© 2025 IJNRD | Volume 10, Issue 7 July 2025 | ISSN: 2456-4184 | IJNRD.ORG

IJNRD.ORG ISSN : 2456-4184
INTERNATIONAL JOURNAL OF NOVEL RESEARCH AND DEVELOPMENT (IJNRD) | IJNRD.ORG
An International Open Access, Peer-reviewed, Refereed Journal

PRODUCT DEVELOPMENT IN NUTRACEUTICALS AND PROBIOTICS

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Abstract

Multigrain are widely known as rich sources of protein and are commonly used as dietary supplements for protein intake. In this project, selected multigrain such as jowar, ragi, almond, soybean, and oats which are known for their high protein and fibre content are used as base components. Additionally, seaweed and wheatgrass, which are rich in medicinal properties, are included to enhance the nutritional and therapeutic value of the supplement. This project focuses on developing multigrain health supplements by incorporating probiotics isolated from Yakult. The isolated microorganisms are first cultured on MRS agar to promote their growth. Gram staining is then performed to identify the type of bacteria followed by various biochemical tests to study their characteristics. Then the organism was mass produced in the MRS medium and centrifuged at 12000 rpm for three times then remove the supernatant and collect pellets and air dried and incorporating into the multigrain flour to enhance the nutritional value and health benefits. The nutritional value of the formulation was studied and found to be moisture content 3.08%; total ash content 3.75%; protein content 24.35%; fat content 3.73%; carbohydrate content 65.09%; energy content kcal/100g is 391.33; sugar content 2.43%.

INTRODUCTION

Grains are one of the most essential components of the human diet and are widely regarded as a staple food. They contribute to growth and supply the body with nutrients needed for daily activities. Nowadays, consumers eat not just to satisfy hunger, but also to gain additional nutritional benefits. Food today plays a vital role in combating diseases related to nutritional disorders (Behera & Srivastav, 2018).

In the 21st century, human lifestyles have changed significantly. Nowadays, people are more conscious about their health and nutritional intake. They seek foods that offer higher nutritional value in smaller quantities. This has led to the development of foods with added health benefits, commonly known as functional foods. Simply put, functional foods are "foods and food components that provide health benefits beyond basic nutrition."

The bran and germ parts of grains contain most of the essential nutrients. However, during the refining process, these parts are removed, resulting in a significant loss of nutrients. Numerous scientific studies have shown that including whole grains in the diet can reduce the risk of coronary heart disease and various types of cancer. Dietary fiber plays a key role in slowing down the breakdown of glucose, leading to reduced absorption in the body and promoting a steady release of carbohydrates and glucose. (Pande et al., 2017).

According to Dahatonde et al. (2018), A multigrain product is made by combining two or more different grains. Each grain offers unique nutritional benefits, so mixing multiple grains provides greater overall nutrition compared to consuming just one type. In addition to improved nutrition, multigrain products also offer a variety of phytochemicals and enhance the texture and sensory appeal of food. Health-conscious consumers today choose foods not just to satisfy hunger but also to gain added nutritional value. Modern food plays an important role in preventing and managing diseases related to nutritional deficiencies (Behera & Srivastav, 2018).

The variety of multi grains featured in this initiative includes ragi, jowar, millet, soybean, almond, oats, and groundnut.

Aim and Objectives

Aim: The primary aim of product development in nutraceuticals and probiotics is to create products that provide scientifically validated health benefits often beyond basic nutrition such as disease prevention or improved physiological function

Objectives:

1. Formulation of multigrain health supplement product.
2. Isolation of probiotics organisms from natural sources.
3. Biochemical characterisation of the isolated.
4. Characterisation of organisms to fulfil probiotics features.
5. Formulation of multigrain supplements with probiotics isolates and fulfilling its nutritional factors and CFU.

MATERIALS AND METHODOLOGY

Materials: Multigrain (ragi, jowar, millet, soybean, almond, oats, and groundnut), supplements (seaweed, wheatgrass), probiotic (yakult)

Methodology

Selection and Preparation of Raw Material

Selection of multigrain ingredients

To effectively select ingredients for multigrain flour, focus on choosing a variety of grains and legumes known for their nutritional value and complementary properties. Consider factors like gluten content, fibre content, and the overall nutritional profile.

The required multigrain are Ragi, Oats, Millet, Soybean, Almond, Groundnut,

- Ragi considered one of the most nutritious cereals. It contains about 5–8% protein, 1–2% ether extractives, 65–75% carbohydrates, 15–20% dietary fibre and 2.5–3.5% minerals.
- Groundnuts (also known as peanuts) are highly nutritious, offering a good source of protein, healthy fats, and various vitamins and minerals. They contain around 26% protein, 49% fat, and 16% carbohydrates.
- Oats are a nutrient-rich food, providing a good source of carbohydrates, protein, and fibre Oats contain about 60% starch, 14% protein, 7% lipids, and 4% β-glucan.
- Almonds are a nutrient-rich food, 1ounce provides about 165 calories, 6 grams protein, 14 grams fat (80% monounsaturated, 15% polyunsaturated, and 5% saturated), 6 grams carbohydrate, and 3 grams fibre.
- Millets are nutrient-rich grains containing, each 100gram (g) of cooked millet contains the following: 3.51 g of protein. 23.7 g of carbohydrate. 1.3 g of dietary fiber.
- Jowar, also known as sorghum, is a nutrient-rich grain that provides a good source of carbohydrates, protein, fibber, and various vitamins and minerals. Per 100g: Calories: 329 kcal. Carbohydrates: 72.1 g. Protein: 10.4 g.
- Soybeans are a nutrient-rich food, particularly high in protein and fat. A 100-gram serving of boiled soybeans contains approximately 172 calories, 18.2 grams of protein, 8.4 grams of carbohydrates, 9 grams of fat, and 6 grams of fibre.

FORMULATION OF MULTIGRAINS

SL NO	INGREDIENTS	Per 200 g
1	Ragi	26g
2	Jowar	26g
3	Soybean	32g
4	Groundnut	20g
5	Millet	26g
6	Oats	26g
7	Almond	26g
8	Wheatgrass	9g
9	Seaweed	9g

Cleaning, Drying and Milling Process

Cleaning Process

Manual cleaning of grains involves physically removing impurities and unwanted materials by hand or with simple tools. These methods are used to separate grains from chaff, stones, dirt, and other debris, ensuring a cleaner and more uniform product.

Methods of Manual Grain Cleaning:

1. Winnowing:

This involves tossing the grain in the air, allowing wind or a gentle breeze to carry away lighter materials like chaff and husks.

2. Sieving:

Using sieves or screens with different mesh sizes, this method separates grains from smaller debris, stones, and other impurities based on size.

3. Hand-picking:

This is a meticulous process where individual grains are examined and unwanted materials are removed by hand, often used for high-value or specialty grains where precision is crucial.

4. Milling process:

a) Single-Stream Milling: The grain is crushed between Steel rollers or millstones. All parts of the original kernel stay together from the beginning to the end of the milling process.

b) Multiple-Stream Milling with Recombination: The grain is crushed, and different grain fractions are channeled into separate millstreams. The millstreams may be sifted and separated by particle size. Large particles are often returned to the mill for further grinding to attain flours and meals with desired and uniform particle sizes. The last step in the milling process reunites all the flour streams at the mill so that they have the original proportions of bran, germ, and endosperm in the whole grain flour. This process is called recombination.

5. Drying process:

Grain drying is a vital operation in preparing finished grain products such as flour, drinks, confectioneries, and infant food. Drying is one of the most important technologies for the preservation of grains. Grains must be dried to the levels of moisture suitable for storage, without spoilage; the levels depend on the specific grains. Sun and solar drying have been practiced extensively since ancient times. However, the technique is not suited for the large quantities of various grains that, today, must be dried commercially (ARUN S Mujumdar, J Bake 2003).

Isolation of Probiotics Organisms from sample source

Currently, there is an increasing interest in and demand for probiotics, after a long history of safe use in fermented dairy products and an increased recognition of the beneficial effects of probiotics to human gut health. According to the FAO of the UN and the WHO, probiotics are 'live microorganisms which, when administered in adequate amounts, confer a health benefit on the host'. In particular, strains belonging to *Bifidobacterium and Lactobacillus,* the predominant and sub-dominant groups of the gastrointestinal microbiota, respectively, are the most widely used probiotic bacteria and are included in many functional foods and dietary supplements (Nancy To edter Williams, Pharm.D 2010).

Source

Yakult is a popular probiotic drink that contains a specific strain of beneficial bacteria called *Lactobacillus casei* Shirota. It was developed in Japan by Dr. Minoru Shirota in the 1930s and is known for its potential digestive health benefits. Yakult is typically sold in small bottles and is consumed as a daily dietary supplement to support gut health. The probiotics in Yakult are believed to help maintain a healthy balance of gut bacteria, which can have a positive impact on digestion and the immune system (Fuller R. 1989).

Isolation as well as culturing of LAB was done using the De Man Rogosa Sharpe (MRS) media. Isolation of LAB from the selected sample was carried out using the spread plate method. Appropriate dilutions of the samples prepared in sterile distilled water were pour 0.1 ml and spread it to all the area of the MRS agar plate. The plates were then incubated at 370c, for a period of 24- 48 hrs. Colonies which were picked up and inoculated in agar plates and slants. The isolated LAB was purified using their respective isolation media by re-streaking on plates until only a single type of colony was present (Figure3 Isolated colony).

Identification Lactic Acid Bacteria (LAB) isolated From Yakult:

The identification of potent isolated culture done based on the characteristics of *Lactobacillus* as described in Bergey's manual of Systematic Bacteriology. The culture was subjected to a battery of biochemical tests which included fermentation of different carbon sources, acid and gas production from glucose, catalase test.

Morphological Characterization (Gram Staining):

Sterile grease free glass slides were taken and disinfected with 70% ethyl alcohol. A loop-full of isolated culture was taken and a smear was made on the glass slide. The smear was heat fixed. The smear was flooded with 2-3 drops of crystal violet and washed after 1 minute. After washing 2-3 drops of gram's iodine was added and washed after 1 minute. And the smear was decolorized with grams decolorizer (70% ethyl alcohol). The smear was counter stained with safranin and washed after 30 seconds. The smear was air dried and viewed under 40x and 100x (Figure4 Gram staining)

Features;

- Rod-shaped (bacilli); may appear long and slender or short and plump, non-motile (do not possess flagella).
- Gram-positive (appear purple under microscope after Gram staining).
- Often found singly, in pairs, or in short chains, non-spore-forming.
- Typically, 0.5–1.2 µm in width and 2–9 µm in length.
- On agar: Small, round, convex colonies; creamy to white in color.
- Facultative anaerobes or microaerophilic.

Biochemical Characterization:

a. Indole production test

Tryptophan, an essential amino acid, is oxidized by some bacteria by the enzyme tryptophan are resulting in formation of indole, pyruvic acid and ammonia (Figure5 Indole test).

Procedure;

- Preparation of (1%) tryptone broth: dissolve 1g of peptone in 10ml of distilled water. Sterilize in the autoclave at 121c for 15min.
- Inoculate one tryptone broth with lactobacillus and keep another tube as an control.
- Incubate the tubes 35ºc for 48hrs.
- After 48hrs of incubation, add 1ml of Kovacs reagent to each tube.
- Shake the tubes gently after intervals for 10-15min.
- Allow the tubes to stand to permit the reagent to come to top.

Interpretation: A red or pinkish-red ring at the top of the medium indicates a positive result, signifying indole production.

A yellow or amber-coloured ring indicates a negative result, meaning the organism does not produce indole.

b. Methyl Red and Voges-Proskauer tests

The methyl red and the Vogues Proskauer test are used to differentiate two major type of facultatively anaerobic enteric bacteria that produce large amount of acid and those that produce the neutral product acetoin end product (Figure6 Methyl red test).

Procedure;

- Prepare of MRVP broth and pour 5ml broth to each tube and sterilize by autoclaving for 15min.
- Inoculate MR-VP broth with the test organism and incubate at 35-37°C for at least 48 hours.
- After incubation, add 3-6 drops of methyl red indicator to each tube (MR Test).
- After incubation, add Barritt's reagent A (α-naphthol) and then Barritt's reagent B (potassium hydroxide) to the culture (VP Test).

Interpretation: A red colour indicates a positive MR Test,

While a yellow or no colour change indicates a negative VP Test.

c. Citrate utilization test

Citrate test is used to differentiate among enteric bacteria on the basis of their ability to utilize/ferment citrate as the sole carbon source (Figure9 citrate test).

Procedure:

- Preparation of Simmons's citrate agar (pH 6.9) slants, dissolve all the constituents, except phosphates which are to be dissolve separately in 100ml of water, and make volume to 1liter. Set the pH to 6.9. Pour the medium in the tubes and sterilize by autoclaving for 15min and prepare slants.
- Inoculate Simmons's citrate agar slants with lactobacillus by streak inoculation. Another tube kept as an uninoculated for control.
- Incubate all the slants at 37ºC for 48hrs.

Interpretation: The medium remains green, the bacteria cannot utilize citrate, and hence it is negative test.

d. Urease test

Urea is a major organic waste product of protein digestion in most vertebrate and is excreted in the urine. Some microorganisms have the ability to produce the enzyme urease. The urease is a hydrolysis enzyme which attacks the carbon and nitrogen bond amide compounds with the liberation of ammonia (Figure7 Urease test).

Procedure,

- ❖ Preparation of urea agar media, add all the ingredients into tubes and dissolve by heating, adjust the pH to 6.8 and autoclave at121ºC for 15min and cool to 50ºC.
- ❖ Then add 3-6 drops of phenol red indicator to each tube.
- ❖ A bacterial sample (e.g., from a pure culture) is introduced into the medium, by streaking on a slant.
- ❖ The inoculated medium is incubated at 37°C) for 48 hours.

Interpretation: The medium remains yellow (or the original colour) if urease is not production, hence it is negative test.

e. TSIA [Triple Sugar Iron Agar Test]

The triple sugar iron agar (TSIA) test is a biochemical test used to differentiate bacteria based on their ability to ferment these three sugars and release acid and hydrogen sulphide gas (Figure8 TSIA TEST).

Procedure,

- ❖ A TSI agar slant are prepared, it is specialized medium containing glucose, lactose, sucrose, ferrous sulphate, and phenol red (a pH indicator).
- ❖ Using a sterile inoculating needle, pick a well-isolated bacterial colony from an 18–24- hour culture.
- ❖ Carefully stab the needle into the butt (bottom) of the TSI agar slant, going about 3-5 mm from the base.
- ❖ While withdrawing the needle, streak the surface of the slant in a zigzag pattern.
- ❖ Loosely cap the tube to allow for gas exchange and incubate at 35°C for 18-24 hours.

Interpretation: yellow colour change in the slant and/or butt of the agar indicates sugar fermentation; hence it is positive test.

f. Oxidase test

The oxidase test is a biochemical test used to determine if a microorganism, typically bacteria, possesses the enzyme cytochrome c oxidase. This enzyme plays a crucial role in aerobic respiration, specifically in the electron transport chain, where it facilitates the transfer of electrons from cytochrome c to oxygen, the final electron acceptor (Figure10 Oxidase test).

Procedure,

- ❖ Place a piece of filter paper on a clean, dry slide or in a petri dish.
- ❖ Add one or two drops of the oxidase reagent to the filter paper, ensuring it's moist but not oversaturated.
- ❖ Using a sterile loop, pick a well-isolated colony from your bacterial culture.
- ❖ Gently smear the colony onto the moistened area of the filter paper.
- ❖ Watch for a colour change on the filter paper. The reaction should be observed within 10- 30 seconds.

Interpretation: No colour changes or a change after 30 seconds indicates a negative result..

g. Catalase test

During aerobic respiration in the presence of oxygen microorganisms produce hydrogen peroxide (H2O2) which is lethal to the cell. The enzyme catalase present in some microorganisms breaks down hydrogen peroxide to water and oxygen (Figure10 Catalase test)

Procedure:

- ❖ Obtain a glass slide and a bottle of hydrogen peroxide.
- ❖ Using a sterilized inoculating loop, smear a small amount of bacterial culture onto the dry slide.
- ❖ Place a drop of hydrogen peroxide on top of the bacteria.
- ❖ Observe the slide immediate for appearance of gas bubbles.

Interpretation: No bubbling on the slide indicates a negative result. If bubbles formed it is positive result.

Acid tolerance test

An acid tolerance test evaluates a microorganism's ability to survive and function in acidic environments, particularly relevant for probiotics and starter cultures, often involving incubation in acidified broth or simulated gastric juice at various pH levels and durations, with or without the presence of pepsin. The test quantifies survival rates to determine a strain's suitability for applications requiring acid resistance, such as passage through the gastrointestinal tract or use in fermented products (Figure11 Acid tolerance test).

Procedure for Acid Tolerance Testing:

1. Culture Preparation:

Grow the bacterial strains, often lactic acid bacteria (LAB), in an appropriate growth medium (e.g., MRS broth) overnight at a suitable temperature (e.g., 37°C or 42°C).

2. Acidic Medium Preparation:

Adjust the pH of the growth medium to desired acidic levels using strong acids like HCl. Common pH levels tested include 3.0, 4.0, 5.0, 6.0, 7.0, simulating different levels of stomach acidity.

3. Enumeration:

Plate Count Method: Make serial dilutions of the samples and plate them onto an appropriate agar medium (e.g., MRS agar). Incubate the plates to allow for bacterial growth and colony formation.

Bile Salt Tolerance Test

It typically refers to assessing its bile salt tolerance or bile salt hydrolase (BSH) activity, Bile Salt Tolerance Testing:

- Incubation in Bile-Containing Media*:* *Lactobacillus* strains are incubated in a growth medium like MRS broth supplemented with varying concentrations of bile salts (e.g.,0.3%, 0.5%, 1%)
- Then add 1ml of probiotics solution into tubes contains bile salt.
- Then take the OD at 600nm (0^{th} hr, 6^{th} hrs).

Mass Production of *Lactobacillus* strains,

Mass production of *Lactobacillus* (commonly used in probiotics, fermented foods, and pharmaceuticals) involves large-scale fermentation using biotechnological techniques to grow, harvest, and preserve viable bacterial cells.

- Prepare 200ml of MRS broth then add the culture media.
- Incubate at room temperature (37ºC) for 72hrs.
- After the growth of organism keep the broth media in water bath for 1hrs.
- Then centrifuge the media, take 8 tubes and fill equally and set the centrifuge at 12000 rpm for 10min.
- After centrifuge discarded the supernatant and add 5ml of distilled water to all tubes mix thoroughly.
- Again centrifuge at 12000 rpm for 10min and discard the supernatant.
- Again add 5ml of distilled water and mix thoroughly and centrifuge at 12000 rpm for 10min.
- Then discard the supernatant and collect the pellets from the tubes.
- Dry the pellets and make into powder form (Figure12 Mass production).

Incorporation of Probiotics into the formulation

Incorporating probiotics into multigrain formulations offers a way to enhance the nutritional value and potential health benefits of these foods.

Steps involved in incorporating probiotics into multigrain formulations:

1. **Strain Selection:**

It includes that probiotic must be taxonomic defined to the strain level, the probiotic genome sequence should be available, the strain must have demonstrated specific evidence of the health benefits, the probiotic must be alive in the food and in the necessary levels to provide the benefit (Marco et al., 2021).

2. **Cultivation and Fermentation:**

Once the strains are selected, they are cultivated in a controlled environment. This process, known as fermentation, involves growing the bacteria in a nutrient-rich medium. The conditions are carefully monitored to ensure optimal growth.

During the initial stage, the conditions are controlled to favour cell growth and after obtaining the desired cell concentration, the conditions are changed to favour product formation. For flask cultures, the temperatures are controlled by cultivating in temperature-controlled incubators or culture rooms.

3. **Harvesting and Concentration:**

After the harvesting of each strain, they are freeze-dried and mixed to produce a probiotic formulation for food or therapeutic applications. An important aspect of probiotic production is the efficient growth of bacterial cells and subsequent preservation of their viability.

4. **Formulation**

Direct Addition: In some cases, probiotics can be added directly to the multigrain mixture, especially if the processing is gentle. Microencapsulation: This involves coating probiotic cells with protective materials like polysaccharides or proteins to shield them from harsh processing conditions and improve their survival in the gastrointestinal tract. Spray drying is a common method for microencapsulation.

5. **Mixing and Processing:**

The probiotic formulation (either free or microencapsulated) is then added to the multigrain dough or other components during the formulation process (Sie Huey Lee, Dave Siak-Wei Ow 2005).

Result

Isolation and Characterization of Probiotic Organism.

While we experimenting, the colonies were isolated for probiotic sample in MRS agar for selective isolation of lactic acid bacteria. Isolated colonies were preliminarily screened based on Gram's staining and biochemical test to identify potential probiotic bacteria.

Colony characters of Lactobacillus isolated

Colony no	Size	Shape	Elevation	Texture	Margin	Colour	Gram character
1	Small	Rod	Flat	Dry	Wavy	Purple	Gram positive

Biochemical Characters of *Lactobacillus* isolated

Biochemical test	Result	Interpretation
Citrate test	Negative	The medium remains green, indicating *Lactobacillus* cannot utilize citrate.
Indole test	Negative	When bacteria species is indole negative (indicating no tryptophanase activity) the Kovacs reagent will produce a dark yellow ring.
Methyl Red test	Positive	This test indicates the presence of mixed acid fermentation bacteria
Triple sugar iron test	Positive	An alkaline/acid (red slant/yellow butt) reaction is indicative of dextrose fermentation only.
Urea test	Negative	Lactobacillus species, in general, do not produce urease
Oxidase test	Negative	This means that when the oxidase test is performed, there is no colour change to a deep purple or blue within the typical timeframe
Catalase test	Negative	It will not produce bubbles (oxygen) in the presence of hydrogen peroxide
Voges-Proskauer	Negative	the test indicates that a bacteria culture does not produces acetoin, a neutral end product of glucose fermentation

Acid Tolerance Test

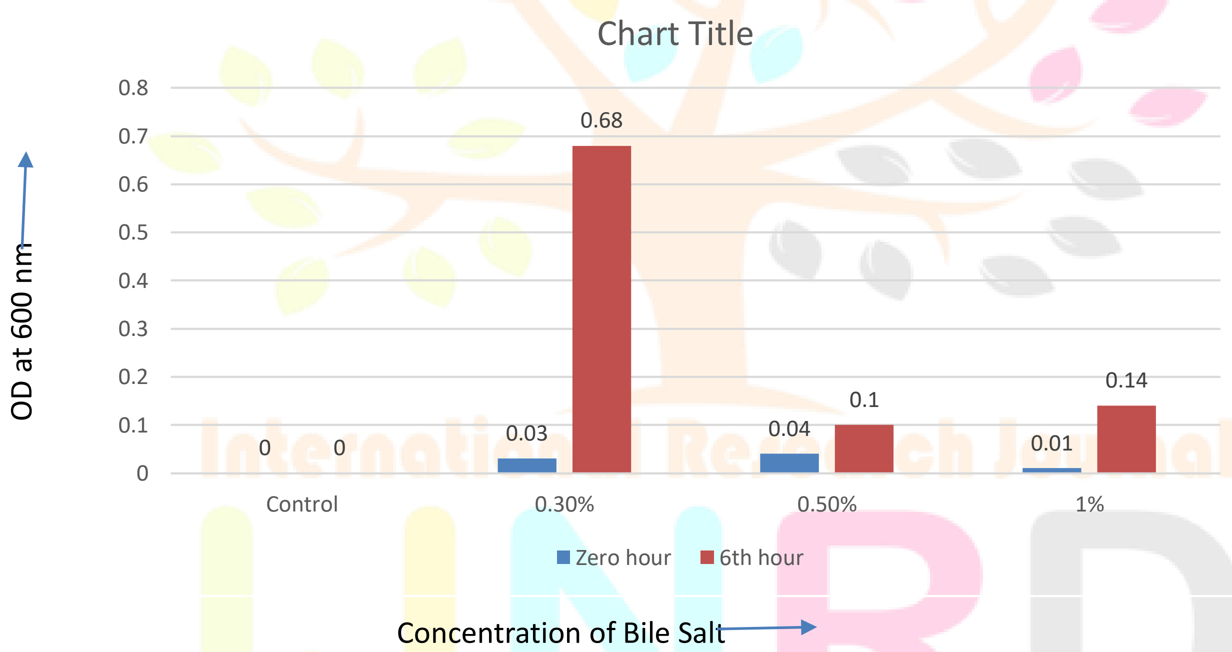
The test indicating varying levels of survival at different pH levels with some strains showing good tolerance at pH 3 and 4, while pH 5shows medium growth, while others exhibit poor growth or population at pH 6 and 7. The tolerance is highly strain specific, with some strain showing considerable higher tolerance at low pH value (3 and 4) compared to other. Generally, most lactobacillus strains can survive in acidic environments, but their ability to tolerate low pH in strain dependent.

Bile Salt Tolerance

Bile Tolerance of Isolated *Lactobacillus*

Sl.no	Concentration of bile salt	OD value at 600nm	
		0^{th} hour	6^{th} hour
1	Control	0.0	0.0
2	0.3%	0.03	0.68
3	0.5%	0.04	0.10
4	1%	0.01	0.14

Graph 1: Bile Salt Tolerance



Nutritional Value of final product

1. Moisture FSSAI Manual % 3.08
2. Total Ash FSSAI Manual % 3.75
3. Protein FSSAI Manual % 24.35
4. Fat FSSAI Manual % 3.73
5. Carbohydrates FSSAI Manual % 65.09
6. Energy FSSAI Manual Kcal/100g 391.33
7. Total sugar FSSAI Manual % 2.43

Discussion

The development of nutraceutical and probiotic products requires a multidisciplinary approach that integrates knowledge from nutrition, microbiology, and biochemistry. In our study, we focused on evaluating the nutritional profile of a product designed to offer health benefits beyond basic nutrition. The formulation incorporated key functional ingredients, such as prebiotics and specific probiotic strains, which have demonstrated positive effects on gut health and overall well-being. We utilized advanced biochemical assays to assess the composition and quality of the product, ensuring its efficacy and safety. The primary nutritional aspects of interest in our study were protein content, dietary fiber, and caloric energy. High protein content is crucial for muscle repair and growth, while dietary fiber contributes to digestive health and metabolic regulation.

Furthermore, the caloric energy provided by the product was analyzed to ensure it could serve as a beneficial supplement to a balanced diet, particularly for individuals seeking to enhance their nutritional intake. Through comprehensive testing and validation, our study demonstrates the potential of this nutraceutical product to support health in a targeted and scientifically-backed manner, aligning with growing consumer demand for functional foods.

Conclusion

The development of nutraceuticals and probiotics is gaining momentum due to their potential to address growing healthcare challenges and the rising demand for preventative care. These bioactive compounds, which include vitamins, minerals, enzymes, and beneficial bacteria, are recognized for their role in supporting health and well-being. As more consumers turn to natural, science-backed alternatives, future research must delve deeper into the mechanisms of action of these substances. Understanding how specific probiotics and nutraceuticals interact with the human body at a molecular level will enable the development of more targeted and effective therapies for a range of conditions, including digestive disorders, immune dysfunction, and even mental health issues.

Moreover, personalized medicine is likely to play a significant role in the future of nutraceuticals and probiotics. By tailoring these products to an individual's genetic makeup, microbiome, and specific health needs, researchers can enhance their efficacy and maximize health benefits. This shift toward precision nutrition requires more robust clinical trials and studies to validate the safety and effectiveness of specific strains and compounds for targeted health outcomes.

Equally important is ensuring the quality and stability of these products throughout their lifecycle. As the market for nutraceuticals and probiotics grows, there will be a need for stringent regulatory standards to maintain product consistency and consumer safety. Research into better preservation methods, stability, and bioavailability will be critical in ensuring that these products retain their effectiveness from production to consumption.

In conclusion, the future of nutraceuticals and probiotics is promising, but it depends on continued innovation in research, improved manufacturing processes, and a deeper understanding of how these compounds work within the body. As the sector matures, it will likely become an integral part of healthcare strategies focused on prevention, personalized treatment, and holistic well-being.

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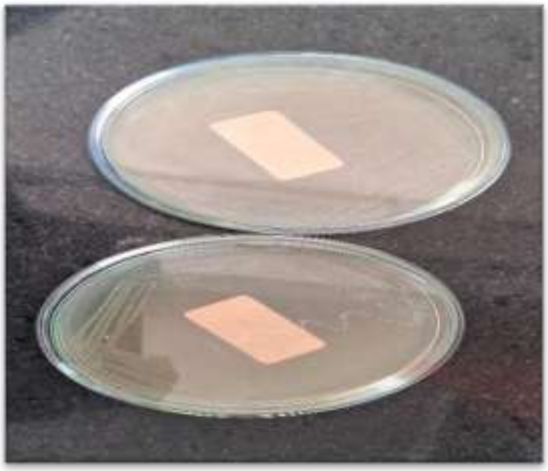
Figure1: Selected Multigrain

Figure2: Selected Probiotic



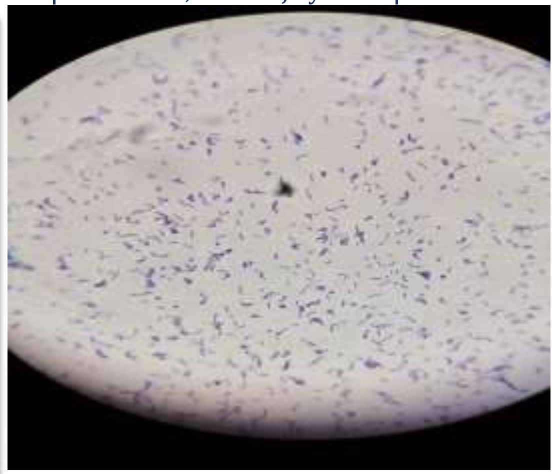
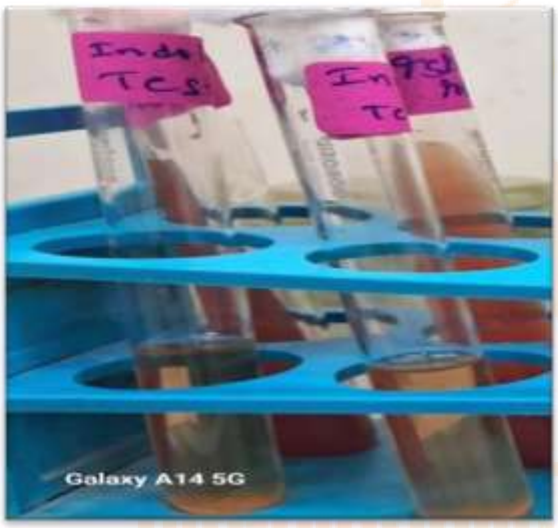


Figure3: Isolated colony

Figure4: Gram staining



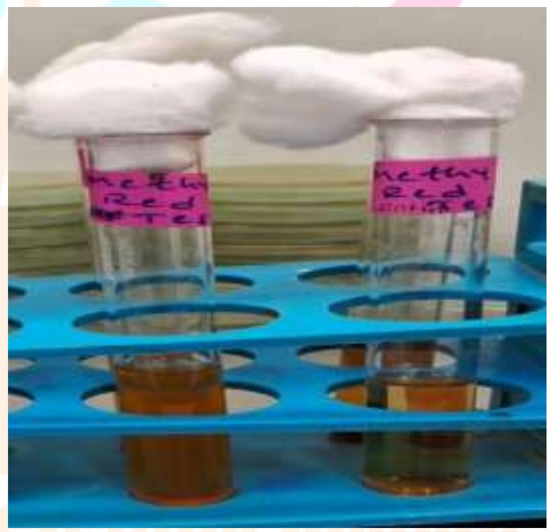


Figure5: Indole test

Figure6: Methyl Red test

Figure7: Urease test

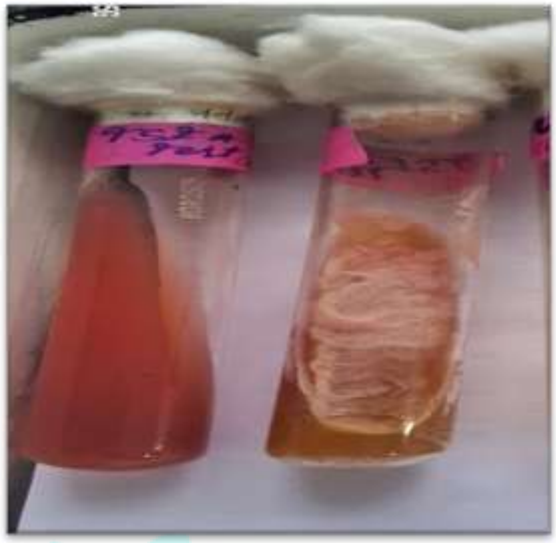


Figure8: TSIA test

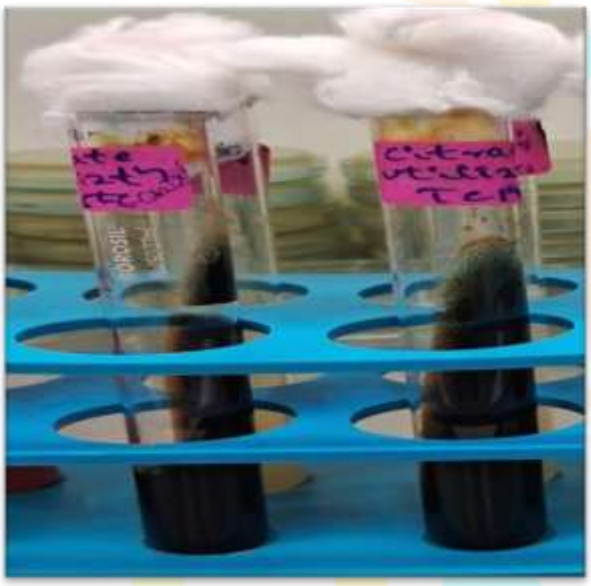


Figure9: Citrate test

Figure10: Catalase and Oxidase test

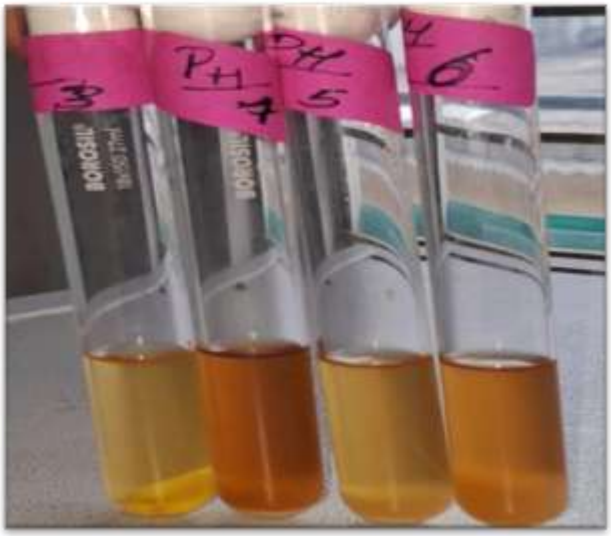


Figure11: Acid Tolerance test

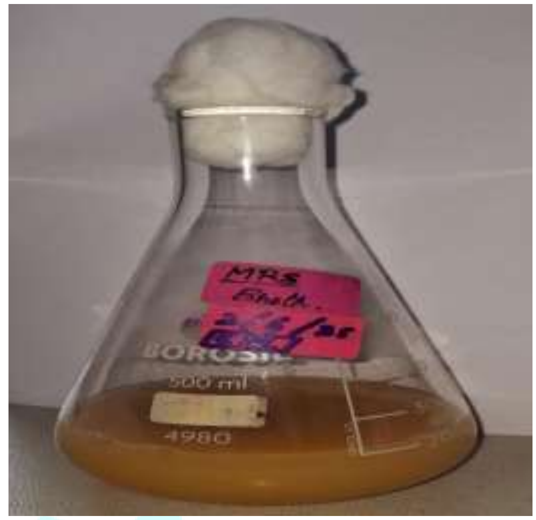


Figure12: Mass production