Effect on the growth of *Aspergillus flavus* during interaction with *Saccharomyces*

A major project report submitted in partial fulfilment of the requirement for the award of a degree of

Bachelor of Technology

In

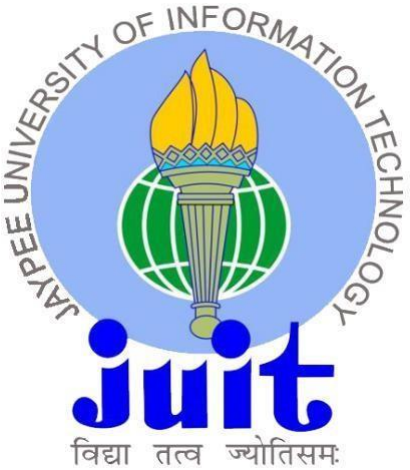
Biotechnology

Submitted by

Ayushi (201814)

Under the guidance & supervision of

Prof. (Dr.) Jata Shankar



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CERTIFICATE

This is to certify that the work which is being presented in the project report titled **'Effect on the growth of *Aspergillus flavus* during interaction with *Saccharomyces*'** in partial fulfillment of the requirements for the award of the degree of **Bachelor of Technology in Biotechnology** and submitted to the Department of Biotechnology & Bioinformatics, Jaypee University of Information Technology, Waknaghat is an authentic record of work carried out by **Ayushi (201814)** during the period from August 2023 to May 2024 under the supervision of **Dr. Jata Shankar** (*Professor*, Department of Biotechnology and Bioinformatic, Jaypee University of Information Technology, Waknaghat)

Submitted by:
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This is to certify that the above statement made by the candidate is correct to the best of my knowledge.

Supervisor Signature with Date

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CANDIDATE'S DECLARATION

It is to declare that the work presented in this report entitled **'Effect on the growth of *Aspergillus flavus* during interaction with *Saccharomyces*'** in partial fulfillment of the requirements for the award of the degree of **Bachelor of Technology in Biotechnology** submitted in the Department of Biotechnology & Bioinformatics**,** Jaypee University of Information Technology, Waknaghat is an authentic record of my own work carried out over a period from August 2023 to May 2024 under the supervision of **Dr. Jata Shankar** (*Professor*, Department of Biotechnology and Bioinformatic, Jaypee University of Information Technology, Waknaghat).

The matter embodied in the report has not been submitted for the award of any other degree or diploma.

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Ayushi
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This is to certify that the above statement made by the candidate is true to the best of my knowledge.

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Ayushi
(201814)

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LIST OF ABBREVIATIONS

S. Boularidii : *Saccharomyces boulardii*

C. difficile : *Clostridium difficile*

SCFAs : Short Chain Fatty Acid

CT : Cholera toxic

EHEC : Enetrohemorrhagic E. coil

EPEC : Enterpathogenic E. coil

ERK : Extracellular single-regulated kinase

A. flavus : *Aspergillus flavus*

PDA : Potato Dextrose Agar

PDB : Potato Dextrose Broth

ABSTRACT

This project dives into the complex and interesting connection between *Saccharomyces boulardii* which is a non-pathogenic yeast with probiotic properties and has *Aspergillus flavus* of both toxigenic and atoxigenic strains. *Aspergillus flavus* is a type of ordinary fungus which is very well known for its ability to produce mycotoxins especially aflatoxins and they are responsible for some major and significant dangers towards the health of living beings such as humans, animals and even plants.

We came to know via different studies about these *Saccharomyces boulardii* that they have multiple impacts on the growth dynamics and toxin generation of these *Aspergillus flavus* strains. Researchers examines the dose dependent effect of these *Saccharomyces boulardii* on *Aspergillus flavus* and came to know that they have some potential as a biocontrol agent.This report cover a wide range of techniques for allowing us to gain a full understanding of the complicated relationship between *Saccharomyces boulardii* and *Aspergillus flavus*.

This research's implication extends to the potential application of *Saccharomyces boulardii* as a sustainable and environment friendly methods for minimising mycotoxin contamination. This report not only emphasises the need or importance of understanding the dynamics between beneficial microbes and some dangerous fungi but it will also suggest that *Saccharomyces boulardii* may be integrated into some existing agricultural practices so that we can improve disease and toxic management.

Chapter 1: Introduction

1.1 Introduction

As we know in our everyday foods which we eat such as cereals, nuts, etc have some potential troublemakers such as aflatoxins which are begin produced by the *Aspergillus flavus* & *Aspergillus parasiticus*. These toxins have more than just an inconvenience and they may cause some major health problems in both of the humans and the animals. Such unhealthy problems consist of liver cancer, low immune system and retarded development in both humans and animals. In the food business we are currently trying to prevent fungal mischief by using chemical preservatives but these preservatives might not be best as they may be harmful to human health and environment.

Researchers are searching more and more natural and ecofriendly food preservatives approaches in the hope to keep out food safe and toxin-free. *Saccharomyces cerevisiae* is a type of common yeast which generally works in the background in baking and fermentation. Some recent researches have suggested that there are certain strains of this yeast which have good and powerful abilities against some pathogens such as *Aspergillus flavus* which are in the spotlight as they can be potential biocontrol agents.

Here in this report, we will try to discover some mysteries on *Saccharomyces cerevisiae* and *Saccharomyces boulardii*. We will try to find some new approach for food safety rather than just fighting off bad fungi. We already know that by using chemical preservatives we can have a speedy solution which is not safe.Therefore, we are trying to find some more natural and organic solution via *Saccharomyces cerevisiae* and *Saccharomyces boulardii*.

Our goal is to find safe food preservatives rather than potentially dangerous chemical preservatives. It’s like reimagining our industry by promoting organic practises which will contribute to a healthier and more sustainable future.

1.2 Problem Statement

As we already know that the contamination of many food products via using mycotoxins such as aflatoxins has some concerns majority in the food safety and health of the public. These aflatoxins have some toxic metabolites which are being produced via some certain types of strains consists of the *Aspergillus flavus* & *Aspergillus parasiticus*. These aflatoxins can easily contaminate some wide ranges of food industry products such as cereal, nuts & spices. These toxins are being responsible in causing liver cancer, low immune system in humans and animals.

Therefore, we must control growth of some of these fungi and must reduce levels of the aflatoxins in all food products which will be of great importance. Use of these chemical preservatives have already raised some concernes about potential health risks.

So now after all this there is a great need of developing natural and eco-friendly methods for food preservation. Recent researches have shown certain strains of *Saccharomyces cerevisiae* have antifungal activity against *Aspergillus flavus* and also other mycotoxigenic fungi. But due to use of *Saccharomyces cerevisiae* as the biocontrol agent which is against the *Aspergillus flavu*s is still in the early stages. Therefore, more research is must needed so that we can evaluate its effectiveness and safety. In addition to this the working management is not very fully understood of *Saccharomyces cerevisiae* against *Aspergillus flavus*, so further studies are much needed for investigation.

Therefore, the problem statement is to evaluate these novel isolates of the *Saccharomyces cerevisiae* and *Saccharomyces boulardii* against the *Aspergillus flavus* and to investigate the action mechanism of the yeast.

1.3 Objectives

1) To evaluate and investigate the potential inhibitory effect of *Saccharomyces* on some growth of the toxigenic & atoxigenic strains of the *Aspergillus flavus*.

2) To evaluate the expression of genes encoding for heat shock proteins during co culture.

1.4 Motivation of the Project Work

The project work which is presented in this article is totally motivated by the need to find different and alternative treatments in our food industry so that we can easily promote the production of organic food which are free of those chemical preservatives. As we all know that chemical preservatives have been linked to a number of health and environmental issues when they are used in different types of foods, animal and fish, food and cereal grains.

Therefore, now is a peak time to have a growing interest in developing natural and eco-friendly methods through which we can control food borne pathogens and harmful microorganisms. This report will try to investigate the potential of new *Saccharomyces boulardii* isolates which acts the biocontrol agent against the aflatoxigenic *Aspergillus flavus*.

The *Aspergillus flavus* & *Aspergillus parasiticus* creates some type of aflatoxins which are very dangerous and also poisonous that can easily contaminate varieties of foods, including the cereals and also dried fruits. As compared to chemical preservatives using *Saccharomyces boulardii* as a bio control agent which will provide a number of benefits such as sustainability and safety. The use of natural and eco-friendly methods to control food, borne pathogens and micro-organisms which are spoiled. Can you contribute to the production of organic food free of chemical preservatives which will promote a healthier and more sustainable food system.

1.5 Organization of Project Report

The report is divided into several sections such as an abstract, an introduction, literature, survey, key gaps in the literature survey, feasibility, study, materials and methods, results, conclusion, future, work etc. Here, the abstract will provide a brief summary of the report in which the main objectives, methods and findings will be highlighted. Our introduction section will provide a background of the research problem, highlighting the significance and motivation of the study. This report will also consist of a detailed review of the literature, discussing some currently states of knowledge regarding the use of the bio control agents in our food industry. Also, some challenges which are associated with chemical preservatives. Our literature survey will provide a detailed introduction on the topic with some key gaps in the literature which will help us later in results, conclusion and future work to work upon. In the feasibility study, we will add some references and their little summary so that we can know

in which direction our project will direct into. The result section presents some main findings on the project including some effects of *Saccharomyces boulardii* on growth of the *Aspergillus flavu*s and its aflatoxin production.

1.6 Technical Requirements

To implement our *Saccharomyces boulardii* as a biocontrol agent requires an advanced microbial cultivation and fermentation technologies. They are some types of analytical tools which are very crucial for assessing its effectiveness in suppressing *Aspergillus flavus* and reducing the aflatoxin. For better food safety and sustainability there are some technologies like metagenomics aid in understanding all the microbiome dynamics. There are some advance studies which demands some sophisticated laboratory equipments. Integrating some methods which are ecofriendly have some necessary technology which supports organic farming and sustainable food processing. In conclusion, successful utilisation requires a suite of advanced technologies spanning some cultivation, analytics, genomics and sustainable methods.

Chapter 2: Literature Survey

2.1 Overview of Relevant Literature

The *Saccharomyces boulardii* was firstly isolated from the litchi fruit in the Indo china and then it was used to treat diarrhoea in France in 1950s. At 37 degree celsius *S. boulardii* had an unusually high optimal growth [1].

Actions of the *Saccharomyces boulardii*

The luminal Action

A) Some Anti-microbial activities

- Inhibits the growth of some of the bacteria and some parasites.
- Reduces the gut trans-location of the pathogens.
- The neutralisation of the bacterial virulence factors.
- Supression of the host cell that somehow interferes with establishing bacterial colonization.

B) The Anti-toxin effects

- Inhibits the toxin receptor of the binding sites [2].
- The stimulation of these antibody productions which are against *Clostridium difficile* toxin.
- The direct proteolysis of some of the patho-genic toxins of these enzymatic proteins [3].

C) The Cross-talk with some of the normal microbiota

When the *S. boulardii* was first it given to the antibiotic-exposed mice or the patients suffering with diarrhea, the normal microbiota was re-established at a rapid rate.

Saccharomyces boulardii exerts multiple beneficial actions within the gastrointestinal lumen [4]. Firstly, it demonstrates antimicrobial activity by inhibiting some of the growth of these bacteria and the parasites, reducing the translocation of the pathogens across gut lining, neutralizing bacterial virulence factors, and suppressing the host cells adherence, which also interferes with the colonisation of the bacteria. Additionally, it exhibits the antitoxin effects by inhibiting the toxin receptors binding sites, stimulating the antibody production against the *Clostridium difficile* toxin [5] and directly proteolyzing pathogenic toxins through the secretion of enzymatic proteins.

Moreover, *S. boulardii* engages in some cross-talks with these normal microbiota. When closely administered to the antibiotic-exposed mice/patients suffering with the diarrhea, it helps in the rapid re-establishment of normal microbiota [6].

Saccharomyces boulardii has a significant positive impact on the intestinal mucosa. It reduces number of the infected cells that encourages the growth and also differentiation of the intestinal cells by responding to the trophic factors. It helps preventing the cell death & the synthesis of TNF-alpha, which reduces the mucositis and also restores the fluid transport pathways. This yeast also boosts proteins and the energy production, restoring the metabolic activity in the colony of epithelial cells [7]. By secreting some mitogenic factors, it enhances cells restitution and promotes the releasing of enzymes in the brush-border membrane. *S. boulardii* helps in restoring normal levels of the colonic (SCFAs) and also stabilizes the gastrointestinal barrier functions, and helps in strengthening the tight junctions between enterocytes. It also reduces the crypt hyperplasia and all the cell damages in the colitis models and decreases the intestinal permeability in the Crohn's disease patients[8]. Mycobiota composition largely depends on food habits. Diet habits such as plant- or animal-based, phytoestrogens-enriched plant products, fat-rich diets also influence the colonization of certain fungal species in the mammalian gut that include Saccharomyces [9]. Study found AFB1 production proportional to conidial concentration, optimized mass spectrometry for sensitive AFB1 detection in *A. flavus* [10]. Saccharomyces cerevisiae inhibited *Aspergillus flavus* and Aspergillus parasiticus growth, with varying effects at different temperatures [11]. *Saccharomyces boulardii's* effects on gut health and its efficacy in gastrointestinal disorder prevention and treatment [12]. Saccharomyces cerevisiae as the valuable organism but advises considering strengths and limitations. It uses functional classification to select appropriate models for specific pathways, improving research efficiency [13]. *S. boulardii* treatment majorly reduced the antibiotic associated with diarrhea in kids by 56% [14].
This study contradicts a prior one, asserting that *S. boulardii*, not *S. cerevisiae*, effectively treats *C. difficile* diarrhoea, challenging previous recommendations [15]. *S. boulardii* is effective for diarrhea and infectious types, with the potential for other conditions [16].

The success of our *S. boulardii* which as the probiotic also involves some of the factors which are included in these above paragraphs and they have some products to product variations and the number of these strains are being used in preparation of probiotic and the dose of the probiotic which is used. *S. boulardii* is generally available in the capsules of either lyophilised or heat dried preparation.

The choice of a very high quality of the probiotic product is one of most necessary factors in the food industry which determines the success of inhibit the toxin producing Aspergilli [16, 17].

Saccharomyces boulardii is effective in managing both acute and chronic diseases of the gastrointestinal system. In chronic diseases, *S. boulardii* is beneficial for the Crohn's disease, ulcerative colitis and the irritable bowel syndrome, and some parasite infections including amebic colitis/ giardiasis, and Blastocystis hominis. Additionally, it helps manage diarrhea associated with human immunodeficiency virus (HIV). Aflatoxin producing *Aspergillus flavus* is a prominent contaminant in crops consumed by human and germination of conidia key event to producing mycelia that produces Aflatoxin B1 & B2 [12, 18]. *Saccharomyces boulardii* has several beneficial actions against various infections:

The *Clostridium difficile* Infection

- Reduces diarrhea and intestinal inflammation and some histological damages by decreasing toxin A receptor binding.
- Releases a protease which cleaves the *C. difficile* toxins and their intestine receptors.
- Also increases the levels of some of the specific type of intestinal antitoxin A immunoglobulins [18].
- Also reduces the IL-8 manufacturing and the activation of the MAP kinases induced by the *C. difficile* toxin A in the human colonocytes.
- Significantly helps in reducing the mortality in animals challenged with *C. difficile* compared to controls [19].

Helicobacter pylori infection

- Alters H. pylori structure: Modifies the structure of Helicobacter pylori.

Vibrio cholerae infection

- Inhibits toxin effects: Reduces the effects of the V. cholerae toxin and the hydro electrolytic secretions by decreasing cAMP activities.
- Similarities to mammalian CT receptors: *S. boulardii* and mammalian cholera toxin receptors which may be structurally and also functionally similar, allowing yeasts to bind cholera toxin [20].

Amebic Dysentery

- Reduces red cell adhesion: Decreases the numbers of RBC (red blood cells) adhering to the amoebae.

- Reduces amoebae bearing the red cells: Decreases the numbers of amoebae with adhered red blood cells.

Infection with Enterohemorrhagic *E. coli* (EHEC)

- Modifies the host signals: Alters the NF-kB associated with the pathways which are activated by the invasion of the bacteria.
- Reduces MLC phosphorylation: Lowers MLC phosphorylation and also decreases transepithelial resistance in the colonocyte monolayers in response to EHEC [21].

Infection with Enteropathogenic *E. coli* (EPEC)

- Acts as a receptor decoy: Modifies the EPEC infection and also acts as the receptor decoy for the EPEC.
- Decreases intracellular EPEC: Lowers the numbers of intra-cellular EPEC.
- Prevents the resistance & permeability changes: Prevents in blocking transepithelial resistances and the permeability changes which reverses the impaired ZO-1 distributions, and also delays the apoptosis of epithelial cells in response to EPEC.
- Dephosphorylates LPS: Dephosphorylates lipopolysaccharides (LPS) from E. coli strain O55:B5

Saccharomyces boulardii has several key characteristics that enable it to survive and function effectively within the human gastrointestinal tract. Despite much of these oral doses being destroyed, with stool level typically 100 to1000 times less than the ingested doses, the surviving organisms are effective and usually presents at levels over 10 organisms per gram of stool. *S. boulardii* thrives at the body temperature (37°C), which is a good advantage as few yeasts perform well at this temperature. In its lyophilized form, it survives gastric acid and bile. Naturally resistant to antibiotics, *S. boulardii* also withstands proteolysis and exists competitively within intestinal tracts. Its level is notably high in patients with the disturbed intestinal microbiota, often because of antibiotic exposure, comparing to those without such exposures.

When administered orally, *S. boulardii* achieved steady-state concentrations within just three days and was cleared from the body within 3-5 days after the discontinuation.
Certain types of fiber, such as psyllium, can increase *S. boulardii* levels by 22%, while others, like pectin, show no effect [21].

Research on the aflatoxin of B1 biosynthesis in *Aspergillus flavus* also isolates from the central India has provided valuable insights into how aflatoxin is produced and identified atoxigenic isolates. Through careful experiments, the study determined the best conditions for extracting aflatoxin and developed a new mass spectrometric method for accurate detection. The study also examined how temperature and pH affect aflatoxin production, finding that higher temperatures and slightly acidic pH levels increased production in the tested isolates. This highlights the importance of environmental factors in fungal toxin synthesis and the need to understand these variables to reduce aflatoxin contamination in agriculture. The identification of non-toxigenic isolates, such as MTCC11580 and MTCC11588, shows promise for biocontrol strategies to reduce aflatoxin levels in crops, using naturally occurring non-toxic strains as biocontrol agents. Overall, this study not only enhances our understanding of aflatoxin biosynthesis but also emphasizes the practical importance of identifying atoxigenic isolates for food safety and improved agricultural practices to combat mycotoxin contamination [22].

The reference explores how yeasts can be used as biological agents to enhance plant disease protection and improve crop yields in sustainable agriculture. Yeasts like Pichia kudriavzevii can switch from a yeast-like form to a biofilm, which increases their antioxidant enzyme activity, stress tolerance, and biocontrol effectiveness. This adaptability and multifunctionality make yeasts valuable in agricultural settings [23].

The study also highlights the importance of the microbial volatile organic compounds being produced by yeasts and bacteria in plant protection. Bacterial volatiles have shown promise in enhancing plant defense mechanisms. Additionally, these volatile organic compounds from the antagonistic yeasts which are immobilised on the hydrogel spheres have been effective against some of various postharvest decays. For example, yeasts like Sporidiobolus pararoseus have been evaluated as the biocontrol agents against the pathogens such as Botrytis cinerea on post-harvest of the strawberry fruits [24].

The effect of *Saccharomyces cerevisiae* yeasts extracted on the butter lettuce demonstrates the potential of yeasts to enhance these health promoting compounds, antioxidants, and some anti

inflammatory properties. The study also explores the use of foliar sprays for microbial inoculants in sustainable agriculture and environmental health. Plant growth-promoting microbes, including yeasts, are recognized for their potential in sustainable agriculture and environmental management [25].

The review discusses the practical use of the beneficial micro-organisms required for plant nutritions and their protection, highlighting the possibilities and hopes of harnessing these microbes' multiple functions. Understanding the role of the microbial volatile organic compounds in plant-microbe interactions is crucial for improving plant defense mechanisms and communication [20].

In conclusion, it gives some valuable insights into the different roles of yeasts in the agriculture, from biocontrol and stress tolerance to enhancing plant nutritional quality and supporting sustainable agricultural practices. The benefits of yeasts in crop protection and yield improvement emphasize their potential to meet the needs of sustainable agriculture and consumer preferences for biocontrol products In a detailed review of how biocontrol yeasts can protect plants from diseases. It stresses the importance of understanding the complex interactions between biocontrol yeasts, plant pathogens, and plants to effectively manage diseases and promote plant health. [19, 23]. In addition, This may allow crucial and successful application of prokaryotic system such as Yeast [27].

The review points out that identifying how biocontrol yeasts work in the lab is a critical first step in developing these strategies. Advances in system biology, molecular biology, and computational tools have helped researchers uncover the very structural and some functional traits of these potential biocontrol agents [28]. Using these types of the tools, researchers can study and predict how these agents interact, leading to the creation of combinations of microbial antagonists that work together to control plant pathogens [30]. The document also mentions the commercial availability of strain mixtures for biocontrol, such as Blossom- and Boni-Protect®, and discusses the benefits of using diverse but complementary strains in biocontrol strategies. It highlights that a deep understanding of biocontrol mechanisms is essential for developing and registering biocontrol products. However, it notes that there is still much to learn about how biocontrol yeasts work, which hampers efforts to improve them using selection or molecular techniques [28].

The review also references on induced system resistance by beneficial microbes and also on viral kill system in yeast, emphasizing the ongoing research in this field [29].

In conclusion, the review underscores the need for fundamental research on biocontrol mechanisms to ensure successful biocontrol applications. Further exploration of the interactions between biocontrol yeasts, plant pathogens, and plants is crucial for advancing the field of biocontrol [33].

2.2 Key Gaps in the Literature

As we further studied the research papers, we found that there were some key gaps in those papers which are as follows: -

- **Standardisation of Research methods**: - There were many studies which lacked standard methodologies for evaluation of the safety and the mechanisms of action of probiotic agents. There was also some lack of consistency in those study designs, sample sizes, and outcomes, which measures the ability to compare and generalise findings [30].

- **Population specific studies**: - in many studies, we found out that there was a need for more research, targeting specific populations, such as paediatric patients or some individuals which have low immune system. According to some studies, the current gaps in the knowledge may exist due to a lack of diversity in the study population [31].

- **Real-world application of biocontrol strategies**: - While some study suggests that the biocontrol potential of some certain probiotic strains against contaminations like aflatoxigenic *Aspergillus flavus*, there is still some gap in the understanding to implement real-world applications based on these strategies. We need more further research for the effectiveness of bio control agents in different food products for a better environmental condition [32].
- **Long-time safety and side effects**: - The majority of the studies focused on short-term effects and benefits of probiotic interventions. There is still some literature gap regarding the long-term safety of these prolonged probiotic use and their potential side-effects [33].

- **Mechanistic understanding of probiotic action**: - While many studies highlighted the positive effects of probiotics, there’s some significant gaps in the understanding of these

underlying working of action. Thus, we need more research on how probiotics interact with the hosts system and exert their beneficial effects, which will result in contributing to a more informed and targeted use of probiotics [34].

- **Interaction between Probiotics and host Microbiota**: - There is also a gap in understanding the interactions between probiotics and the existing post microbiota. Many research or focusing on how probiotics influences the composition and function of the gut microbiome [35].

- **Dose-Response Relationships**: - There were many studies which provided information on the benefits of probiotics but they also lacked detailed exploration of dose response relationships. We have to understand that the optimal dosage for different probiotic strains in different conditions is very crucial for ensuring success and avoiding potential adverse effects but still this area remains under explored till this day [36].

- **Effectiveness in clinical subgroups**: - There was a gap in the knowledge regarding the effectiveness of probiotics in some specific clinical subgroups where the individuals with pre-existing medical conditions or those which are undergoing some specific medical treatments. The tailored use of probiotics for these subgroups may provide some valuable insights in the diverse health contexts [37].

Chapter 3: Feasibility Study

Table 1: Feasibility Study

S. No	Paper Title	Journal (Year)	Results	Limitation and other findings
[1]	Yeasts as a Potential Biological Agent in Plant Disease Protection and Yield Improvement	Agriculture (2022)	Yeasts show biocontrol potential by colonizing plants, producing compounds, inducing immunity, and enhancing plant growth and health.	Limited understanding of yeast mechanisms, complex genome organization, lack of plasmids.
[2]	Aflatoxins biodetoxification strategies based on probiotic bacteria	Toxicon (2020)	It reviews Aflatoxin toxicity, proposes probiotic bacteria for detoxification.	Relies on previous studies and lack of specific probiotic strain for aflatoxin degradation.
[3]	Insight into the metabolic changes during germination of *Aspergillus niger* conidia using nLC-qTOF	Biologia (2022)	Application technology	Secondary pathway
[4]	*Saccharomyces boulardii*: What makes it tick as successful probiotic?	Journal of Fungi (2020)	More research needed to understand *S. boulardii's* mechanisms and optimize its use	More research to understand *S. boulardii's* mechanisms
[5]	The action mechanism and biocontrol potentiality of novel isolates of *Saccharomyces cerevisiae* against the aflatoxigenic *Aspergillus flavus*	Letters in Applied Microbiology (2019)	The study suggests *Saccharomyces cerevisiae* as the biocontrol agents against aflatoxigenic *Aspergillus flavus*, improving food safety & sustainability.	Limitations included for more research in different food products, strain variations, and real-world applicability.

Table 2: Feasibility Study

S. No	Paper Title	Journal (Year)	Results	Limitation and other findings
[6]	Activity and anti-aflatoxigenic effect of indigenously characterized probiotic lactobacilli against *Aspergillus flavus*	Animals (2019)	Lactobacilli isolates effectively removed AFB1 aflatoxin in poultry feed	Further research needed for in vivo effectiveness.
[7]	The action mechanism and biocontrol potentiality of novel isolates of *Saccharomyces cerevisiae* against the aflatoxigenic *Aspergillus flavus*	Letter in Applied Microbiology (2019)	The study suggests *Saccharomyces cerevisiae* as the biocontrol agents against aflatoxigenic *Aspergillus flavus*, improving food safety & sustainability.	Limitations include the need on more research in different food products, strain variations, and real-world applicability.
[8]	Biocontrol yeasts: mechanisms and applications	World Journal Of Microbiology and Biotechnology (2019)	Biocontrol yeasts produce antifungal compounds, compete with pathogens, induce plant resistance.	Secondary pathwayLimited research on biocontrol yeast mechanisms and products hinder their development.
[9]	Food habit associated mycobiota composition and their impact on human health	Frontiers in Nutrition (2021)	*Saccharomyces* as important probiotic source	Largely depends on the food habits

Table 3: Feasibility Study

S. No	Paper Title	Journal (Year)	Results	Limitation and other findings
[10]	Evaluation of aflatoxin B1 biosynthesis in *A. flavus* isolates from central India and identification of atoxigenic isolates	Biotechnology And Bioprocess Engineering (2014)	Study found AFB1 Production proportional to Conidial concentration, optimized mass spectrometry for sensitive AFB1 detection in *A. flavus.*	Study limitations include potential variation in the aflatoxin production among the *A. flavus* which isolates the need for standardized conditions.
[11]	Antagonistic effects of *Saccharomyces cerevisiae* on the growth of *Aspergillus flavus* and *Aspergillus parasiticus* at varying temperatures	Mycology	*Saccharomyces cerevisiae* inhibited *Aspergillus flavus* and *Aspergillus parasiticus* growth, with varying effects at different temperatures	Limited understanding of the exact mechanism of *S. cerevisiae's* antagonistic effects on *Aspergillus species.*
[12]	Efficacy and safety of the probiotic *Saccharomyces boulardii* for the prevention and therapy of gastrointestinal disorders	Therapeutic Advances in Gastroenterology (2011)	It reviews studies on *Saccharomyces boulardii's* effects on gut health and its efficacy in gastrointestinal disorder prevention and treatment.	Caution advised for at-risk patients; individual results may vary. More research needed for *Saccharomyces boulardii's* efficacy and safety.
[13]	*Saccharomyces cerevisiae* as a model organism: A comparative study	PLoS ONE (2011)	The study highlights *Saccharomyces cerevisiae* as the valuable organism but advises considering strengths and limitations.	Static data used, may not capture dynamics;incomplete/ incorrect annotations in some organisms, but minimized with well-annotated *S. cerevisiae.*

Table 4: Feasibility Study

S. No	Paper Title	Journal (Year)	Results	Limitation and other findings
[14]	*Saccharomyces boulardii* in the prevention of antibiotic-associated diarrhoea in children: A randomized double-blind placebo-controlled trial	Alimentary Pharmacology and Therapeutics (2005)	*S. boulardii* treatment majorly reduced the antibiotic associated with diarrhea in kids by 56%, with a 8.5% incidence vs. placebo's 19.6%.	Study limitations include specificity to certain populations and settings, potential lack of generalizability, and unaccounted biases.
[15]	*Saccharomyces boulardii* is not *saccharomyces cerevisiae*	Clinical Infectious Diseases (1996)	This study contradicts a prior one, asserting that *S. boulardii*, not *S. cerevisiae*, effectively treats *C. difficile* diarrhea, challenging previous recommendations.	Current study challenges prior research, citing differences between the *S. boulardii* & the *S. cerevisiae*, lack of controlled experiments, and potential patient risks.
[16]	Edible mushroom: occurrence, management and health benefits	Food Materials Research	Mushroom- A good source prebiotics	Require specific condition

Chapter 4: Requirement Analysis and Methodology

4.1 Problem Definition

As we already know that the contamination of the various food products by mycotoxins, in particular aflatoxins which acts as a very significant threat on both food safety & public health. Aflatoxins which are being produced by specifically strains such as the *Aspergillus flavus* and the Aspergillus parasiticus can easily infiltrate a diverse range of food products mainly as cereals, nuts, and some spices. This will result in health effects such as liver cancer and weak immune system in both humans and animals. We already know that chemical preservatives have been used in the food industry for many decades and therefore the potential health risks also have emerged. Therefore, there is a very urgent need to develop some natural and eco-friendly methods so that we can use them in food preservation. Recent studies have indicated that some certain strains of *Saccharomyces boulardii* can exhibit antifungal activity against *Aspergillus flavus* and other mycotoxin fungi. The utilisation of *Saccharomyces boulardii* as a Bio control against *Aspergillus flavus* is still in its early stages so further exploration is required for its effectiveness and safety. The problem definition will provide a clear overview of the issues which are surrounding aflatoxin contamination and will further highlight the potential of *Saccharomyces boulardii* as a biocontrol agent.

4.2 problem Analysis

- **Aflatoxin contamination**: - This aflatoxin contamination in various food products poses dangerous threat to public health which causes liver cancer and weak immune system in humans and animals.

- **Traditional Preservatives Concerns**: - The constant using of these chemical preservatives have raised some valid concerns on potential health risks and environmental impact. We need to explore some alternate natural methods for food preservation to ensure long-term sustainability.

- **Potential of *Saccharomyces Boulardii***:- some previous researches have showed that the potential of certain strains of *Saccharomyces boulardii* may act as a bio control agents against *Aspergillus flavus* . However, the success and safety of this certain strain in practical applications is not very well established.

- **Limited understanding of Mechanisms**: - The specific mechanism by which *Saccharomyces boulardii* inhibits the growth of *Aspergillus flavus* still remains not understood completely. Therefore, we are in a desperate need of detailed investigation into the molecular and psychological interactions between these yeasts strains so that the target fungi is very essential for optimising the food applications.

- **Need for novel isolates**: - We already know that some certain strains of *Saccharomyces boulardii* showed some results but still it's under exploration. Therefore, we are unable to know the true potential of their efficacy against both toxigenic and atoxigenic strain of *Aspergillus flavus* which is very crucial for fungal control.

- **Research Gap**: - The use of *Saccharomyces boulardii* which acts as the biocontrol agent against these *Aspergillus flavus* is still in early stages thus, indicating significant research gap.

4.3 Requirements

- **Laboratory Facilities**:- As we already know that to perform an experiment, a laboratory is the first essential priority to have with sterile conditions for cultivating and studying our *Saccharomyces boulardii*. Facilities for fungal culture which includes growth media, and specialized equipment for monitoring fungal growth.

- **Fungal Strains**:- To perform this miracle, we will be needing toxigenic and atoxigenic strains of *Aspergillus flavus* for some comprehensive evaluation. Also to know the aflatoxin production capabilities we need to have authentication and documentation of the fungal strains.

- **Yeast Isolates**:- We need to make *Saccharomyces boulardii* for testing against *Aspergillus flavus*. We have to measure quality controls to ensure the purity and validity of the yeast isolates.
- **Experimental Design**:- We need to have well-defined experimental protocols for assessing these antifungal activities of the yeasts which isolates against *Aspergillus flavus*. We will be doing randomised controlled trials to minimise bias and must ensure statistical validity.

- **Documentation and reporting**:- A very well-maintained documentation of experimental procedures, results and analysis.

4.4 Solutions

- **Isolates selection and cultivation**:- We have to carefully select novel isolates of *Saccharomyces boulardii* for their unique antifungal properties. We have to cultivate yeast isolates under controlled laboratory conditions to ensure purity and viability.

- **Fungal strain preparation**:- We have to obtain toxigenic and atoxigenic strains of *Aspergillus flavus* for evaluation. We also have to characterise and authenticate fungal strains so that we can document their production capabilities.

- **Experimental design**:- We have to develop well defined experimental protocols including dose response studies, time course of experiments and controls.

- **Suitable environment**:- We have to use suitable growth media and conditions so that we can mimic real world scenarios.

- **Use of probiotic capsules**:- We will be using probiotic capsules (Entroflora) to extract *Saccharomyces boulardii* to observe the effects of *Saccharomyces boulardii* on Aspergillus flavus.

4.5 Material Needed

Table 5: Chemicals

S.No	Chemical	Manufacturer
1	Potato Dextrose Agar (PDA)	HIMEDIA
2	Potato Dextrose Broth (PDB)	HIMEDIA
3	Potassium chloride	HIMEDIA
4	Sodium phosphate dibasic	HIMEDIA
5	Potassium phosphate monobasic	HIMEDIA
6	Tween 20	Merck
7	Lactophenol cotton blue	HIMEDIA
8	Trizol Reagent	Takara
9	Chloroform	HIMEDIA
10	Isopropyl alcohol	Loba Chemie
11	Ethanol	HIMEDIA
12	Nuclease free water	Affymetrix
13	Agarose (1.2%)	HIMEDIA
14	Tris	HIMEDIA
15	Glacial acetic acid	Merck
16	EDTA (0.5M)	Sigma-Aldrich
17	EtBr	HIMEDIA
18	6X DNA loading dye	Thermo Scientific
19	DNA ladder	Thermo Scientific

Table 6: Instruments Used

S.No	Instrument	Manufacturer
1	Laminar air flow	Microsil India
2	Incubator with shaker	Labnet
3	Weighing balance	Denver
4	Centrifuge	HITACHI, Eppendorf
5	Autoclave	SANYO
6	Agarose gel electrophoresis setup	Bio-RAD
7	Gel doc. System	Bio-RAD
8	Freezer (4℃, -20℃, -80℃)	Celfrost/Vestfrost solutions
9	Spectrophotometer	Thermo Fisher scientific
10	Haemocytometer	Rohem India
11	PCR	Applied Biosystem
12	Ice machine	Manitowac
13	Vortex	REMI

Table 7: Equipment and other requirements

S.No	Equipment and other requirements	Manufacturer
1	Micropipettes	Eppendorf/Thermo Scientific
2	Microtips	Tarson
3	Conical flasks	JSGW
4	Microcentrifuge tubes (1.5ml and 2ml)	Eppendorf
5	Falcon tubes (50ml, 15ml)	Tarson
8	Reagent bottles (1 litre and 100 ml)	JSGW
9	Mortar and pestle	JSGW
10	Spatula	JSGW
11	Surgical blade	JSGW
12	Forceps	JSGW

4.6 Methodology

- **Prepared PDB and PDA media:-**
- For example, In preparing 10 plates of PDA media and 10 slants we used 400ml of dH2O, 15.6gm of PDA. Then we autoclaved the media and poured them carefully under laminar airflow.
- We used sterile loop and under sterile conditions we streaked the plates and slants accordingly. We grew BT01,BT03 and BT05 *Saccharomyces boulardii* and *Saccharomyces cervisiae* and these were grown in PDB media and then further spreaded over PDA plates. After growth we observed them under microscope. Then we calculated the cells using hemocytometer.
- The formula to count the cells is :-

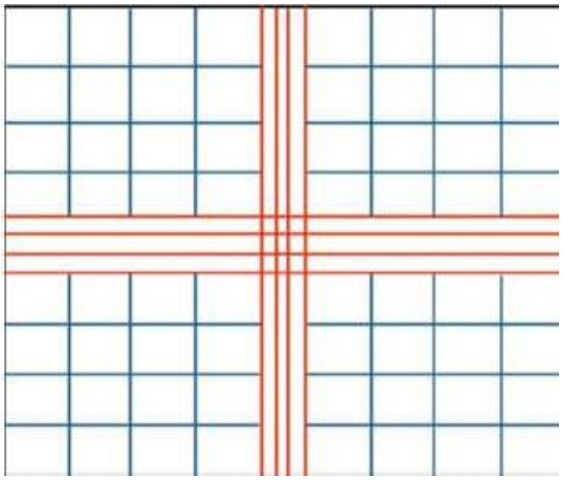

Fig:- 1 Reference image of hemocytometer

- In *Saccharomyces cervisiae* we got 1.5*106 no. of the cells in just 1 µL sample.
- In *Saccharomyces boulardii* we got 6*106 no. of the cells in just 1 µL sample.
- In *Aspergillus flavus* BT01 we got 1*103 no. of the cells in just 1 µL sample.

- **Preparation of co-cultures:-**

Prepared different ratios of cells to see if *Saccharomyces boulardii* is inhibiting the growth of toxigenic strain i.e., BT01

Table:- 9 Inhibiting ratio of *Saccharomyces boulardii* :: BT01

Sr. No.	*Saccharomyces boulardii*	BT01	INHIBITING
1	10 µL	100 µL	No
2	10 µL	1000 µL	No
3	1 µL	1 µL	No
4	100 µL	1000 µL	Yes
5	10 µL	100 µL	No
6	1 µL	1 µL	No
7	1 µL	10 µL	No
8	10 µL	1 µL	Yes

Growth of culture broth:-

After 2 days of growth of co-culture of *Saccharomyces boulardii* and BT01 spores were harvested.

RNA extraction:-

RNA extraction was done by using TRIZOL-CHLOROFORM method at time point of 24 hour for both treated and control samples. RNA extraction process is as follows:-

1. After the growth of 24 hour the cell suspension in the media transferred to the 50ml tarsons and centrifuged for 6000 rpms for almost 10 minutes.
2. Discarded the media by avoiding the cell loss.
3. 1ml of the Trizol reagent is being added to cell pellet, mixed & then transferred to the chilled mortar and pestle.
4. Liquid nitrogen is added to the cells to homogenized cells until forms powder.
5. 2-3ml of Trizol reagent is added to the mortar, mixed using pestle.
6. The homogenised sample which was incubated for 5 minutes at the room temperature will convert in liquid form used.
7. 0.2 milli-litre of the chloroform was then added per 1 ml of that Trizol reagent.
8. The tubes were shaken vigorously by hands for almost 15 seconds, then incubated for 2-3 minutes at basic room temperature.
9. Samples were then centrifuged at the 12000 ×g for around 15 minutes at 2- 8℃.
10. After centrifugation mixture separates into the lower red organic phase, interphase and upper colourless aqueous phase, RNA resides in the upper aqueous phase.
11. Aqueous phase was carefully transferred in the fresh tube.

12. 0.5 milli-litre of isopropyl alcohol was then added to freshly transferred sample per 1 milli-litre of that Trizol reagent. Then sample was incubated for 45 min at -20℃.
13. The sample was then centrifuged at 12000 rpms at the 2-8℃ for almost 10 min.
14. RNA gets precipitated and forms a gel like pellet at the bottom.
15. The supernatant was then removed and the pellet was washed with around 75% of ethanol.1 milli-litre of that 75% ethanol was added per 1ML of that Trizol reagent.
16. Further it was mixed using vortex and centrifuged at 7500 ×g at 2-8℃ for around 5 min.
17. Remove the supernatant and left the pellet air dry.
18. Stored in 25 µl of nuclease free water.

The agarose gel electrophoresis:-

Once the RNA is extracted gel electrophoresis was run to check its quality and yield. To achieve this 450 ml of 1X TAE buffer working solution was prepared in distilled water from 50X TAE buffer stock. For 0.6% agarose, 0.6 gms of agarose was weighed & dissolved in 50 ml of 1X TAE buffer, heated till become transparent, followed by the addition of 1µl of EtBr when the flask of gel is bearable to hold. Gel was carefully poured on the casting tray with comb in and allowed to solidify for 15 minutes. Once the gel is solidified, it was put in the buffer chamber and remaining 400ml of the buffer poured in, carefully remove the comb. Loading of samples involved placing 6×DNA ladder in the first well and RNA samples in the remaining wells. The Setup was ran initially at 50V for 10 minutes when the samples was ran out from the wells then at 100V for 45 min followed by visualisation of the RNA bands in gel doc. System.

Sample preparation

DNA ladder preparation - 3 µL ladder

RNA sample preparation - 2 µL loading dye and 5 µL RNA sample

450 ml of 1× TAE Buffer preparation from stock

For preparation of 450ml of 1X working from 50× stock of TAE buffer, 9ml of the stock was added to 441ml of distilled water.

Table:-10 Litre of 50X TAE buffer preparation (stock)

Components	**Quantity**
Tris	242g
Anhydrous Acetic Acid	57.1 ml
0.5 M EDTA	100 ml
Deionized Water	Up to 1L

RNA Quantification using Spectrophotometer:-

RNA quantification, also known as nucleic acid quantification. It determines the concentration of the RNA which is presented in a sample.

Following the visualisation of the bands, a spectrophotometer was utilised to quantify the RNA. Spectrophotometer analysis provides average concentrations of the DNA or RNA presented in the mixture as well as determine the purity on the fact that the nucleic acids absorb UV light in a specific pattern in the case of DNA and RNA the absorbance take place at 260nm. The analysis is totally based on this fact that nucleic acids absorb UV light in specific pattern, in case of DNA and RNA the absorbance take place at 260 nm while absorbance at 280nm is used to estimate the amount of Protein in the sample.

The Beer-Lambert law makes a linear relation between the absorbance and concentration the, therefore can be used to further relate at the amount of the light being absorbed to the concentration of that molecule.

Before analysis of the RNA sample was diluted as, in a cleaned quartz cuvette, 5µl of RNA sample added to 995µl of distilled water and the optical density was determined at 260nm and 280 nm using spectrophotometer.

Once the OD was known, 260/280 ratio was calculated. Ratio
approximately or near to 1.8 indicates the purity for DNA
near to 2.0 indicates purity for RNA
lower than or equal to 1.6 that may indicate presence of protein contamination.

The formula to calculate the amount of RNA is as follows-

40 µg/ml * Abs at 260nm * Dilution Factor

cDNA Synthesis :-

It is also known as copy DNA or we can also say it as complementary DNA. It is a synthetic DNA molecule which is transcribed from mRNA. cDNA was synthesised samples using Thermo scientific cDNA synthesis kit. cDNA is synthesised on based on the amount of RNA (100ng) obtained as described in previous reported literature (38) using 1 µl of RNA.

Table:- 11 Reaction mixture concentration of different components

Components	**Volume (in µl)**
5x cDNA Synthesis buffer	4
dNTPs	2
Oligo dt	1
Random Hexamers	1
RT enhancer	1
Enzyme mix	1
Template RNA	1-5
Nuclease free water	Up to 20

The concluding volume of reaction mixture was kept at 20 µl

Table:- 12 Cyclic program for cDNA synthesis

Steps	Temperature	Time	No. of cycles
cDNA synthesis	42ºC	30	1
Inactivation	95 ºC	2	1

- **qRT-PCR :-**

  Firstly, the primer which we used was diluted 100 times (10 µl stock primer and 990 µl nuclease free water). Then qRT- PCR was performed for the selected genes i.e tubulin, Hsp90. Tubulin is a type of housekeeping gene which we used as reference gene. In total there were 12 reaction mixture which were prepared (3 NTCs, 3 positive controls, 1 sample). All the components were then carefully added in the PCR vials, according to the manual. iQ™ SYBR® Green supermix was used. Protocols were already set according to the manual itself.

Table:- 13 20 µl qRT-PCR reaction mixture concentration

Components	**Volume (in µl)**
Green master mix 2×	10
Forward primer	1
Reverse primer	1
DNA template	2
Nuclease free water	6

Table:- 14 Cyclic program for qRT-PCR

Steps	**Temperature**	**Time**
Initial denaturation	95°C	3 min
Denaturation	95°C	10 sec
Annealing	60°C	30 sec
Extension	72°C	30 sec
Melt curve	65°C to 95°C	0.05 sec

Table:- 15 Annealing temperature for selected genes

Selected genes	**Annealing temperature**
Tubulin (Positive control)	56°C
Hsp90	60°C

Primarily, Hsp90 among the heat shock family proteins from *A. flavus* was selected RT-PCR analysis to determine the stress, regulated in aflatoxigenic and non-aflatoxigenic *A. flavus* isolates and as a potential drug targets during the interaction with *Saccharomyces*, and tubulin gene as a reference gene (38, 40, 41).

Table:- 18 List of primers used in quantitative Real-time PCR

Gene	**Sequence 5' to 3'**	**GI. NO**	**Product size (bp)**
β-tubulin	F:GGAATGGATCTGACGGCAAG	480538393	122
	R: GGTCAGGAGTTGCAAAGCG		
hsp90	F:CGTCAAGTCCATCACTCAGC R:GCTTGTGGATGCGCTCGGC	238503320	98

Chapter 5: Results

5.1 Results

Fig:- 2 Different strains of *Aspergillus flavus* and *Saccharomyces*

Fig:- 3 Entroflora (Probiotic) capsules isolates *Saccharomyces boulardii* strain

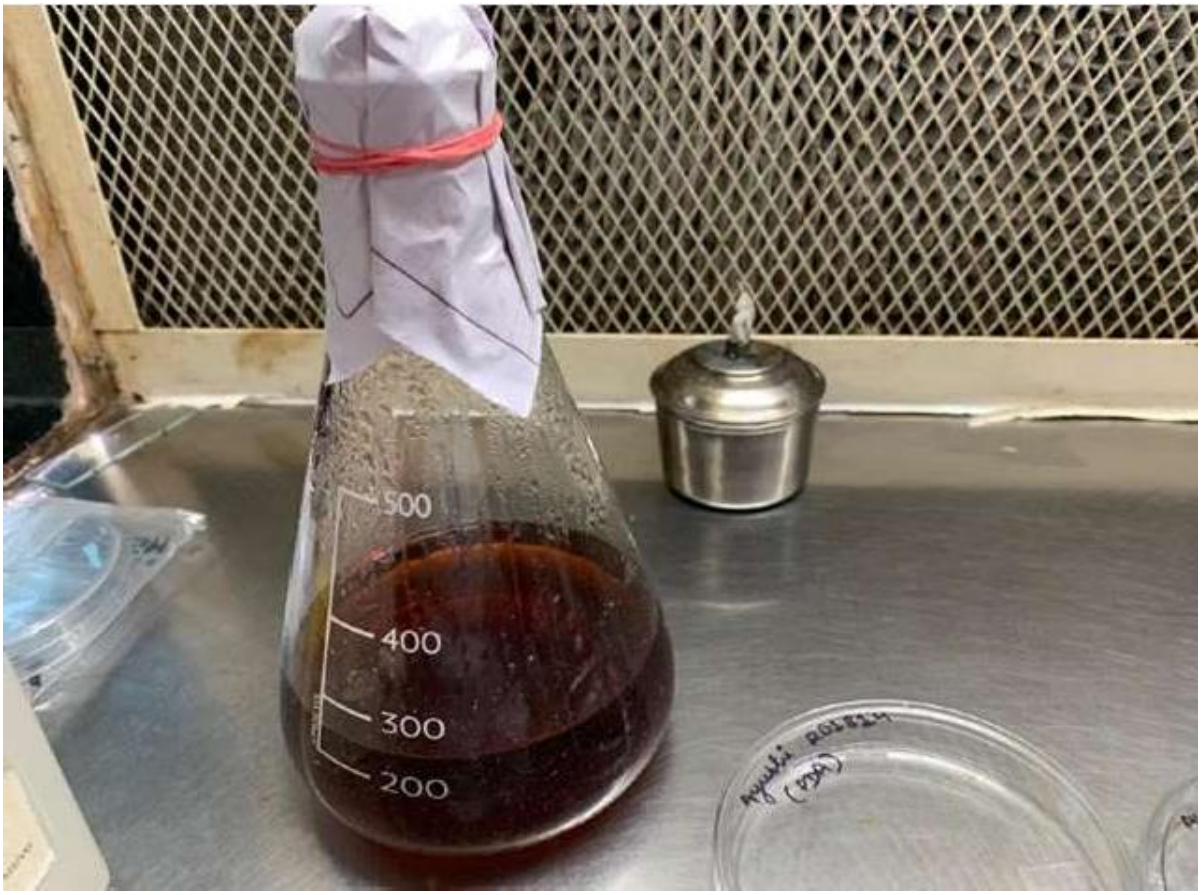

Fig:- 4 Prepared PDB media for growing of *Saccharomyces boulardii*

Fig:- 5 Prepared PDA plates for growing of *Saccharomyces boulardii*

Fig:- 6 Plates prepared from Entroflora probiotic capsules

Fig:- 7 Plates prepared from isolated colonies of *Saccharomyces boulardii*.

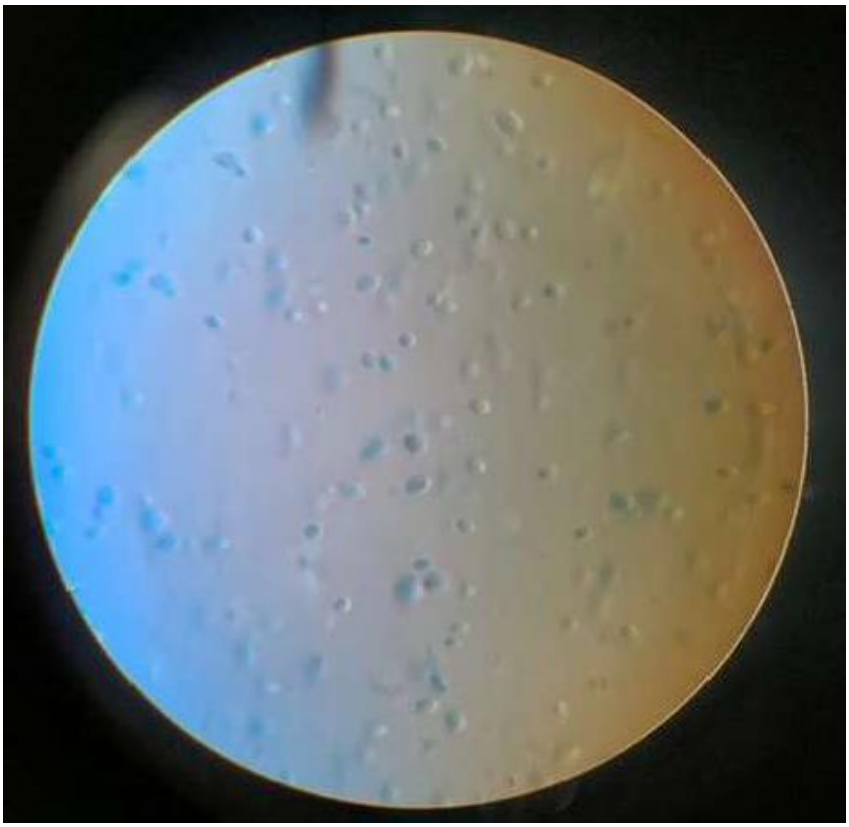

Fig:- 8 Microscopic view of *Saccharomyces boulardii*

Fig:- 9 Plates prepared by spreading baker's yeast.

Fig:- 10 Plates prepared by streaking baker's yeast.

Fig:- 11 Plates prepared from isolated colonies of *Saccharomyces boulardii*

Fig:- 12 Plates of BT01 (isolated from soil)

Fig:- 13 Plates of BT03 (isolated from chilli fruit)

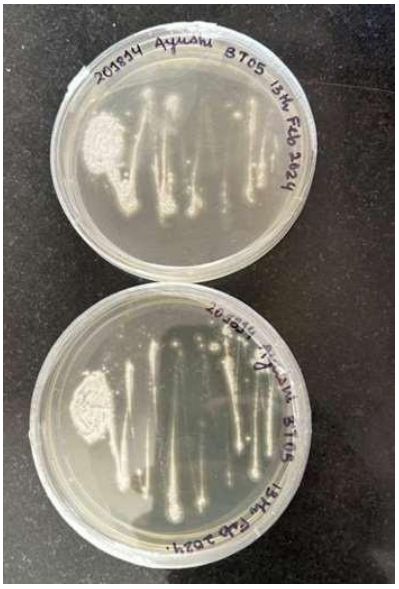

Fig:- 14 Plates of BT05 (Isolated from rice)

Fig:- 15 Cultures of *S. boulardii* and *S. cervesiae*

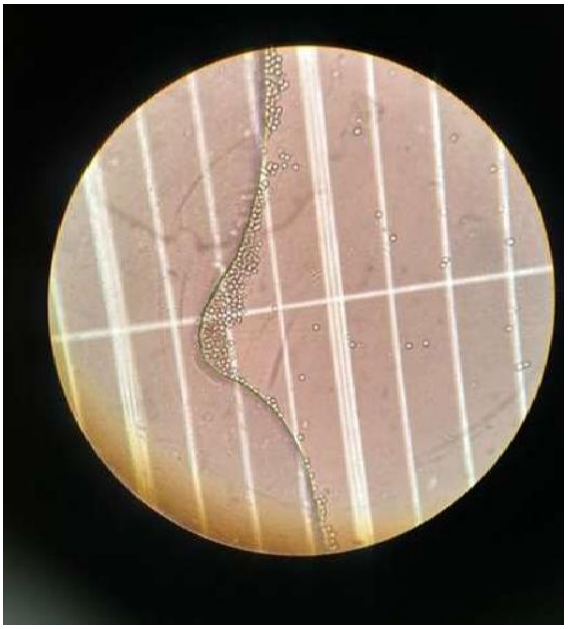

Fig:- 16 Microscopic view of Bakers yeast (In 10 micro Littre = $1.5 * 10^7$ cells)

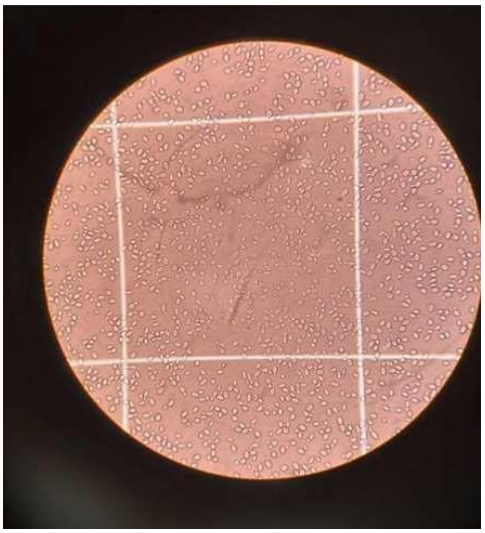

Fig:- 17 Microscopic view of *Saccharomyces boulardii* (In 10 micro litre = $616.75 * 10^5$ cells)

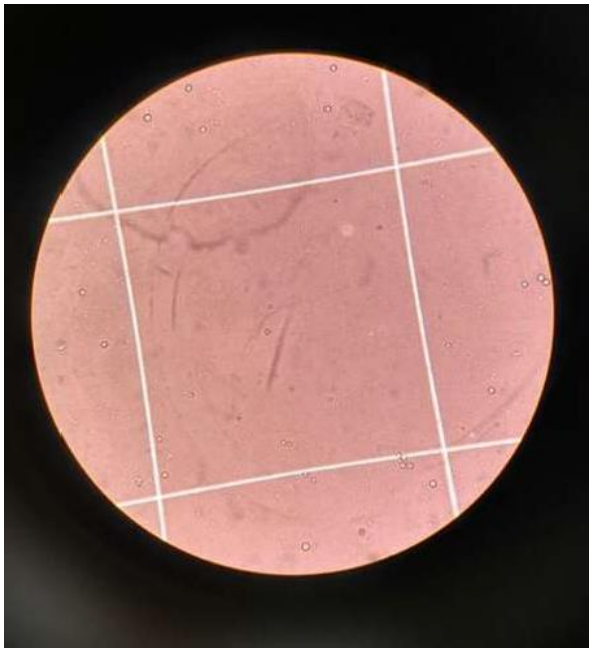

Fig:- 18 *Aspergillus flavus* (BT01) (In 10 micro Littre = $1 * 10^4$ cells)

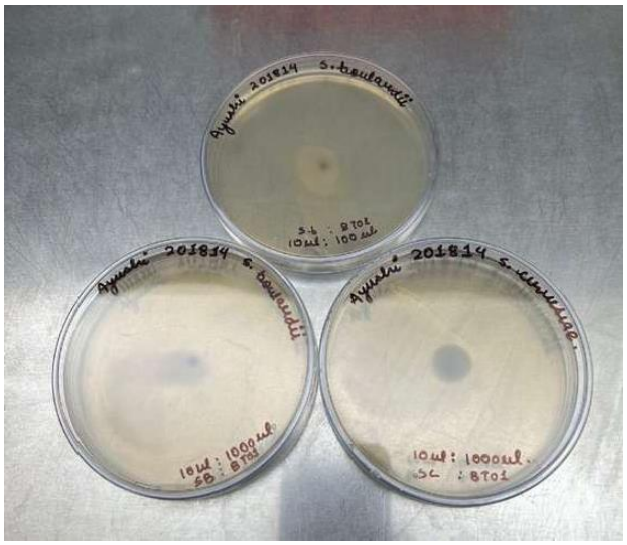

Fig:- 19 Day 1 – Co-culture of BT01 along with *Saccharomyes boulardiii* and *Saccharomyces cerevasiae*

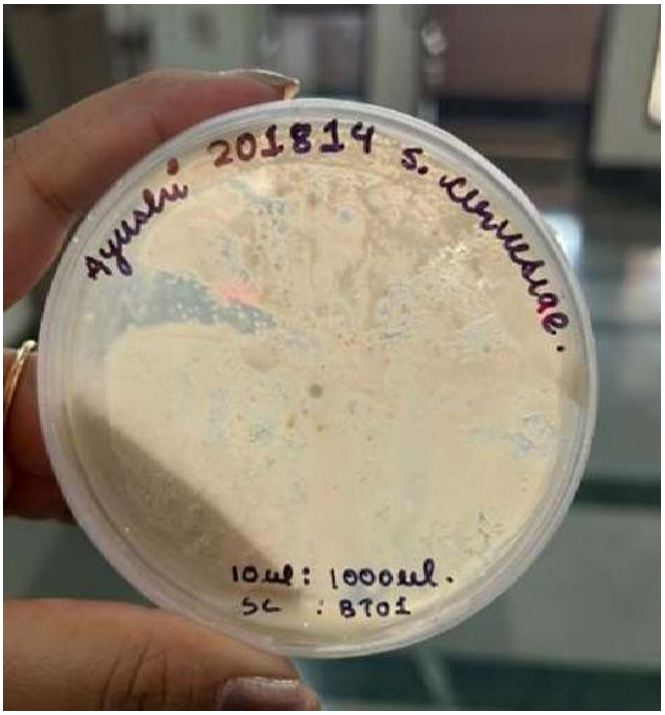

Fig:- 20 Day 2 - *Saccharomyces cerevesiae* couldn't inhibit the growth of BT01

Fig:- 21 *Saccharomyces boulardii* inhibiting the growth of BT01 (1)

Fig:- 22 *Saccharomyces boulardii* inhibiting the growth of BT01 (2)

Fig:- 23 Plates of co-culture of *Saccharomyces boulardii* and BT01 of different ratios(1)

Fig:- 24 Plates of co-culture of *Saccharomyces boulardii* and BT01 of different ratios (2)

Fig:- 25 Broth of co-cultures of *Saccharomyces boulardii* and BT01 of different ratios

Fig:- 26 Settled down cell after centrifugation

Fig:- 27 Settled down cells after discarding the media (1)

Fig:- 28 Settled down cells after discarding the media (2)

Fig:- 29 After phase separation

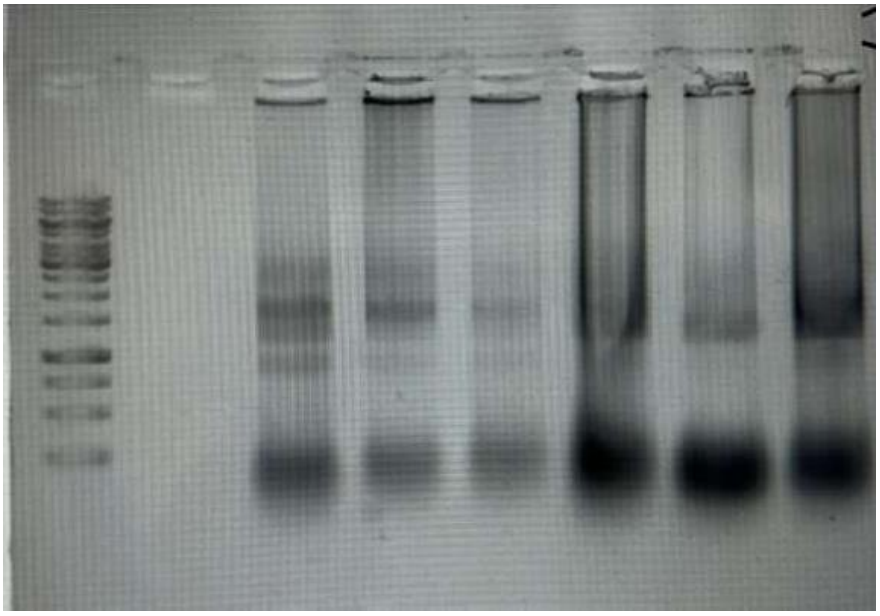

Fig:- 30 Visualisation of RNA bands in gel doc. System (1)

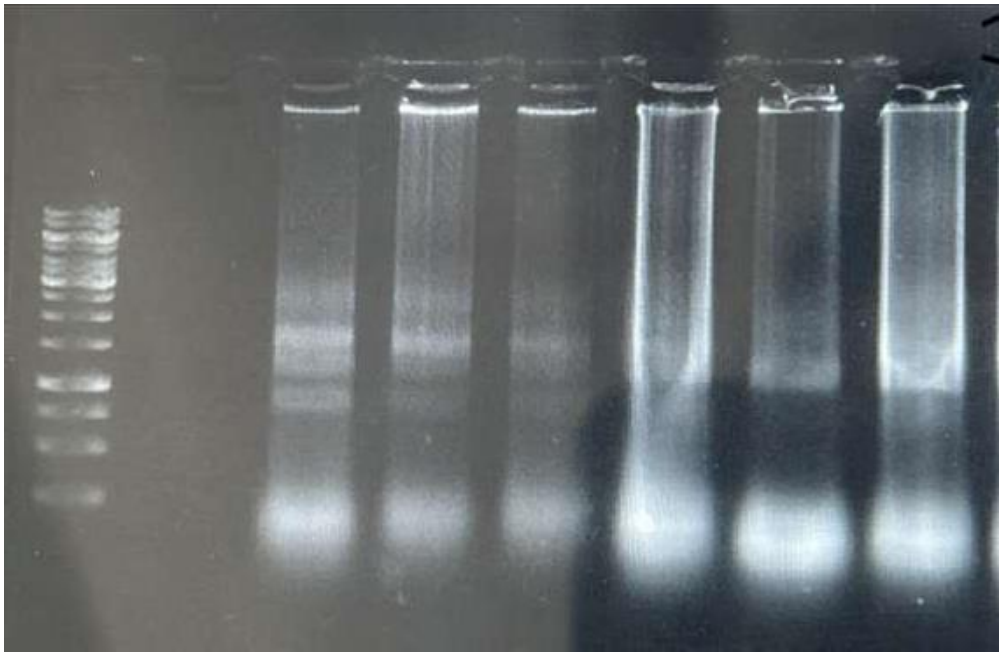

Fig- 31 Visualisation of RNA bands in gel doc. System (2)

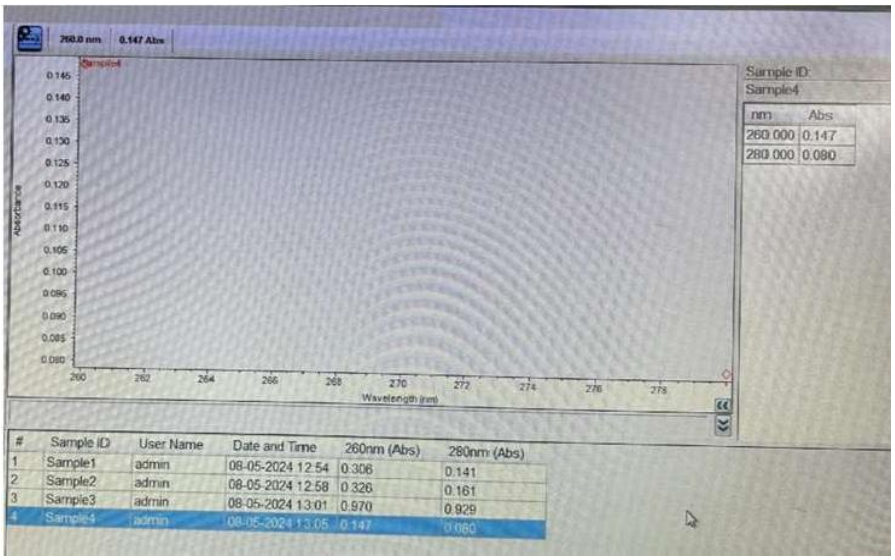

Fig:- 32 RNA quantification using spectrophotometer

Concentration (*µg*/*ml*) = 40*µg*/*ml* * *Absorbance* 260* *Dilution Factor*

Table 19 Absorbance table of different samples

	A260	A280	A260/280
Sample 1 (Lane 1)	0.306	0.141	2.1702
Sample 2 (Lane 2)	0.326	0.161	2.0248
Sample 3 (Lane 3)	0.970	0.929	1.044
Sample 4 (Lane 5)	0.145	0.080	1.8375

- Sample 1, 2 and 3 are the co-cultures of *Saccharomyces boulardii* [[1000 µL] :: *Aspergillus flavus* (BT01) [10 µL].

- Sample 4 are the co-cultures of *Saccharomyces boulardii* [1000 µL] :: *Aspergillus flavus* (BT01) [1 µL]

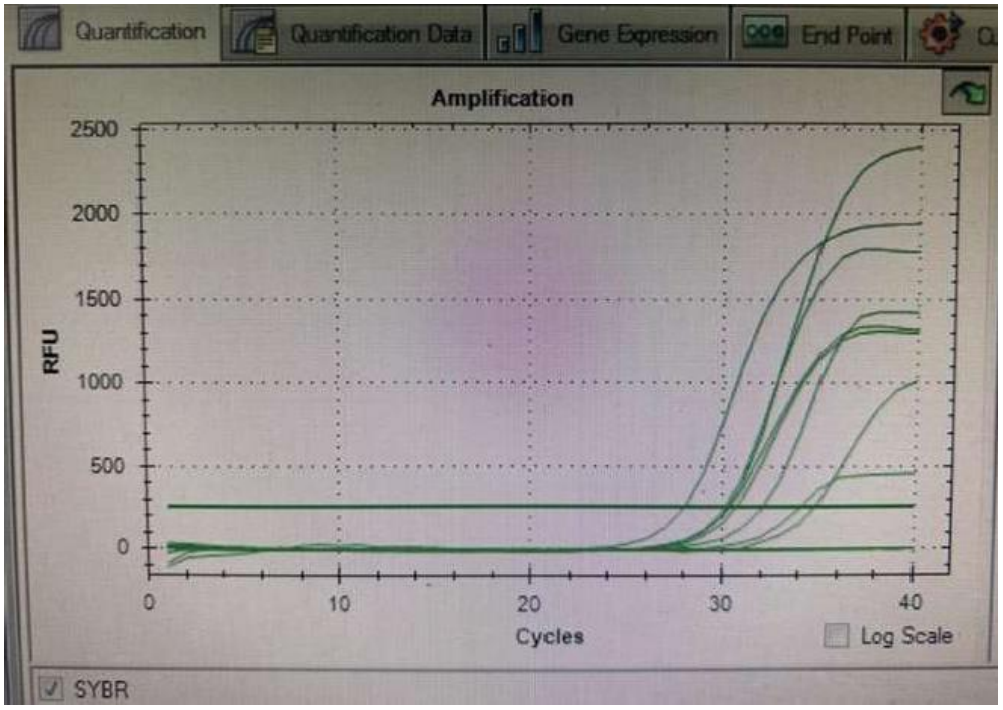

Fig:- 33 Graph of the RT-P**C**R of the **co**-cultures of the *Aspergillus flavuss* and *Sacchromyces boulardii*

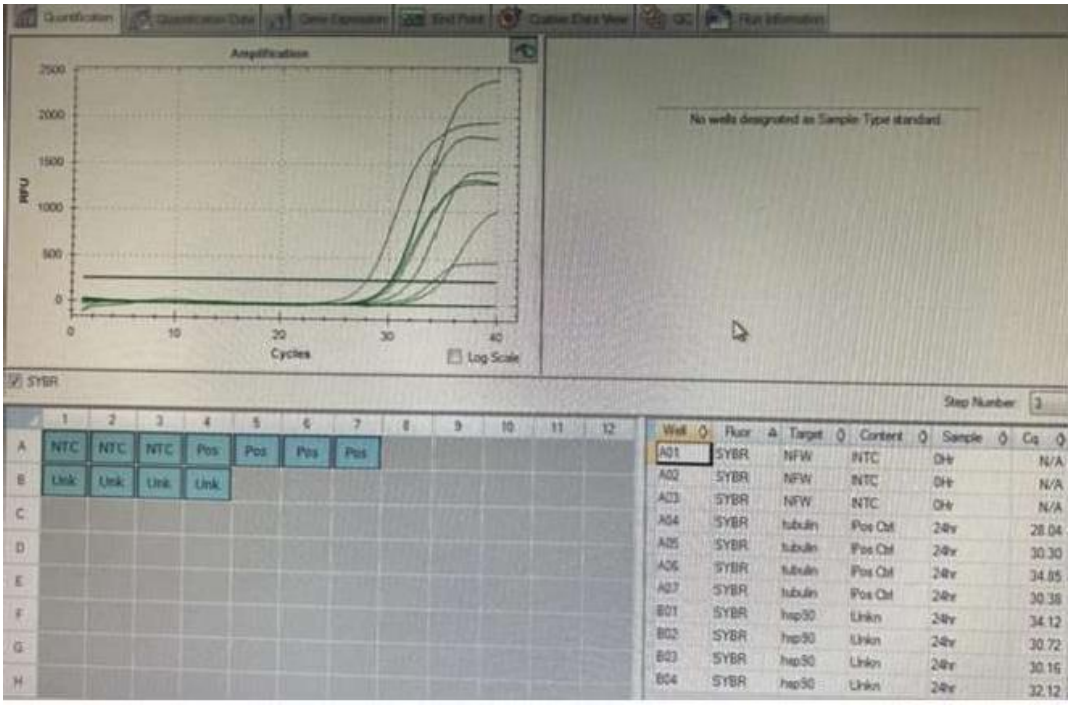

Fig:- 34 Graph of the RT-P**C**R of the **co**-cultures of the *Aspergillus flavuss* and *Sacchromyces boulardii* along with their values.

Chapter 6: Conclusions and Future Scope

6.1 Conclusion

Concluding the project work which is presented in this report will provide a very significant contribution to the field of food safety and sustainability. Our study also explored the potential of isolates of *Saccharomyces* as the biocontrol agent against the aflatoxigenic *Aspergillus flavus* which is a major food-borne pathogen which can easily contaminate a very wide range of foods. *Saccharomyces* was also able to suppress these growths of the *Aspergillus flavus* and further reduced its aflatoxin contents by up to 98%. The mode of action of our *Saccharomyces* acts by reducing the pH of the medium resulting in the production of organic acids and competing for nutrients with *Aspergillus flavus*. The use of *Saccharomyces* as a potential biocontrol agent includes many good advantages over chemical preservatives, including safety, effectiveness and sustainability. The Study also has some significant indications for food, safety and sustainability, as the use of natural and eco-friendly methods which can control food-borne pathogens and microorganisms which can contribute to the production of organic food which are free of chemical preservatives, thus, promoting a very healthier and more sustainable food system.

In the above experiments, we also found *Saccharomyces* inhibiting the growth of *Aspergillus flavus* (BT01). Also, in RT-PCR, the expression analysis for genes encoding for tubulin and Hsp90 from *Aspergillus flavus* were observed during the interaction of *Aspergillus flavus* with Saccharomyces.

6.2 Future Scope

From this report we already came to know that *Saccharomyces boulardii* as the biocontrol agent against the aflatoxigenic *Aspergillus flavus* provides a robust foundation for our future research in food safety and sustainability. Future research can dive into optimising the application methods, exploring some additional screens and accessing the scalability of *Saccharomyces boulardii* in diverse food and feed commodities.

We also have to address some safety considerations through comprehensive toxicity, studies and navigating pathways will be very crucial for industry acceptance. Moreover, expanding the scope to include other foodborne pathogens can enhance the versatility of this Biocontrol strategy. Real-world applicability, industry scale trials and consumer perceptions are also essential in aspect of future research which will ensure a holistic approach to integrate *Saccharomyces boulardii* as an eco-friendly solution for organic and chemical free food preservatives.

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