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Development of synbiotic powder preparation from reduced starch and fermented soyabean to improve gut health

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Abstract

Synbiotics are combination of both prebiotics and probiotics containing product mainly involved in the improving of gut health. These are emerging high demand nutraceuticals commercially around the world which was expecting of gaining 1.5 billion USD during the year 2023. Various countries like North America, UK, Germany, France, China and India are focused on this product development. Synbiotics plays a major role in the prevention of various gut related disorders like irritable bowel syndrome, gastro esophageal reflux disease, lactose intolerance etc. Our study was aimed to prepare the synbiotic by using soya bean and reduced starch from the rhizome of banana. Firstly, the soyabean paste was prepared by through cleaning of soyabeans then it was soaked, boiled and thrashed into paste. Add barley to this paste and mix well. Then the mixture was left for fermentation by using the Bacillus subtilis in a clean dry porcelain container for about 3 months. After the through fermentation the product was dried and powdered along with addition of reduced starch isolated from the rhizome of banana. After the formulation the product was evaluated for stability, growth rate of beneficiary bacteria, and reduction activity against pathogenic bacteria like E. coli. The results shows that the stability of the product was satisfactory. The colony forming units of bacteria was increased and the product shows the inhibition of pathogenic bacteria. Now a days because of adultrated food, antibiotic therapy gut health was disturbing synbiotics are major hope in future nutraceuticals commercially plays vital role in gut health maintenance.

Keywords: Synbiotics; Nutraceuticals; Gut Health; Reduced Starch; Fermented Soya

1. Introduction

In the earlier days humans are consumed healthy fermented foods and beverages which helps in the improvement of beneficiary bacteria in the intestine. Gradually as the many numbers of changes occurred in the dietary aspects leads to decrease in reduction of gut bacteria. Due to this condition debiosis occurs which will lead to various GIT disorders like indigestion, abdominal discomfort, flatulence.[1] Prolonged imbalance of gut bacteria leads to many numbers of pathological conditions like constipation, neurological disturbances, immunity related problems, increased blood glucose levels etc. [2] To overcome this condition synbiotics are only approach to improve the gut health. Synbiotics are combination of the prebiotic and probiotics components which shows the beneficiary effects on both growth and maintenance of beneficiary bacteria. Various fermented products used since from the ages like yogurt, cheese, kimchi, miso products are having this synbiotic activity because of fermentation due to good bacteria.[3] Now a days various products like carbonated beverages, irregular dietary aspects, excessive usage of antibiotics and intake of low fiber foods leads to decrease in the gut bacteria. As per the recent statistics synbiotic products research and usage was increased around worldwide.[4] Many countries like east America, Japan, china and india are drastically used these synbiotics from the past few years. Commercialization of synbiotics is come into existence and it was commercialized now a days. Even in the geriatrics, neonates also these prebiotics are prescribed during the usage of antibiotics which

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will gives the commercial importance and the marketing values as per the year wise areanticipated to be worth US $638.2 million by 2023. [5] With sales increasing at a compound annual growth rate of 7.5% throughout the forecast period, the market size will reach US $1116.9 million by 2033. Our study aims to develop a stable synbiotic powder using fermented soya beans and reduced starch for enhanced gut health which enhances the probiotic viability from fermented soya beans using *Bacillius subtilis* and prebiotic rich content to help the gut health. [6-7]

2. Materials and Methods

2.1. Selection of Probiotics

Bacillus subtilis probiotic should Survive stomach acid (pH 1.5 - 3.5), Tolerate bile salts (0.3 - 2%) in the intestine, Survive pancreatic enzymes (lipases, proteases, amylases). must adhere to the intestinal epithelial lining for long-term benefits. Adhesion helps in colonization and competition with pathogens.

2.2. Selection of Prebiotics

The prebiotic must resist digestion by human enzymes and reach the colon intact. Prebiotic Should not be absorbed or hydrolyzed in the stomach or small intestine. Stimulate Beneficial Gut Bacteria. The prebiotic must promote the growth of probiotics. Resistant starch, Inulin, FOS, Arabinoxylan etc.

2.3. Preparation of the Reduced Starch (prebiotic) from the Banana Rhizome

The Banana rhizome was collected, washed thoroughly with clean water to remove soil, debris and unwanted out layers from the banana rhizome before processing and peeled using a knife. The out layers are discarded as they contain more fibrous materials and fewer starch reserves. The inner part of the rhizome which is rich in starch is cut in small pieces to facilitate further processing. It is important to cut banana into small pieces because small pieces increase the surface area for better grinding and to make extraction easy. banana rhizome pieces were placed in a blender or grinder. Water was added to facilitate blending and to create a slurry. The blended mixture was left undisturbed for a specific duration to allow sedimentation. Sedimentation is an effective method to collect pure starch without the need for chemical processing. Sedimentation separates the higher starch granules from the lighter fibers and other impurities. To separate the unwanted fibers and coarse particles from the starch rich liquid. The supernatant liquid is passed through a muslin cloth to remove the excess fiber content. The liquid that passes through the muslin cloth contains dissolved starch, while the retained fibrous material is discarded. removal of remaining impurities after washing the starch solution was left undisturbed for second sedimentation process. The purified starch settles at the bottom and the remaining liquid is carefully removed. The drying process takes approximately five hours at 55^0C until the starch reaches a dry and powder consistency to prevent microbial growth and spoilage.[8]

A) B) C)

Figure 1 Raw materials used a) Soyabean b) Barley c) Rock salt

2.4. Preparation of Probiotic Using Soya Beans and Barley

Collect the soya beans from the local market and rinse the soya beans under cold water to remove any impurities. Place the beans in a large bowl and cover them with plenty of water. Soak the beans for at least 12 hours or overnight, at room temperature, covering them with a lid. After soaking, Heat the soya beans along with water until they become soft. Drain the beans and transfer them to a pot. Add fresh water to the pot, ensuring the beans are submerged. Bring the water to a boil, then reduce the heat and simmer for about 2-3 hours, or until the beans are soft and easily mashed. Add more water as necessary to keep the beans covered. Once the soya beans are cooked, drain them and let them cool slightly. Using a mortar and pestle, mash the Soya Beans, Barley until smooth. In a large mixing bowl, combine the mashed soya beans, cooked barley, and salt. Mix thoroughly until well combined. Transfer the mixture into a clean glass or ceramic container, pressing it down firmly to eliminate air pockets. Smooth the top and cover it with cheesecloth or a breathable cloth to allow air circulation while preventing contaminants. Add Bacillus subtilis as the fermenting agent. Place the fermentation container in a cool, dark place for fermentation. The ideal temperature is around 15-20°C (59-68°F). Allow fermentation to continue for 2 to 6 months. Stir the mixture occasionally to ensure aeration and prevent uneven microbial growth. Monitor the fermentation process periodically by observing the formation of bubbles, a sour smell, or a tangy flavour for any surface mold, which can be scraped off if it appears. After the fermentation period, check for a rich savory aroma and a thick paste-like consistency. Blend the mixture using a blender or food processor to form a smooth paste. Filter the paste using cheesecloth or a fine mesh sieve to remove any solid particles. Transfer the Product to smaller containers for storage, and keep it refrigerated to prolong its shelf life. [9-10]

2.5. Functional Assay of Synbiotic Product

2.5.1. Viability assay

Invitro Viability Assay [11]: For this assay suitable culture media is required for the growth of *Bacillus subtillis* bacteria. the suitable media for this assay was simple Agar media. The composition of agar media was shown in the Table 01 along with their composition.

Table 1 Composition of media

Ingredients	Quantities (100ml)
Agar	2 gm
Beef extract	1gm
Peptone	1 gm
Sodium Chloride	0.5gm
Distilled water	100ml

The above ingredients are weighed and dissolved in boiling water and stirred continuously by maintaining pH of 7 by adding acids or bases to it. Constant heat is supplied to obtain the clear solution. After obtaining clear solution it was transferred into sterile test tubes which are preheated in the hot air oven at 25^0C temperature for one hour. Transfer was done in a sterile condition to prevent any microbial contamination. The test tubes are placed in the autoclave and heated for about one hour at 121^0C temperature 15 pressure [psi]. After the completion of auto clave process the tubes are cooled at room temperature for about 24 hours. During this period the agar media was solidified and clear culture media was visible. Place all the tubes in the incubation chamber at 37.2^0C-37.8^0C temperature for about 3 days to identify any contamination and growth of other microorganisms.[12]

Prepare the agar medium as mentioned above by maintaining the sterile Conditions. Now pour the agar medium in the completely sterile petridishes and allow it to solidify. Now sterilize the agar plate by using autoclave for at 121^0C temperature 15 pressure [psi]. Inoculate the microorganism on to the surface of the agar medium by using an inoculation loop. Spread the microorganism eventually on the surface of the agar medium with the help of sterile spreader. Now create a small well in the surface of the agar medium by using cup plate or agar cup. [13] The well should be about diameter 1-2 cm. Now add small amount of the synbiotic powder solution to the well. The synbiotic powder solution is prepared by dissolving in distilled water. Ensure the synbiotic is evenly distributed within the well.

Incubate the agar plate at optimal temperature 37^0C for 24 hrs. After the incubation measure the diameter of the clear area around the well [inhibition zone].

The size of the inhibition zone is directly proportional to the antimicrobial activity of the substance.

Table 2 Zone of Inhibition Values

S.no	Microorganisms	Concentrations (µg/ml)	Zoneof inhibition (mm)
1.	*Bacilius subtilis*	1µg/ml	6mm
		10µg/ml	11mm
		100µg/ml	17mm
		1000µg/ml	22mm
2.	*E. coli*	1µg/ml	4 mm
		10µg/ml	8mm
		100µg/ml	15mm
		1000µg/ml	19mm

3. Results and Discussion

The antimicrobial activity of the synbiotic powder preparation was evaluated against *Bacilius subtilis* (pathogenic) and *E. coli* using the agar well diffusion method. The results indicate a concentration dependent increase in the zone of inhibition for both microorganisms. *Bacilius subtilis* showed significant inhibition, with zones increasing from 6 mm at 1µg/mL to 22 mm at 1000 µg/ml. *E. coli* exhibited a similar trend, with inhibition zones ranging from 4 mm at 1 µg/mL to 19 mm at 1000 µg/m. These findings suggest that the synbiotic preparation possesses antimicrobial properties, with stronger activity against Pseudomonas aeruginosa compared to E. coli. The results highlight its potential as an antimicrobial agent, possibly due to the combined effects of probiotics and prebiotics in the formulation. Further studies on its mechanism of action and spectrum of activity are recommended.

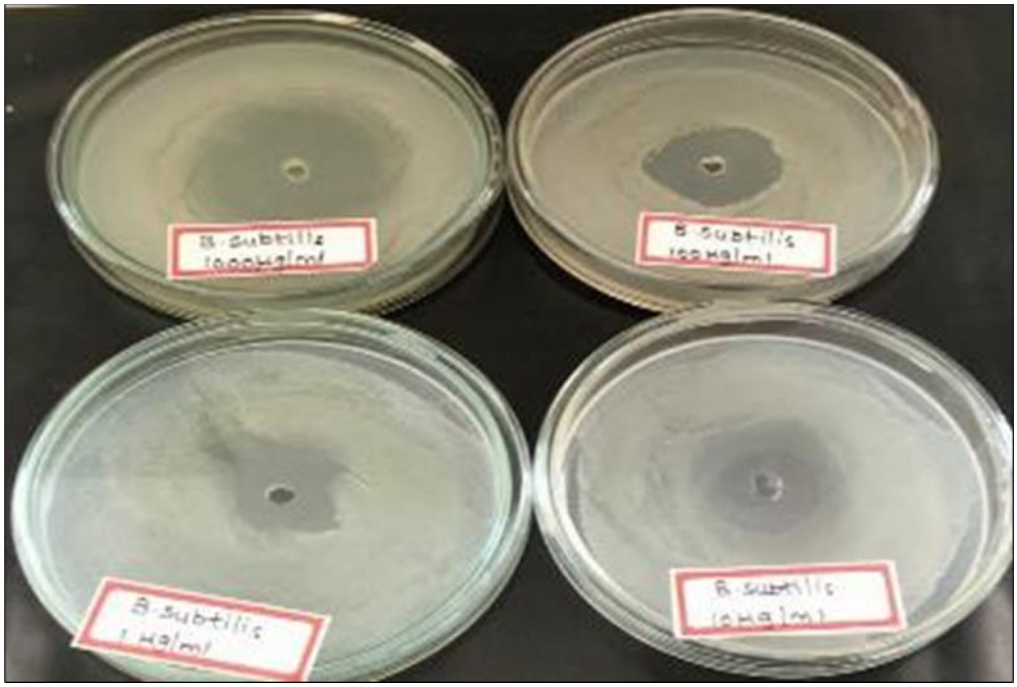

Figure 2 Zone of inhibition of *Bacilius subtilis*

4. Conclusion

Synbiotics are the novel products which plays a major role in maintenance of the gut health. It comprises natural existing bioactive ingredients from the plant source mixed with microbial organisms involved in fermentation. The sources

derived from plants are acted as gut microbial growth stimulators which in turn produces various metabolic enzymes results in absorption of vitamins, carbohydrates and other energy source. Synbiotics are commercially manufactured by various countries like Japan, China and Africa. Most of these products are ancient and utilized by the primitive civilization to secure healthy gut condition. Various microorganisms like *Lacto bacilli*, *Bacillis subtilis*, bacterium species. In our study *Bacillis subtilis* are choosen as Probiotic and resistant starch as prebiotic. The basic source of resistant starch was Banana rhizome discarded after collecting the fruits. These rhizomes are considered as biodegradable waste materials inturn can be used as healthy product. Synbiotics are not only involved in the maintenance of the gut health and also involves in inhibition of pathogens growth in intestine. They also prevent the attachment of pathogenic microorganisms to the mucal membrane of the intestine. The present study includes the extraction of reduced starch from banana rhizome and mixed along with the fermented paste containing soya beans, Barley and *Bacillis subtilis* bacteria. Various functional parameters are evaluated regarding the growth of bacteria and antimicrobial activity. Finally, the product exhibit significant functional activity and to be further investigated regarding *invivo* efficiency and enzymatic degradation.

Compliance with ethical standards

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Disclosure of conflict of interest

No conflict of interest to be disclosed.

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