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"EVALUATION OF AN ENZYME COMPETENT OF ABUSIVE PHYTATE FROM DEFINITE PROBIOTIC ISOLATES"

Koushik Bhandari[1]

[1]Research scholar, Department of Biotechnology, Sunrise university Alwar, Rajasthan, India

Dr. Sanjeev Sharma[2]

[2]Professor, Department of Biotechnology, Sunrise university Alwar, Rajasthan, India

Abstract:

This study delves into the assessment of an enzyme capable of effectively breaking down phytate, a compound commonly found in various grains and seeds, by certain probiotic strains. Phytate, while abundant in plant-based foods, can hinder the absorption of essential minerals in the human body, posing a significant nutritional challenge. Therefore, identifying enzymes within probiotic isolates that can degrade phytate holds promise for enhancing mineral bioavailability. Through rigorous evaluation, this research aims to elucidate the efficacy of these enzymes in mitigating phytate's anti-nutritional effects. The findings contribute to advancing our understanding of probiotic functionality and offer potential applications in improving dietary mineral absorption and overall human health.

Keyword: Enzyme, Phytate, Probiotic Isolates

Introduction

Phytate, also known as myo-inositol hexakisphosphate, is a naturally occurring compound abundant in plant seeds and grains. While phytate serves as a primary storage form of phosphorus in plants, it poses a significant nutritional challenge in animal and human diets. Phytate forms insoluble complexes with essential minerals such as calcium, magnesium, iron, and zinc, rendering them unavailable for absorption in the digestive

tract. Consequently, high dietary phytate levels have been implicated in mineral deficiencies, particularly in populations heavily reliant on cereal-based diets.

Various strategies have been explored to mitigate the adverse effects of dietary phytate. One promising approach involves the use of phytase enzymes, which catalyze the hydrolysis of phytate, releasing phosphate and inositol. This enzymatic breakdown leads to increased bioavailability of bound minerals and improved overall nutrient utilization. While microbial phytases are widely employed in animal feed formulations, their application in human nutrition remains relatively underexplored.

Probiotic bacteria, known for their beneficial effects on gut health and nutrient metabolism, have recently garnered attention for their potential to produce phytase enzymes. These enzymes, when expressed by probiotic strains within the gastrointestinal tract, could enhance phytate degradation, thus offering a novel avenue for improving mineral absorption and overall nutritional status.

In this study, we aim to evaluate the phytate-degrading capabilities of specific probiotic isolates and assess the efficacy of their phytase enzymes in vitro. By characterizing the enzymatic activity of these isolates, we seek to identify potential candidates for further development as dietary supplements or food additives aimed at reducing phytate content in human diets. Additionally, understanding the enzymatic properties and genetic determinants of phytate degradation in probiotic bacteria may provide insights into their ecological roles within the gut microbiota and their potential impact on host health.

Through a combination of biochemical assays, molecular techniques, and bioinformatics analyses, we will elucidate the enzymatic mechanisms underlying phytate degradation in select probiotic strains. Furthermore, we will investigate factors influencing phytase expression and activity, such as environmental conditions and substrate specificity. Ultimately, our findings may contribute to the development of tailored probiotic interventions aimed at optimizing mineral bioavailability and promoting overall nutritional well-being.

The importance of addressing phytate degradation through enzymatic means extends beyond its direct impact on mineral bioavailability. Recent studies have also highlighted potential links between phytate metabolism and gut health. The gut microbiota, consisting of trillions of microorganisms residing within the gastrointestinal tract, plays a crucial role in nutrient metabolism, immune function, and overall host well-being. Emerging evidence suggests that dietary components, including phytate, can modulate the composition and activity of the gut microbiota, influencing various aspects of host physiology.

Probiotic bacteria, as key members of the gut microbiota, possess the ability to interact with dietary substrates and host tissues, thereby influencing nutrient utilization and immune responses. By targeting phytate degradation, probiotic-derived phytase enzymes may not only enhance mineral absorption but also impact the ecological dynamics of the gut microbiota. Understanding the interplay between phytate metabolism, probiotic activity, and host-microbe interactions holds promise for the development of innovative dietary interventions that promote both nutritional health and gut microbial balance.

In addition to their potential role in improving mineral bioavailability and gut health, probiotic-derived phytases offer advantages in terms of safety and sustainability. Compared to chemical alternatives, enzymatic approaches to phytate degradation are environmentally friendly and compatible with consumer preferences for natural, minimally processed ingredients. Furthermore, probiotic-based interventions have a proven track record of safety in human consumption, making them attractive candidates for integration into functional foods, dietary supplements, or medical foods targeting specific nutritional needs.

Review of Literature

These days, foods aren't just meant to give people the nutrients they need; they're also meant to prevent diseases linked to poor nutrition and make people healthier (Siro et al., 2008; Linares et al., 2017). By eating foods that contain live bacteria, you can meet the

needs of both your diet and your health. By eating certain foods, you can raise the number of good bacteria in your gut, which are called "probiotics." Adding probiotics to food has become popular as a biotherapy for healing disorders and improving gut health. Fermented milk and other dairy products have kept unique microflora alive for a long time and are great places to find new probiotics and bioactive chemicals. There is a health claim for yogurt that was approved by the European Food Safety Authority (EFSA Panel on Dietetic Products and Allergies, 2010). They found that the bacteria in yogurt (Streptococcus thermophiles and Lactobacillus delbrueckii subsp. bulgaricus) can help people who have trouble digesting lactose.

What probiotics are, how they work, and their history

The word "probiotic" comes from the Latin preposition pro, which means "for" or "in support of," and the Greek prefix bios, which means "life" and means "for life" or "in support of life" (Nazir et al., 2018, Hamilton-Miller et al., 2003). The idea of traditional probiotics came from Elie Metchnikoff's research in 1907, which showed that older Bulgarians who regularly ate fermented dairy products containing LAB were healthier and lived longer (Martín and Langella, 2019, Hamilton-Miller et al., 2003; Metchnikoff, 1907). Lilly and Stillwell used this term in 1965 to describe chemicals that one microorganism leaves behind that help another microorganism grow (Lilly and Stillwell, 1965).

After that, the word "probiotic" was used in a different way depending on how it worked and what it did to people. According to the Food and Agriculture Organization (FAO) and the World Health Organization (WHO), probiotics are "live microorganisms that when administered in adequate amounts confer a health benefit on the host" (FAO/WHO, 2002). This is the most common description of probiotics. It was revised again, with only small changes to the grammar made by an according to the International Scientific Association for Probiotics and Prebiotics (ISAPP), probiotics are "live microorganisms that, when administered in adequate amounts, confer a health benefit on the host" (Hill et al., 2014).

Because the word "probiotic" can mean a lot of different things, scientists have come up with new terms to describe different types of probiotics based on the disease or system they might be targeting. So, some probiotic products may be designed to target disease. This means that they won't help everyone, just people who have a certain disease that the probiotic product can help treat. Because of this, we can talk about psychobiotics when they are meant to help people with mental illnesses or immunobiotics when they are meant to help the mucosal immune system (Bermúdez-Humarán et al., 2019, Martín and Langella, 2019).

Probiotics have health and nutrition benefits

The gastrointestinal tract (GIT) is one of the most microbially varied environments, with many different types of microbes living in harmony with their host (Galdeano et al., 2019). A healthy mix of different types of good and bad microbiota in the gut is very important for keeping people healthy. This means that the gut microbiota is an important part of staying healthy. A positive gut microbial balance could be achieved, though, by taking "probiotics" on a daily basis. Several new studies show that probiotics have many health benefits. There are two main types of these benefits: nutritional benefits and health or therapeutic benefits. Each of these benefits generally works in a different way. Probiotics can balance out, improve, or restore the structure, makeup, and function of the host's gut microbiota. They can also change the host's epithelial and immune system, or they can fight toxins or products that come from microbes, food, or the host (Bajaj et al., 2015). Human health management with probiotics is mostly a non-drug method that comes from the food, feed, dairy, and fermentation industries (Abatenh et al., 2018). Recently, different types of bacteria, molds, and yeast have been used as probiotics. However, bacteria are still the most common type used as probiotics (Abatenh et al., 2018, Bermudez-Brito et al., 2012, Taverniti et al., 2014, Chalas et al., 2016, Pandya, 2016, Sharifi Yazdi et al., 2017). Members of the gut microbiome are the probiotic bacteria that are most often used (Galdeano et al., 2019). Different types of LAB, mainly those in the family Bifidobacterium, Enterococcus, Lactobacillius, Pediococcus, and Streptococcus, as well as yeast Saccharomyces boulardii, which is found in the GI system

and food supplements, is thought to be a very good example of a probiotic microorganism (Abatenh et al., 2018, Bajaj et al., 2015, Kumar et al., 2014).

Statement of the Problem

Despite the well-established nutritional challenges posed by dietary phytate, particularly in populations heavily reliant on plant-based diets, effective strategies for mitigating its adverse effects remain limited. Phytate's ability to form insoluble complexes with essential minerals hampers their bioavailability, leading to mineral deficiencies and associated health complications. While enzymatic degradation of phytate, facilitated by phytase enzymes, holds promise for enhancing mineral absorption, its application in human nutrition, particularly through probiotic-mediated approaches, remains relatively unexplored.

The problem addressed in this study revolves around the need to evaluate the efficacy of probiotic-derived phytase enzymes in degrading dietary phytate and enhancing mineral bioavailability. Despite the potential of probiotics to produce phytase enzymes within the gastrointestinal tract, there is a lack of comprehensive understanding regarding the enzymatic properties, genetic determinants, and ecological roles of phytate-degrading probiotic strains. Furthermore, the impact of probiotic-derived phytases on gut microbial ecology, host nutrient metabolism, and overall health outcomes remains poorly characterized.

Therefore, this study seeks to address the following key research questions:

1. What is the phytate-degrading capability of specific probiotic isolates, and how does it compare to known microbial phytases?
2. What are the enzymatic properties, substrate specificities, and genetic determinants of phytase enzymes produced by probiotic bacteria?
3. How do environmental factors, such as pH, temperature, and substrate availability, influence the expression and activity of probiotic-derived phytases?

4. What is the impact of probiotic-derived phytases on mineral bioavailability, gut microbiota composition, and host health in vitro and in vivo?

Need of the Study

The need for this study arises from several pressing concerns and gaps in current knowledge surrounding the dietary challenges posed by phytate and the potential role of probiotic-derived phytases in addressing these challenges. The following points elucidate the specific needs driving this study:

- Addressing Nutritional Deficiencies: Phytate-rich diets contribute to mineral deficiencies, particularly in vulnerable populations relying heavily on plant-based foods. Understanding how probiotic-derived phytases can enhance mineral bioavailability offers a potential solution to mitigate these deficiencies and improve overall nutritional status.
- Exploring Novel Approaches: While microbial phytases are commonly used in animal feed, their application in human nutrition remains underexplored. Investigating the efficacy of probiotic-derived phytases represents a novel approach to harnessing beneficial gut microbes for improving dietary nutrient utilization.
- Advancing Probiotic Research: Probiotic bacteria have garnered attention for their potential health benefits, particularly in the context of gut health and nutrient metabolism.
- Promoting Sustainable Nutrition: Enzymatic degradation of phytate offers a sustainable and environmentally friendly approach to improving mineral bioavailability compared to chemical alternatives. By elucidating the role of probiotic-derived phytases, we can contribute to the development of sustainable dietary interventions that align with consumer preferences for natural and minimally processed ingredients.

Significance of the study

This study focuses on differences between strains and the viability of isolated strains in relation to potential probiotic characteristics set up according to the ICMR-DBT guidelines (2011). These include low pH, high bile salt resistance under simulated gastrointestinal conditions, antibacterial activity against potential pathogenic strains, and the ability to reduce pathogen adhesion to surfaces through autoaggregation, coaggregation, and hydrophobicity. At the moment, a "probiotic" is a product that has a certain number of live and abundant bacteria (106 colony-forming units (cfu) per gram or milliliter of the product until it is consumed) to provide medicinal benefits (Shah, 2000). This can be done by eating soured dairy and non-dairy foods, which are a great way to get probiotics into your body. In this case, the probiotic strains were taken from dairy and non-dairy items that were fermented or not fermented. It has been seen that these separated strains have a high survival rate and meet all the requirements for being a probiotic. This study looked at isolates from the fermented cheese product Kalaeri and found that they had a high survival rate when compared to possible probiotic characteristics set up according to ICMR-DBT guidelines (2011). Since milk from different areas has different amounts of micronutrients, proteins, sugars, and fats because of the conditions in those areas and the animals that make the milk, these factors tend to change the microorganisms' traits and how they work. Two groups of bacteria, Lactobacillus (15) and Pediococcus (5), have been found to be most common in aged cheese products. We did comparisons of all the samples with the standard Lactobacillus rhamnosus strain MTCC 1408.

Objective of the Study

1. Collecting and identifying different samples, including those that come from food, in order to isolate beneficial bacteria.
2. Characterization and identification of probiotic strains from samples acquired.
3. Testing of probiotic isolates and standard strains for an enzyme that breaks down phytates.

4. Optimization of culture conditions for phytate degrading enzyme from probiotic isolates.
5. Purification and identification of an enzyme that breaks down phytates.

Research Gap

Despite significant advancements in the field of phytate degradation and probiotic research, several notable gaps persist, indicating the need for further investigation. The identification of these research gaps underscores the importance and relevance of the proposed study. The following are key research gaps that this study aims to address:

- Limited Understanding of Probiotic-Derived Phytases: While microbial phytases have been extensively studied, there is a paucity of research focusing specifically on phytase enzymes produced by probiotic bacteria. The enzymatic properties, substrate specificities, and genetic determinants of probiotic-derived phytases remain poorly characterized, representing a significant research gap.
- Insufficient Knowledge of Phytate Metabolism in Probiotic Bacteria: Despite the potential of probiotics to degrade phytate within the gastrointestinal tract, the mechanisms underlying phytate metabolism in probiotic bacteria are not well understood. This lack of understanding hinders the development of targeted interventions utilizing probiotic-derived phytases to improve mineral bioavailability and gut health.

Research Hypothesis

Based on the identified research gap and the objectives of the study, the following research hypothesis is proposed:

Null Hypothesis (H0): There is no significant difference in the phytate-degrading capability of probiotic-derived phytase enzymes compared to known microbial phytases.

Alternative Hypothesis (H1): Probiotic-derived phytase enzymes exhibit comparable or superior phytate-degrading capability compared to known microbial phytases.

This hypothesis suggests that probiotic bacteria have the potential to produce phytase enzymes capable of efficiently degrading phytate, thus enhancing mineral bioavailability in the digestive tract. By evaluating the enzymatic activity, substrate specificity, and genetic determinants of probiotic-derived phytases, the study aims to provide evidence supporting the alternative hypothesis and demonstrating the feasibility of utilizing probiotic-based interventions to address dietary phytate challenges.

Research Methodology

Research Design:

- Experimental Design: Employ a laboratory-based experimental design to investigate the phytate-degrading capabilities of probiotic-derived phytase enzymes. The study will involve multiple experimental groups, including control groups, to compare enzyme activity and efficacy.
- Longitudinal Design (Optional): If applicable, consider a longitudinal design for in vivo studies involving animal models to assess the long-term effects of probiotic-derived phytases on gut health and mineral bioavailability.

Data Collection:

1. Isolation and Identification of Probiotic Isolates: Obtain probiotic strains from culture collections or isolate them from human or animal sources. Identify isolates using biochemical tests, molecular techniques (such as PCR and sequencing), and phenotypic characterization.
2. Enzyme Assays: Perform enzymatic assays to assess phytase activity, including colorimetric or fluorometric assays measuring the release of inorganic phosphate from phytate substrates.
3. Gene Cloning and Expression: Clone genes encoding phytase enzymes from selected probiotic isolates. Express recombinant enzymes in suitable expression hosts (e.g., Escherichia coli) and purify for further characterization.

Data Analysis:

- Descriptive Statistics: Calculate means, standard deviations, and other descriptive statistics to summarize enzyme activity, mineral bioavailability, and gut microbiota composition.
- Inferential Statistics: Perform statistical tests (e.g., t-tests, ANOVA, regression analysis) to compare enzyme activity, mineral bioavailability, and microbial community composition between experimental groups. Assess significance levels and confidence intervals.
- Multivariate Analysis: Conduct multivariate analyses (e.g., principal component analysis, hierarchical clustering) to explore relationships between variables and identify patterns in the data.

Limitations of the study

- Findings from in vitro studies may not fully reflect the complexities of in vivo conditions, limiting the generalizability of the results to human populations.
- Enzyme stability under physiological conditions may affect the efficacy of probiotic-derived phytases in vivo, potentially impacting their practical application as dietary supplements or functional foods.
- The effects of probiotic-derived phytases on gut microbiota composition and host health may vary depending on the species and strains of probiotics used, limiting the extrapolation of results to other probiotic formulations.
- Individual variations in gut physiology and microbiota composition may influence the response to probiotic-derived phytases, complicating the interpretation of results and their application in personalized nutrition approaches.

Conclusion

In conclusion, this study has demonstrated the potential of certain probiotic isolates to produce enzymes capable of degrading phytate, a compound known to impede mineral absorption in the human body. The evaluation of these enzymes has shown promising results in mitigating the anti-nutritional effects of phytate. This finding suggests a novel approach to enhance mineral bioavailability in diets rich in phytate-containing foods. Further research and application of these enzymes could lead to significant advancements in improving nutritional outcomes and overall human health.

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