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In vitro Activity of Encapsulated Lactic Acid Bacteria on Aflatoxin Production and Growth of *Aspergillus* Spp

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Abstract

This study investigated the potential of simultaneously using *Lactobacillus acidophilus* (LA-5), *Lactobacillus rhamnosus* (LGG), and *Lactobacillus casei* (LC-01) in both encapsulated (E) and non-encapsulated (NE) forms to inhibit the growth of *Aspergillus* spp. and aflatoxin production by *A. flavus*. The agar well diffusion method was used to assess fungal growth inhibition. Aflatoxin quantification was performed via HPLC. The lactic acid bacteria showed high antifungal activity and significantly reduced the production of AFB1, AFB2, AFG1, and AFG2 in both forms compared to the control. The total aflatoxin reduction was 94.1% (E) and 95.5% (NE). These results suggest that these lactic acid bacteria, used simultaneously, can prevent the growth and aflatoxin production of toxigenic *Aspergillus*.

Keywords: Aflatoxin, Antifungal Activity, *Aspergillus*, Lactic Acid Bacteria

1. Background

Fungi are major contaminants that compromise food quality, safety, and shelf-life, causing significant global economic losses annually. It is estimated that 5–10% of the world's food supply and up to 50% of fruits and vegetables in tropical regions are lost to fungal spoilage. Many fungi produce hazardous mycotoxins, such as aflatoxins, ochratoxins, and fumonisins, which pose serious threats to food safety.[1] Among these, *Aspergillus* species are particularly concerning due to their production of aflatoxins (AFs). Aflatoxin B1 (AFB1) is the most carcinogenic, leading to both acute and chronic health issues in humans, including acute hepatotoxicity (aflatoxicosis), liver cancer, immune suppression, and nutritional disorders. These toxins, primarily produced by *Aspergillus flavus* and *Aspergillus parasiticus*, result in severe health risks and substantial financial losses. Various methods, including chemical, physical, and biological approaches like oxidation, irradiation, and nanoparticles, have been used to control fungi and mycotoxins. However, many of these methods have drawbacks, such as high cost, reduced nutritional value, altered food properties, and potential unknown health effects. Consequently, there is growing interest in using beneficial indigenous microorganisms, traditionally present in foods, as a safe and effective biological strategy to inhibit fungal growth and prevent mycotoxin production, offering a promising alternative to conventional techniques.[2-4]

2. Methods

The study utilized *Lactobacillus acidophilus* (LA-5), *Lactobacillus rhamnosus* (LGG), and *Lactobacillus casei* (LC-01) from CHR-Hensen and *Aspergillus* test fungi from IROST. Encapsulated (E) and non-encapsulated (NE) LAB mixtures were prepared. For encapsulation, a bacterial suspension was mixed with a coating material (whey protein and inulin) and skim milk, then spray-dried at a low inlet temperature (120 °C) to preserve viability. The encapsulation yield was calculated. Antifungal activity was assessed using the agar well diffusion method against *Aspergillus niger*, *Aspergillus flavus*, and *Aspergillus parasiticus*. Fungal inhibition percentages and dry mycelial weight were measured. To evaluate aflatoxin (AF) inhibition, LAB (E and NE) were co-cultured with *Aspergillus flavus* in broth for 10 days. AFs (B1, B2, G1, G2) were extracted using an immune-affinity column and quantified via HPLC with fluorescence detection. All experiments were performed in duplicate or triplicate. Data were analyzed using ANOVA and Tukey's test ($P < 0.05$) in SPSS software.

3. Results

This study reports an encapsulation yield (EY) of 96.8% for spray-dried *Lactobacillus paracasei* using whey protein, a favorable biomaterial that protects bacterial cells. Both encapsulated (E) and non-encapsulated (NE) lactic acid bacteria (LAB) exhibited significant antifungal activity against bread spoilage fungi and a toxigenic

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Aspergillus flavus strain. Notably, they drastically reduced aflatoxin (AF) production (94.1% and 95.5% reduction for E and NE, respectively), with no significant difference in efficacy between the two forms. While NE bacteria more effectively reduced fungal mycelial dry weight, encapsulation did not significantly diminish LAB's inhibitory or anti-mycotoxin potential. The findings confirm that spray drying with appropriate materials like whey protein is a viable method for producing functional probiotic powders with preserved bio-preservative properties against fungi and mycotoxins

4. Discussion and Conclusion

In the present study, antifungal and anti-aflatoxin effects of encapsulated LAB bacteria are tested for the first time, and results show that spray drying is a useful method for large-scale preparation of encapsulated bacteria, aiming antifungal properties in food systems. This study is currently in progress to examine the effectiveness of such encapsulated bacteria on fungal contaminated bread.

5. Policy Recommendations

1. Promote the Adoption of Spray-Dried Probiotic Encapsulation in Food Preservation: Given the study's demonstration that spray-dried encapsulation effectively preserves the antifungal and anti-aflatoxin activity of lactic acid bacteria (LAB), food safety regulators and industry stakeholders should encourage the use of this technology. Policies should support the integration of encapsulated LAB as natural bio-preservatives in high-risk food products, such as bread and grains, to enhance shelf life and reduce mycotoxin contamination.

2. Establish Standards for Probiotic Encapsulation in Food Applications: To ensure consistency, safety, and efficacy, regulatory bodies should develop and enforce standardized guidelines for the encapsulation of probiotics using spray drying. These standards should specify optimal encapsulating materials (e.g., whey protein concentrate), process parameters, and viability thresholds to maximize the functional benefits of LAB in inhibiting fungal growth and aflatoxin production.

3. Support Research and Development for Bio-Preservative Solutions: Public and private funding should be directed toward further research on the application of encapsulated LAB in various food matrices and against a broader range of mycotoxigenic fungi. Policies should incentivize collaborations between academic institutions and the food industry to translate these findings into scalable, cost-effective solutions for global food safety challenges, particularly in developing regions prone to aflatoxin exposure.

4. Incorporate Encapsulated LAB into Food Safety and Public Health Programs: Health and agricultural ministries should consider incorporating encapsulated LAB-based interventions into national food safety strategies and public health campaigns. This includes educating producers on the use of LAB as a preventive measure against aflatoxin contamination and promoting consumer awareness about the benefits of bio-preserved foods for reducing mycotoxin-related health risks.

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Author Contributions

Authors contributed equally to this work.

Conflict of Interest Disclosures

All authors declared that they have no conflict of interest.

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