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Exploring the Microbial Interferences in Overripe Rice Grains: Implications for Food Safety and Preservation

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ABSTRACT

This study investigates the microbial interferences occurring in overripe rice grains and their implications for food safety and preservation. Overripe rice grains, often discarded due to potential spoilage, present a unique environment where various microbial communities interact. This research work aims to identify the dominant microorganisms present in overripe rice and to evaluate their roles in grain deterioration and quality management. Using culture-dependent and bio-chemical analysis, we isolated bacteria from rice samples at different stages of over ripeness. The findings reveal that the microbial communities present in the vicinity of overripe rice are actively takes part in plant growth promoting activities as MS-01 and 03 shows positive results for Catalase, HCN and IAA activity. Apart from that MS-05 only gives fruitful results during siderophore analysis. Specific species of *Bacillus* and *Pseudomonas* are likely to be there predominantly suppressing the growth and pathetic nature of *Aspergillus*, and other fungal species, which contribute to enzymatic breakdown and off-flavor production. Furthermore, the isolated microorganism MS-05 was gone through with HPLC and FTIR analysis respectively and reflects the traces of 2-4 DAPG (retention time 20.7 and 20.6 for HPLC) a bacterial metabolite responsible for PGP activity.

Key words: overripe rice, food safety &preservation, pseudomonas, PGPR, HPLC, FTIR

INTRODUCTION

Rice, as one of the world's staple foods, plays a crucial role in global food security. However, the quality of rice can be compromised by various factors, including over ripeness, which can lead to spoilage and degradation of nutritional content. Overripe rice grains are particularly susceptible to microbial colonization due to their altered biochemical composition and increased susceptibility to environmental contaminants. (Shivappa et al., 2021). Understanding the microbial communities inhabiting overripe rice grains is essential for ensuring food safety, preventing foodborne illnesses, and improving rice storage and preservation practices. Microbial communities present in food matrices are diverse and dynamic, comprising bacteria, fungi, yeasts, and other microorganisms (Wang et al., 2023). These communities play a significant role in food quality, safety, and shelf-life through their metabolic activities, interactions, and potential pathogenicity. In the case of overripe rice grains, the presence of specific microbial species may accelerate spoilage processes, produce harmful toxins, or contribute to undesirable changes in taste, texture, and aroma. The microbial ecology of overripe rice grains remains relatively understudied compared to other food matrices (Brandalise et al., 2023). While extensive research has been conducted on fresh rice grains and their associated microbial communities, fewer studies have focused specifically on overripe rice grains and the unique microbial dynamics associated with their deterioration. Investigating the microbial diversity in overripe rice grains is crucial for filling this knowledge gap and elucidating the complex interactions between microorganisms and their host environment. Furthermore, understanding the microbial composition of overripe rice grains has practical implications for food safety management and agricultural practices. With the increasing demand for sustainable food production and reduced food waste, effective preservation strategies for overripe rice grains are paramount (Shukla & Srivastava, 2024) By characterizing the microbial communities present in overripe rice grains, researchers can identify potential spoilage microorganisms, develop targeted preservation methods, and optimize storage conditions to prolong shelf-life and minimize food losses. Moreover, the

microbial diversity of overripe rice grains may vary depending on factors such as geographical location, agricultural practices, storage conditions, and rice varieties. Therefore, comprehensive studies encompassing diverse sampling sites and experimental conditions are needed to capture the full spectrum of microbial diversity associated with overripe rice grains.

In this research paper, we aim to present a comprehensive analysis of the microbial community found in overripe rice grains, employing a multidisciplinary approach combining microbiology, molecular biology, and food science. Through our findings, we seek to contribute to the development of effective strategies for preserving the quality and safety of overripe rice grains, thereby advancing food security and sustainability goals.

Thus, investigating the microbial diversity in overripe rice grains is essential for understanding the complex interactions between microorganisms and their host environment, elucidating the mechanisms of rice spoilage, and developing practical solutions for food preservation and safety. By bridging the gap between fundamental research and practical applications, this study aims to address key challenges in rice storage and contribute to the advancement of food science and technology.

METHODOLOGY

Sample Collection

Overripe rice grains were collected from different sources to ensure representativeness of the microbial community. The preferred area for this study is natural agriculture, environmental and storage conditions. The targeted experimental fields are located in adjacent areas of Saharanpur, Uttar Pradesh, India. The quantity of the samples were approx. 500 grams and were collected in sterile zip poly begs and stored under room temperature for further analysis.

Sample Preparation

Remove any debris or foreign material from the rice grains. Homogenize the grains to obtain a representative sample for analysis.

Isolation and Characterization of Rhizobacteria and Their Plant Growth Promoting Traits

Bacterial colonies were isolated from overripe rice grains by using serial dilution method dilutions from 10^{-1} to 10^{-6} were prepared (Dubey and Maheshwari, 2012). Then spread 100 µl from each dilution on Nutrient agar medium (Hi-media) plates in triplicate further incubate the plates at 29 °C for 48 hours. Seven bacteria were isolated and purified thorough streak plate method. All the isolated bacteria were checked for fluorescence property under UV light. Further, biochemical tests on the isolates were completed through Hi-media specific biochemical test kit (KB-002) for Gram negative rod shaped bacteria.

All isolates were screened for siderophore production was determined by the chrome azurol S (CAS) assay (Arora and Verma, 2017), Catalase, HCN and IAA production was analyzed as per (Shao et al., 2015).

High Pressure liquid Chromatography Analysis

Analyze the extracted metabolites using high-performance liquid chromatography (HPLC). Use appropriate columns and mobile phases to separate and detect metabolites present in the samples (Bultreys et al., 2003).

Fourier Transforms Infrared Spectroscopy (FT-IR)

The functional group of metabolites in the sample (MS-05) was analyzed via FT-IR as per (Prakash and Arora, 2021). briefly, the dried sample of metabolites was mixed with analytical grade potassium bromide (KBr) (IR Grade; purity C 99%) (1:30 ratio) and the reaction mixture was thinly ground and further fused into a thin pellet (13 mm × 1 mm) by hydraulic pressure under vacuum. Afterwards, spectra absorbance was observed by FT-IR (Model: Nicolet TM 6700, Thermo Fisher Scientific, USA). Further, the result was analyzed in the mid-infrared region from 4000 to 450 cm^{-1} with a resolution of 4 cm^{-1}. For spectrum peak, scanning was done and the pure KBr as a background spectrum was kept to measure in the ambient air. The processing of data was done using OMNICTM (v7.4) software.

RESULTS AND DISCUSSION

Isolation and Characterization

All the isolated bacterial colonies were gone through with various bio-chemical analysis ie. IAA, Sederophore and HCN production in which MS- 01, 03 & 05 shows positive results for Catalase test, HCN and IAA while only one sample (MS-05) shows positive for

Sederophore. (Table 1). On the bases of bio-chemical analysis it was found that there is a maximum probability of plant growth promotion and production enhancement by these microbial community found in overripe rice grains (Table 2). In support of this statement similar results were found by (Arora and Verma, 2017) followed by (Prakash and Arora, 2020) in their research work on sederophore production and PGP activity on stevia plants respectively.

Table 1. PGP activity tests of bacterial isolates.

Sample	Catalase	Siderophore	HCN	IAA
MS01	++	--	++	+++
MS02	++	--	--	--
MS03	++	--	++	++
MS04	--	--	--	--
MS05	++	++	++	+++
MS06	--	--	--	--
MS07	++	--	--	--

++ & +++= Positive results; -- = Negative results.

Table 2. Biochemical tests via KB002 Hi- Media Kit for Identification Index for Gram-negative rods: (A) Reference table (B) Sample Analysis.

(A)

Tests	Citrate utilization	Lysine	Ornithine	Urease	TDA	Nitrate reduction	H_2S production	Glucose	Adonitol	Lactose	Arabinose	Sorbitol
Pseudomonas aeruginosa	+	-	-	v	-	+	-	+	nd	-	-	-
Pseudomonas fluorescens	+	-	-	v	-	v	-	+	nd	v	v	-
Pseudomonas putida	+	-	-	v	-	_	-	+	nd	v	v	nd

(B)

Tests	Citrate utilization	Lysine	Ornithine	Urease	TDA	Nitrate reduction	H_2S production	Glucose	Adonitol	Lactose	Arabinose	Sorbitol
MS-01	+	-	-	v	-	+	-	+	nd	-	-	-
MS-02	-	-	-	-	-	-	-	-	-	-	-	-
MS-03	+	-	-	v	-	_	-	+	nd	v	v	nd
MS-04	-	-	-	-	-	-	-	-	-	-	-	-
MS-05	+	-	-	v	-	v	-	+	nd	v	v	-
MS-06	-	-	-	-	-	-	-	-	-	-	-	-
MS-07	-	-	-	-	-	-	-	-	-	-	-	-

+ = Positive (more than 90%); - = Negative (more than 90%); v = 11-89%; Positive nd = No data available.

HPLC Analysis

In the HPLC analysis of isolates it was found that in crude chloroform extract of (MS-05) the secondary metabolite was produced 2,4-DAPG at retention time at 20.7 and 20.6 (Figure 1). Similarly it was found by (Sharma et al., 2019) in their work on P. fluorescens. (Sageera et al. 2014) also reported the same in their research. Recently, Suresh and his team reviled the nearby observation on the presence of 2,4 DAPG (Suresh et al., 2022).

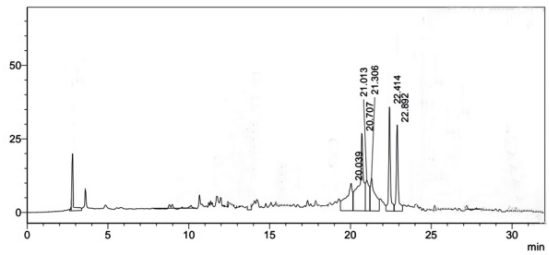

Fig. 1. HPLC Analysis of Pseudomonas fluorescence showing presence of 2,4 DAPG.

FT-IR Analysis

In FT-IR analysis a remarkable difference in proteins/polysaccharide ratio was observed. PF shows strong bands between the range of 1400-1700 cm^{-1} along with between 2800-3400 cm^{-1}, related to protein absorption, and weak polysaccharides absorption. (Figure 2) Further, according to (Vadithe et al., 2021), Infrared absorption spectra revealed that a peak at 1076 cm^{-1} arises mainly from nucleic acid vibrations and carbohydrate. Amide I at 1650 cm^{-1} and amide II at 1553 cm^{-1} are dominant in this region. In continuation, (Chen et al., 2023) completed FT-IR spectroscopy after exposure to different antimicrobial compounds or changes of environmental conditions. Moreover, molecular composition in bacteria can changes including cytoplasmic proteins, cell membrane phospholipids, cell wall polysaccharides, and nucleic acids etc. The wavenumbers denotes changes appears between 3000 and 2800 cm^{-1} reflect lipid regions assigned to the alkyl group of lipids.

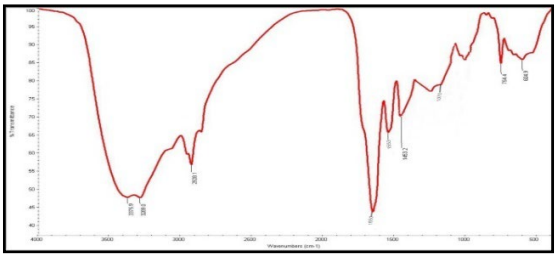

Fig. 2. FT-IR Analysis of Pseudomonas fluorescence showing presence of 2,4 DAPG.

CONCLUSION

In concluding remark, this study resulted a rich microbial diversity within overripe rice grains, highlighting both the potential risks and opportunities for food safety and preservation practices. The presence of diverse microbial communities, including pathogens and beneficial microbes, underscores the need for vigilant monitoring and effective mitigation strategies in rice processing and storage. Furthermore, understanding the dynamics of microbial interactions and their impact on rice quality and safety is crucial for developing targeted interventions that can enhance food preservation methods while ensuring consumer health. Future research should focus on elucidating the specific roles of key microbial species identified in this study and exploring innovative preservation techniques that harness beneficial microbes to improve the safety and shelf-life of rice products. By addressing these challenges, we can advance both the scientific understanding and practical applications necessary to sustainably manage microbial diversity in rice grains and enhance food security globally.

CONFLICT Of INTEREST

The authors declare no financial or personal conflicts of interest related to this research. The authors have disclosed all relevant relationships and affiliations.

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