rate on E. coli [8]

1.5.1. Sauerkraut

Sauerkraut is a traditional vegetable product usually produced by spontaneous fermentation that relies on lactic acid bacteria (LAB) naturally present in white cabbage. The word sauerkraut means Sour cabbage or acidic cabbage. Cabbage is the vegetable used in Sauerkraut preparation, which is a good source of ascorbic acid, vitamins, and minerals and possesses value for its salad and culinary properties. Fermentation has been used since the early days of civilization to preserve food materials and to develop newer products. Cabbage could be preserved as Sauerkraut, which is an acid fermented product. It is a result of natural fermentation by bacteria indigenous to cabbage in the presence of 2-3 % salt [9]. The fermentation yields lactic acid as the major product. The lactic acid, along with other minor products of fermentation gives sauerkraut its characteristic flavour and texture. As no starter referred to as wild fermentation. The normal flora of the cabbage leaves is relied upon to include the organisms responsible for the desired fermentation, one that will enhance preservation and organoleptic acceptability. The floral succession is governed mainly by the pH of the growth medium. Initially, a coliform starts the fermentation. As acid is produced, an environment more favourable for Leuconostoc is quickly formed. The coliform population declines as the population of the strain of Lactobacillus builds. As Leucistic is the heterofermentative lactic acid bacterium, much gas (CO2) accompanies the acid production stage. The pH continues to drop, and a strain of Lactobacillus succeeds in the Leucistic. (Sometimes Pediococcus arises instead of Lactobacillus). The complete fermentation then involves the succession of three major groups of genera of bacteria, a succession governed by the decreasing Ph [10]

1.5.2. Addition of salt

Salting of the cabbage serves two major purposes. First, it causes as an osmotic imbalance, which results in the release of water and nutrients from the cabbage leaves. The fluid expelled is an excellent growth medium for the microorganisms involved in the fermentation. It is rich in sugar and growth factors. Second, the salt concentration used inhibits the growth of many spoilage organisms and pathogens. It does not obviously inhibit desired floral succession. Cabbage is approximately 90 % water, and the salt is dissolved entirely in the water. The actual salt concentration (brine strength) experience by the microorganisms in their aquatic milieu is around 2.8%. Thorough and even distribution of the salt is critical. Pockets of lower high salt concentration would result in spoilage and /or lack of the desired fermentation. [11]

1.5.3. Oxygen supply

Throughout the fermentation it is critical that oxygen is excluded. The presence of Oxygen would permit the growth of some spoilage organisms, particularly the acid-loving moulds, and yeasts. [12]

1.5.4. Temperature

The time required for the fermentation depends on is preferred for the temperature. A temperature of 21c fermentation. If it is favourable, a period of 8-6 weeks will ordinarily sufficient. At the end of this time, the following changes are marked [13].

- The product will have acquired its typical aroma.
- All the fermentable carbohydrates will have been consumed.
- The acid content will have risen to between 1.85 and 2.2 % .
- The pH will be approximate to the range of 3.5 -3.

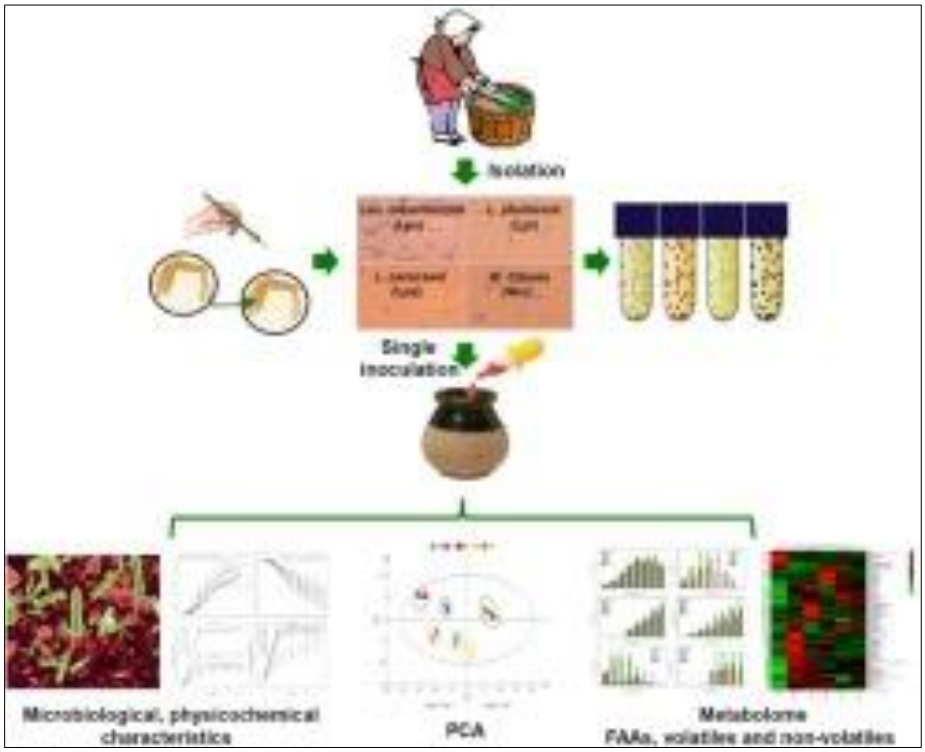

Figure 5 Process of fermentation of sauerkraut

1.6. Isolation and identification of lactobacillus from fermented sauerkraut:

Enumeration and isolation of LAB was performed according to ISO 15214:1998[6]. Dilutions of 10-4,10-5 and 10-6 were used for spread plate inoculations on MRS agar for total LAB and on M17 agar for the isolation and enumeration of cocci. The plates were incubated at 37°C for 48 h. Samples were analyzed in duplicate. From each medium, a number of colonies equal to the square root of the total number recorded in Petri dishes with 15 to 300 CFUs were randomly selected for isolation. The isolated LAB were subjected to phenotypic and genotypic characterization. The phenotypic

characterization of LAB was performed based on cell morphology, Gram reaction and catalase activity. Gram staining was performed for all isolated colonies according to the standard procedure. For the catalase test, a drop of 3% hydrogen peroxide was added to a bacterial colony on a sterile glass slide and mixed well. Production of air bubble indicated catalase positive and no bubble indicated catalase negative activity. Only Gram positive and catalase-negative strains were selected for further characterization. Pure cultures were obtained from the samples and stored at -20°C. [14]

1.6.1. Soyabean

Soyabean (Glycine max (L.) Merrill.) is one of the most important oil crops of the world which also has tremendous importance as a food legume. Soybeans were first known in China, where several fermented soy products were created. They spread to the nearby nations, where other process improvements were developed. These fermented soybean products are considered a valuable source of protein and are widely consumed for their high value in nutrition, functionality, and low cost [15]. After preparation, soybeans are subjected to either solid-state, submerged fermentation processes, or a sequential of both processes. Solid-state fermentation is an old method of subjecting moist solid food particles to fermentation using bacteria, yeast, or filamentous fungi to produce fermented food or metabolites such as enzymes, Flavors, acids, etc. The submerged fermentation is a process conducted in the presence of free excess water The difference between fermented soybeans is based on several parameters, but mainly due to the microorganism used in the process. Thus, fermented soybean products are different in terms of aroma, texture, and therapeutic and nutraceutical values. Some fermentations occur only with bacteria, others using only filamentous fungi, and, in many cases, both these microbial groups are used. Some products are fermented only with Bacillus (natto, kinema, chungkookjang); some are fermented with fungi Aspergillus oryzae, Mucor spp. Rhizopus spp. and Fusarium spp. (douche, tempeh, miso, tofu) and in some cases both microorganisms are used, as in the case of doenjang, where the bacteria involved in this process would be B. subtilis and fungi include Rhizopus spp., Mucor spp., Geotrichum spp., and Aspergillus spc [16].

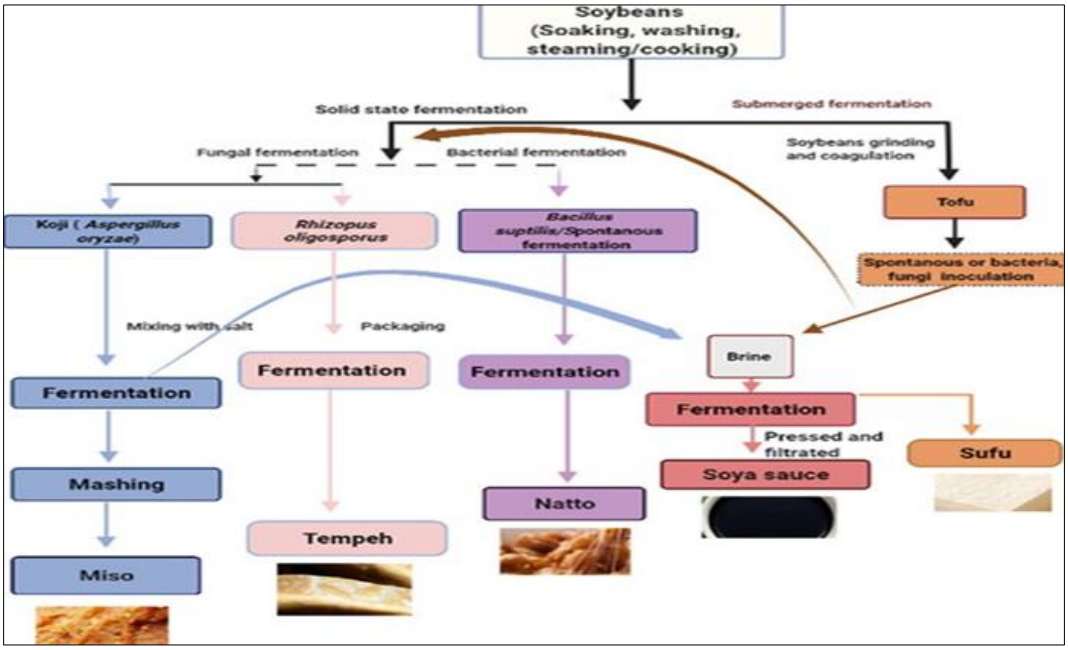

Figure 6 Process of fermentation of soyabean

1.7. Isolation and identification of lactobacillus from fermented soyabean

Several isolates were detected during the soaking process belonging to LAB, yeasts, Enterobacteriaceae, and Bacillus species. Lactcaseibacillus casei, Lactiplantibacillus plantarum, Leconostoc mesenteriodes, Pedicoccus sp., and Enterococcus faecium were the predominant LAB species during the whole soaking process. Enterobacteriaceae such as C. diversus and Klebsiella pneumoniae were also detected during the early stage of the soaking process and declined at the end. Yeasts such as P. burton, C. diddensiae, and R. rubra, Saccharomyces diaresis were also detected with a weak growth rate at the initial of the soaking process. Studies have shown that the ecology of soybeans is the main origin of the leading microorganisms of acid fermentation and not the tap water. In addition, it is believed that the microbial metabolites produced during this fermentation diffuse into the beans and affect their chemical profiles [17]. This

soybean acidification may control microbial pathogens and undesirable microbial growth, ensuring the safety and sensory quality of the final product . Weak acidification was linked to high Bacillus cereus levels in the final soybean product after heat treatment. Endogenous species such as Citrobacter freundii, Pseudomonas fluorescens, K. pneumoniae, and Streptococcus sp. were found to produce high levels of vitamin. Furthermore, the soaking process was reported to reduce the level of water-soluble anti-nutritive factors such as phytic acids, but also reduce other water-soluble important components such as vitamins, minerals, and phytochemicals. Heat treatment of soybean before soaking was reported to eliminate most of the LAB, yeasts, and Enterobacteriaceae species, leaving behind heat resistance spore-forming bacteria such as Bacillus brevis causing acidification failure. This promoted undesirable microbial contaminant growth during the fermentation process and adding acetic and lactic acids into soaking water was recommended as a countermeasure. To the best of the authors' knowledge, limited information is available on the acidic fermentation changes that may occur in the beans during the soaking, as well as possible strategies. Endogenous species such as Citrobacter freundii, Pseudomonas fluorescens, K. pneumoniae LAB or yeasts) that may control this process without interfering with the subsequent processes, as will be discussed later under Bacillus fermentation [18].

1.7.1. Apple cider vinegar

Apple cider vinegar is the fermented juice from crushed apples. It contains acetic acid and nutrients such as B vitamins and vitamin C. It might help lower blood sugar levels after a meal by changing how foods are absorbed from the gut. It is made by crushing apples, then squeezing out the juice. The apple juice is then fermented by yeast which converts the sugars in the juice to ethanol. In a second fermentation step, the ethanol is converted into acetic acid by acetic acid-forming bacteria (Acetobacter species), yielding cider vinegar [19]. Apples are loaded onto a processing belt where they are washed, crushed, pressed, and the juice separated. Autochthonous or inoculated yeasts, mainly Saccharomyces cerevisiae, start the process of alcoholic fermentation which converts the sugars in the juice to ethanol, producing apple cider. The apple cider is then inoculated with either a pure culture of acetic acid bacteria or a proportion of 'mother vinegar', resulting in a secondary acetic fermentation which then converts the ethanol in the cider to acetic acid, yielding apple cider vinegar. The "mother" is an undefined microbial culture left in the vinegar prior to distilling and pasteurization. The ideal fermentation temperature for hard cider is generally between 13°C and 22°C (55°F and 72°F). A lower temperature can produce a cleaner, more refined cider, while a higher temperature promotes fruity, full-bodied flavours [20].

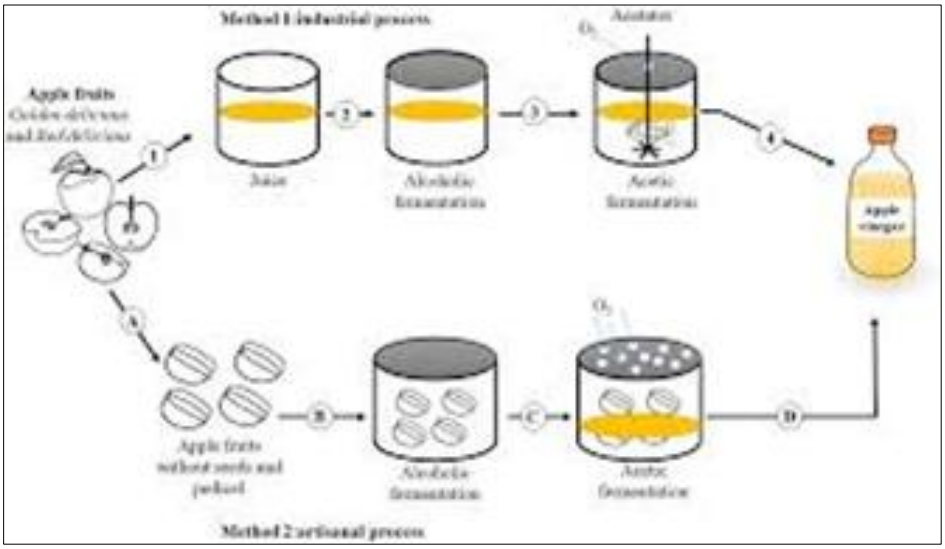

Figure 7 Process of fermentation of apple cider vinegar

1.8. Isolation and identification of lactobacillus from apple cider vinegar

Two grams of each sample was transferred in a flask containing MRS Broth as enrichment media and added distilled water to 100 ml and incubated in 37°C. After 24 h, 100 µg of enriched samples was spread on MRS agar and incubated at 37°C and anaerobic condition for 48 h. Bacterial colonies were purified by subsequent subcultures. Final identification was done using classic microbiology tests including Gram-staining for detecting morphology, catalase and oxidase tests, motility, indole producing, growth at 15°C, and carbohydrates fermentation (arabinose, fructose, galactose, lactose, mannitol, salicin, sucrose, and trehalose) test. All Gram-positive and catalase-negative bacilli were selected for the assessment of antimicrobial ability. Antimicrobial effect of isolates was evaluated by disc diffusion test

on MHA medium plated with three pathogens. For this purpose, fresh culture of isolates was centrifuged (8000 rpm, 15 min) and supernatants were removed. Blank discs were inoculated with 40 µg supernatant of each isolate and were placed on separate MHA medium inoculated with S. aureus (ATCC-6538), B. subtilis (ATCC-12711), and P. aeruginosa (ATCC-27853) strains. Growth inhibition zones of pathogens and isolated lactobacilli inhibitory ability were assessed after incubation of all agar media at 37°C for 24 h [21].

1.8.1. Fermented mustard

Fermented mustard (picked mustard green) is made from green mustard and its production is a spontaneous fermentation process by a mixed microbial population mainly composed of LAB. The aim of this study was to screen lactobacilli with probiotic characteristics isolated from traditionally fermented mustard, and to determine the effect of the screened Lactobacillus strains for their ability to lower cholesterol and the strains were also identified. Lactobacilli with probiotic characteristics isolated from traditionally homemade koumiss, Lactobacillus strains were able to lower cholesterol in vitro. Moreover, L. plantarum, a normal resident of the human gut microflora, is able to adhere to the epithelial cells, with a preference for the small intestine [22]. In the present work, the results showed that strain B0007 and B0022 belonging to L. plantarum displayed marked cholesterol-lowering property and were able to adhere to the epithelial cell. Acid tolerance of the cultures was investigated by incubating the organisms in MRS broth supplemented with 0.30% oxigall. The pH was adjusted to 3.0 and 2.0 with HCl and cultures were incubated at 37 °C for 3 h. Each of the isolated LAB was subculture at least 3 times before experimental use, followed by centrifugation after the final subculture, inoculation (10% v/v) into the broth, and growth The LAB isolates were analysed for their resistance to bile salt. The MRS broths at concentrations of 0%, 0.5% and 1.0% (w/v) of oxigall were prepared and dispensed in 10 mL volumes and sterilized by heating 121 °C for 15 min. Each of the isolated LAB was sub cultured at least 3 times before experimental use, followed by centrifugation after the final subculture, inoculation (10% v/v) into the broth, and growth monitoring using the plate count method. The reaction mixture and MRS broth were incubated at 37 °C for 24 h. All the experiments were repeated twice. monitoring using the plate count method. The experiments were repeated twice [23].

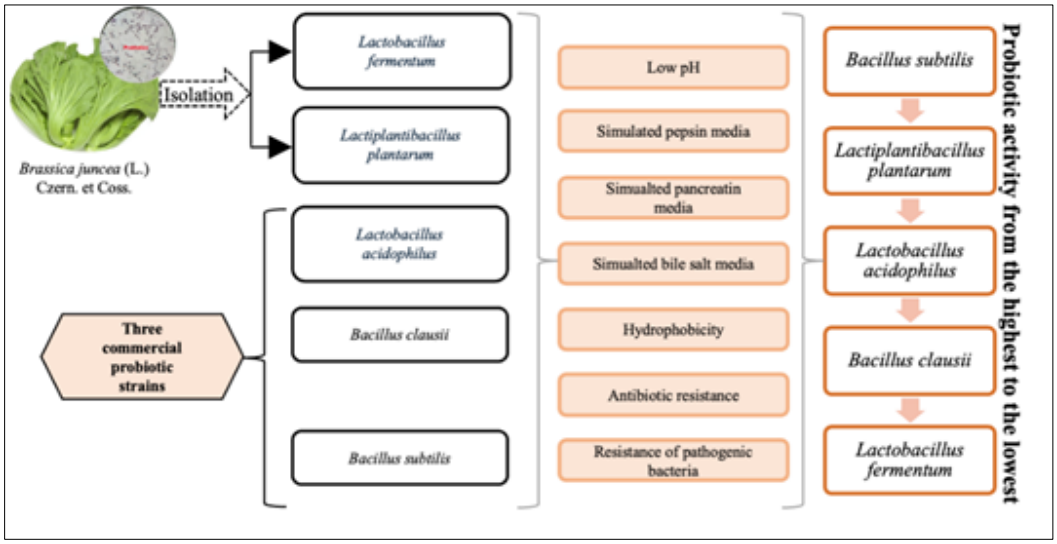

Figure 8 Process of fermentation of mustardss

1.9. Isolation and identification of lactobacillus from fermented mustard

LAB isolates that showed physiological tests as a probiotic, acid tolerance and bile tolerance and lowering cholesterol activity were identified by API 50 CHL fermentation assays (Biome Rieux, S.A., Marcy I'Etoile, France) and 16S rDNA sequence analysis. The primarily confirmed by API 50 CHL fermentation assays were following the instruction procedure. In 16S rDNA sequence analysis, the PCR primers designed from the 16S rRNA genes primers 27F/1492F. For the PCR assay, the method of Michael et al. was followed. The amplification products were purified with DNA purification kit and sequenced by Nucleic acid Synthesis and Analysis Core Laboratory. Sequence homologies were examined by comparing the obtained sequence with those in the DNA data bases (24).

2. Conclusion

- Probiotics have clearly established as an adjuvant in the management of lactose malabsorption and acute diarrhoea, particularly acute infant diarrhoea
- Evidence from well documented clinical trials has revealed that probiotics can potentially alleviate different GI and other disorders.
- Further well-designed clinical trials, involving large numbers of patients, are mandatory to achieve definite evidence of the preventive and curative role of probiotic in medical practice.

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