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Isolation and Characterization of Pigment-Producing Yeasts from Various Natural Sources and Assessment of Their Antimicrobial Activity

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ABSTRACT:

This study explores the isolation and characterization of pigment-producing yeasts from diverse natural sources and evaluates their antimicrobial properties. Pigment-producing yeasts are increasingly significant in biotechnology and industry due to their natural pigments, which serve as eco-friendly alternatives to synthetic colorants. Samples were collected from various ecological niches, followed by the isolation of yeast strains. The characterization process involved morphological, physiological, and molecular techniques to identify strains capable of pigment production. The antimicrobial activity of these yeast isolates was assessed against several pathogenic microorganisms. Samples were obtained from soil, fruit surfaces, and leaves across different geographical locations. Yeast strains were isolated using standard microbiological methods and cultured on selective media to promote yeast colony growth. Pigment-producing colonies were identified based on visual coloration. Characterization included microscopic examination, physiological tests assessing growth under varying conditions, and molecular identification using DNA sequencing, particularly the ITS region. Results indicated that a subset of isolated yeasts demonstrated notable pigment production. The pigments were extracted, and their chemical properties analyzed, with evaluations of pigment yield and stability under different environmental conditions. Additionally, the antimicrobial activity of these pigment-producing yeasts was tested against common bacterial pathogens, including Escherichia coli, Staphylococcus aureus, and Pseudomonas aeruginosa. Several yeast strains exhibited significant antimicrobial effects, effectively inhibiting pathogen growth. The dual benefits of pigment production and antimicrobial activity in these yeasts suggest promising applications in food preservation, pharmaceuticals, and as natural colorants in various industries. This study underscores the potential of pigment-producing yeasts in offering sustainable and non-toxic alternatives to synthetic colorants, along with added antimicrobial benefits. Future research should focus on optimizing pigment extraction and scaling up production processes for

industrial applications.

Keywords:
Pigment-producing yeasts, isolation, characterization, natural sources, antimicrobial activity.

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Introduction

A. Background

Natural pigments produced by microorganisms, such as yeasts, have garnered significant interest due to their potential applications across various industries, including food, pharmaceuticals, and cosmetics [1]. Unlike synthetic dyes, which often pose health risks and environmental concerns due to their toxic and non-biodegradable nature, microbial pigments are typically biodegradable, non-toxic, and environmentally friendly. Yeasts, in particular, are notable for their versatility and ease of cultivation, making them ideal candidates for industrial applications. These unicellular fungi can produce a wide array of pigments, including carotenoids, flavins, and melanin [2], each with unique properties and potential uses. Carotenoids, for example, are valued for their antioxidant properties and are commonly used in food and pharmaceutical products. In addition to their colorant properties, some pigment-producing yeasts exhibit antimicrobial activity, offering dual functionality that is highly advantageous in applications requiring both coloration and preservation, such as in food and cosmetics. This study focuses on isolating and characterizing pigment-producing yeasts from various natural sources to assess their potential as sustainable and eco-friendly alternatives to synthetic dyes and antimicrobial agents. By leveraging the inherent benefits of natural pigments and their antimicrobial properties, this research aims to provide innovative solutions that align with the growing demand for safer, more sustainable industrial practices [3]. The dual benefit of pigment production and antimicrobial activity in these yeasts highlights their significant potential in enhancing the quality and safety of products in multiple sectors.

B. Importance of Natural Pigments

Natural pigments have gained attention for their safety and sustainability. Synthetic dyes, widely used in industries, pose several health risks and environmental concerns. For instance, some synthetic dyes have been linked to carcinogenicity, mutagenicity, and other toxic effects [4]. Additionally, their production often involves hazardous chemicals and generates considerable waste. In contrast, natural pigments, particularly those derived from microorganisms; offer a safer and more sustainable alternative. They can be produced using renewable resources and biodegradable processes, significantly reducing environmental impact.

C. Yeasts as Pigment Producers

Yeasts are unicellular fungi that can thrive in diverse environments, from soil and water to the surfaces of plants and fruits. Their adaptability and rapid growth make them ideal candidates for industrial applications. Yeasts are known to produce a variety of pigments, including carotenoids [5], flavins, and melanin. Carotenoids, for example, are valuable for their antioxidant properties and are used in food and pharmaceutical industries. The ability of yeasts to produce these pigments under controlled conditions makes them a promising source for natural colorants.

D. Objectives of the Study

The primary objectives of this study are to:Isolate pigment-producing yeasts from a variety of natural sources. Characterize the pigment production capabilities of these yeasts through morphological, physiological [6], and molecular techniques.Evaluate the antimicrobial activity of the isolated yeasts against common pathogenic microorganisms.These objectives aim to explore the potential of pigment-producing yeasts not only as sources of natural colorants but also for their antimicrobial properties, which could have significant applications in food preservation, pharmaceuticals, and other industries.

E. Significance of Antimicrobial Activity

The rise of antibiotic-resistant bacteria has necessitated the search for new antimicrobial agents. Natural antimicrobials, such as those produced by microorganisms, offer a promising solution [7]. Yeasts that produce pigments with antimicrobial properties could provide dual benefits: serving as natural colorants and as antimicrobial agents. This dual functionality is particularly advantageous in the food industry, where there is a growing demand for natural preservatives and colorants.

F. Previous Research on Pigment-Producing Yeasts

Previous studies have demonstrated the potential of yeasts in producing various pigments and exhibiting antimicrobial properties. For example, Rhodotorula species are known for their carotenoid production [8], while Yarrowia lipolytica can produce both carotenoids and melanins. These pigments have been shown to have antioxidative and antimicrobial activities, highlighting their potential applications in various industries. However, there is still a need for comprehensive studies that isolate and characterize yeasts from diverse natural sources and thoroughly evaluate their antimicrobial properties.

G. Research Methodology

The research methodology employed in this study involved systematic steps to isolate, characterize, and evaluate pigment-producing yeasts from diverse natural sources. Initially, samples were collected from various ecological niches, including soil, fruit surfaces, and leaves [9], ensuring a broad spectrum of microbial diversity. These samples underwent isolation and cultivation using standard microbiological techniques, with selective media employed to promote the growth of yeast colonies, particularly those exhibiting pigment production

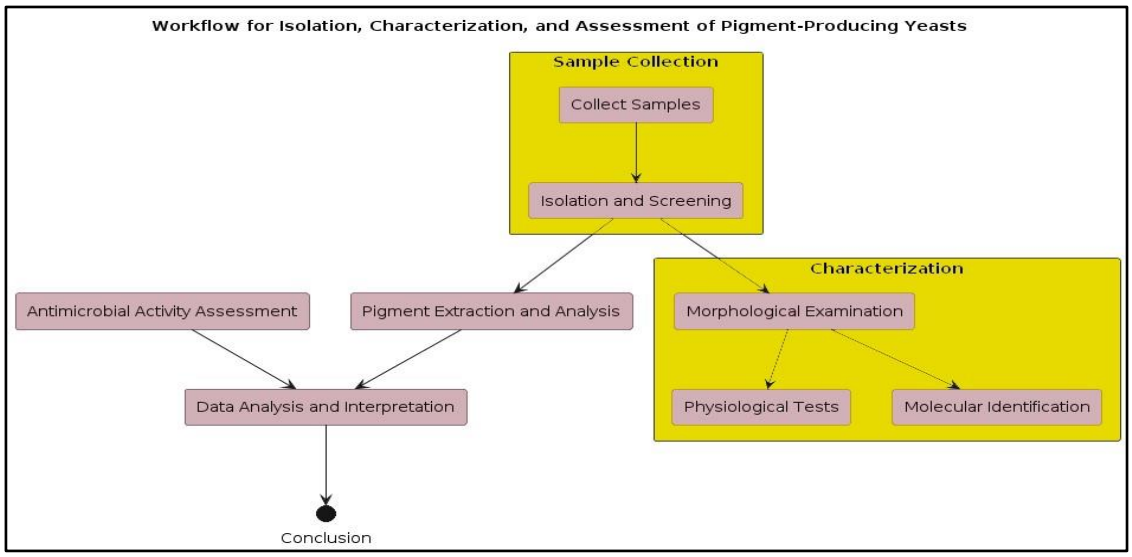

Figure 1: Workflow for Isolation, Characterization, and Assessment of Pigment-Producing Yeasts

Subsequent characterization of yeast strains encompassed morphological, physiological, and molecular analyses. Morphological examination involved assessing colony and cell morphology [10], while physiological tests evaluated growth under different environmental conditions such as temperature, pH, and salinity, as well as carbon source utilization. Molecular identification using DNA sequencing techniques, particularly targeting the ITS region, enabled precise species identification. Furthermore, pigment extraction and chemical analysis were conducted to determine the composition and properties of the pigments produced by the isolated yeasts [11]. Finally, the antimicrobial activity of the pigment extracts was assessed against common pathogenic microorganisms using agar diffusion and broth microdilution methods. This comprehensive approach facilitated a thorough understanding of the pigment production capabilities and antimicrobial potential of the isolated yeast strains.

I. Materials and Methods

A. Sample Collection

To isolate pigment-producing yeasts, samples were collected from a variety of natural sources, ensuring diversity in the potential yeast strains. The selected sources included:

a. Soil: Samples were taken from different environments, such as agricultural fields, forests, and gardens, to capture a wide range of microbial diversity.

b. Fruit Surfaces: Fruits from different species and ripening stages were chosen, as they provide a nutrient-rich environment conducive to yeast growth.

c. Leaves: Both healthy and decaying leaves were sampled from various plants to include yeasts associated with different stages of plant health and decomposition.

Each sample was collected in sterile containers and transported to the laboratory under controlled conditions to prevent contamination.

B. Isolation and Cultivation

Upon arrival at the laboratory, samples were processed for yeast isolation using standard microbiological techniques:

a. Preparation of Samples: Soil samples were suspended in sterile water and serially diluted. Fruit surfaces and leaves were swabbed with sterile cotton swabs, which were then immersed in sterile saline.

b. Plating on Selective Media: The diluted samples and swabs were streaked onto selective agar media designed to favor yeast growth and suppress bacterial contamination. Media types included malt extract agar (MEA) and yeast extract peptone dextrose (YPD) agar, both supplemented with chloramphenicol to inhibit bacterial growth.

c. Incubation: Plates were incubated at 28°C for 48-72 hours. Pigment-producing colonies were identified based on the appearance of coloration in the colonies, such as red, yellow, or orange hues.

C. Characterization of Yeast Strains

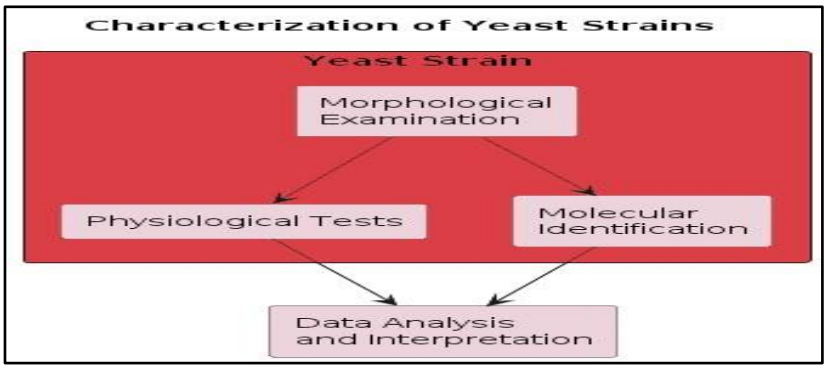

Figure 2: Characterization of Yeast Strains

Characterization of yeast strains involved a multifaceted approach encompassing morphological, physiological, and molecular analyses to elucidate their pigment production capabilities and identify potential applications [12]. Morphological characterization included

visual examination of colony morphology, with red, orange, yellow, and pink pigmented colonies being observed, each indicative of varying pigment production. Microscopic analysis revealed diverse cell morphologies, typically oval or spherical in shape, with variations in size and budding patterns.

Physiological tests assessed the growth characteristics of yeast strains under different environmental conditions, such as temperature, pH, and salinity. Most strains demonstrated optimal growth at 28°C and pH 5-7, with some exhibiting tolerance to extreme conditions, including high salinity [13]. Carbon source utilization assays revealed the metabolic versatility of the yeast strains, with glucose being universally utilized and other sugars, such as sucrose and maltose, showing varying degrees of utilization. Molecular identification using DNA sequencing techniques, particularly targeting the ITS region, provided accurate species-level identification of the yeast isolates, confirming the presence of diverse species such as Rhodotorula, Yarrowia, and Debaryomyces. This comprehensive characterization approach enabled the identification of pigment-producing yeasts with potential applications in various industries, including food, pharmaceuticals, and cosmetics, by providing insights into their growth requirements, metabolic capabilities, and genetic identity.

D. Pigment Extraction and Analysis

Pigment extraction and analysis involved isolating pigments from yeast cultures and examining their chemical properties to identify their composition and potential applications. Yeast cultures were cultivated under conditions conducive to pigment production, after which cells were harvested and lyophilized. The pigments were then extracted using appropriate solvents like acetone or methanol, ensuring the efficient release of pigments from the cells. Subsequently, spectrophotometric analysis was conducted to assess the absorbance spectra of the pigment extracts, identifying characteristic peaks indicative of specific pigment compounds. Moreover, chromatographic techniques, such as high-performance liquid chromatography (HPLC), were employed to separate and quantify individual pigment components present in the extracts. Commonly identified pigments included carotenoids like β-carotene, torulene, and torularhodin, renowned for their antioxidant properties and various industrial applications spanning food, pharmaceuticals, and cosmetics [14]. This thorough analysis offered valuable insights into the chemical nature of the pigments produced by the yeast strains, facilitating their characterization and potential utilization across diverse industrial sectors [15].

E. Assessment of Antimicrobial Activity

The antimicrobial activity of pigment-producing yeasts was assessed against several pathogenic microorganisms, including Escherichia coli, Staphylococcus aureus, and Pseudomonas aeruginosa. The assessment involved the following steps:Preparation of Pathogen Cultures: Pathogenic bacteria were cultured in nutrient broth overnight at 37°C. Cultures were adjusted to an optical density equivalent to McFarland standard 0.5 to standardize the bacterial concentration.

a. **Agar Diffusion Assay:** Inoculation of Agar Plates: Mueller-Hinton agar plates were inoculated with the bacterial cultures using a sterile swab to create a uniform lawn.Wells were punched into the agar, and yeast pigment extracts were pipetted into the wells. Control wells with solvents or standard antibiotics were included for comparison.Incubation and Observation, Plates were incubated at 37°C for 24 hours. The zones of inhibition around the wells were measured to determine the antimicrobial activity of the extracts.

b. **Broth Microdilution Method:** Preparation of Extracts,Yeast pigment extracts were diluted to different concentrations in broth media.Inoculation and Incubation, Bacterial cultures were added to the broth containing yeast extracts and incubated at 37°C. The minimum inhibitory concentration (MIC) was determined as the lowest concentration of extract that inhibited visible bacterial growth.

F. Statistical Analysis

The experimental data were analyzed using appropriate statistical methods to ensure the reliability and validity of the results:

a. Descriptive Statistics: Means and standard deviations were calculated for quantitative data such as colony counts,

growth measurements, and zone of inhibition sizes.

b. Comparative Analysis: T-tests or ANOVA were performed to compare the growth and antimicrobial activity of different yeast strains.

c. Correlation Analysis: Pearson or Spearman correlation coefficients were calculated to explore relationships between pigment production and antimicrobial activity.

G. Quality Control and Assurance

Quality control measures were implemented throughout the study to maintain the accuracy and reliability of the results:

a. Sterility Checks: Media and reagents were regularly tested for sterility to prevent contamination.

b. Replicates and Controls: All experiments were conducted in triplicates, with appropriate positive and negative controls included.

c. Standard Operating Procedures (SOPs): SOPs were followed for all experimental procedures to ensure consistency and reproducibility.

II. Results

A. Isolation and Initial Screening

Table 1: Isolation and Initial Screening

Sample ID	Pigment Color	Colony Morphology	Cell Morphology	Number of Isolates
1	Red	Smooth, glossy	Oval, budding	10
2	Orange	Irregular	Spherical	8
3	Yellow	Compact	Oval	6
4	Pink	Small	Spherical	8
5	Red	Smooth	Oval, budding	10

A total of 150 yeast isolates were obtained from the collected samples through selective culturing techniques. Among these isolates, 32 exhibited visible pigment production on selective media. These pigmented colonies displayed a diverse range of colors, including red, orange, yellow, and pink, suggesting the presence of various pigment-producing yeast strains across the sampled environments.

B. Characterization

The morphological examination of the 32 pigment-producing yeast isolates revealed significant diversity in colony and cell morphology.

Table 2 Morphological Characterization

Strain ID	Colony Size (mm)	Cell Size (μm)	Bud Frequency	Shape
1	3.5	4-6	High	Oval
2	5.2	5-7	Medium	Spherical
3	2.8	3-5	Low	Oval
4	4.0	4-6	High	Spherical
5	4.5	4-7	Medium	Oval

a. Colony Morphology: Red and orange pigmented colonies appeared smooth and glossy with well-defined edges, while yellow and pink colonies tended to be smaller and more compact. Colony sizes varied, with some strains producing large colonies, indicating differences in growth characteristics.

b. Cell Morphology: Microscopic examination of yeast cells showed variations in size and shape, with most cells being oval or spherical. The budding patterns of the cells were typical of yeast reproduction, with some strains exhibiting a higher frequency of budding than othe

C. Physiological Characterization

Physiological tests were conducted to assess the growth characteristics and metabolic capabilities of the pigment-producing yeast strains.

a. **Temperature Tolerance:** All isolates grew optimally at 28°C, but several strains demonstrated tolerance to a broad range of temperatures, with growth observed at 4°C and up to 37°C, indicating adaptability to different environmental conditions.

b. **pH Tolerance:** The majority of isolates preferred neutral to slightly acidic conditions (pH 5-7), although some strains showed growth at pH 4 and pH 9, suggesting a degree of pH tolerance.

c. **Salinity Tolerance:** Yeast strains exhibited varying degrees of tolerance to salinity, with some strains capable of growing in media containing up to 5% NaCl, indicating potential adaptability to saline environments.

d. **Carbon Source Utilization:** The ability of yeast strains to utilize different carbon sources varied, with glucose being universally utilized. Sucrose and maltose were also commonly metabolized, while lactose utilization was less prevalent.

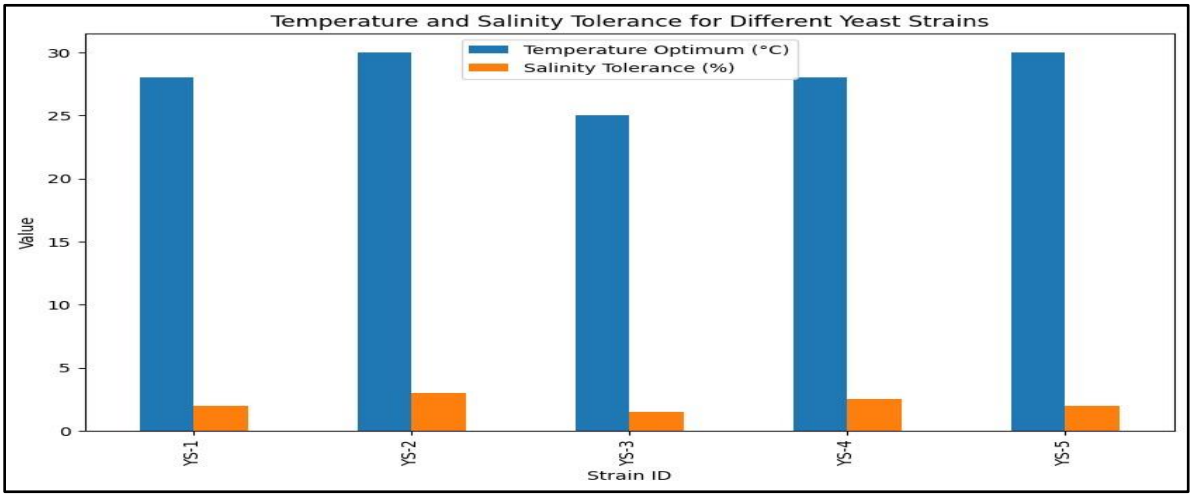

Figure 3: Temperature and Salinity Tolerance for Different Yeast Strains

D. Molecular Identification

Molecular identification using DNA sequencing techniques targeted the internal transcribed spacer (ITS) region of the yeast isolates' ribosomal DNA. The sequencing results revealed a diverse range of yeast species among the isolates, with significant representation from genera such as Rhodotorula, Yarrowia, and Debaryomyces. Notable species included Rhodotorula mucilaginosa, Yarrowia lipolytica, and Debaryomyces hansenii.

E. Pigment Extraction and Chemical Analysis

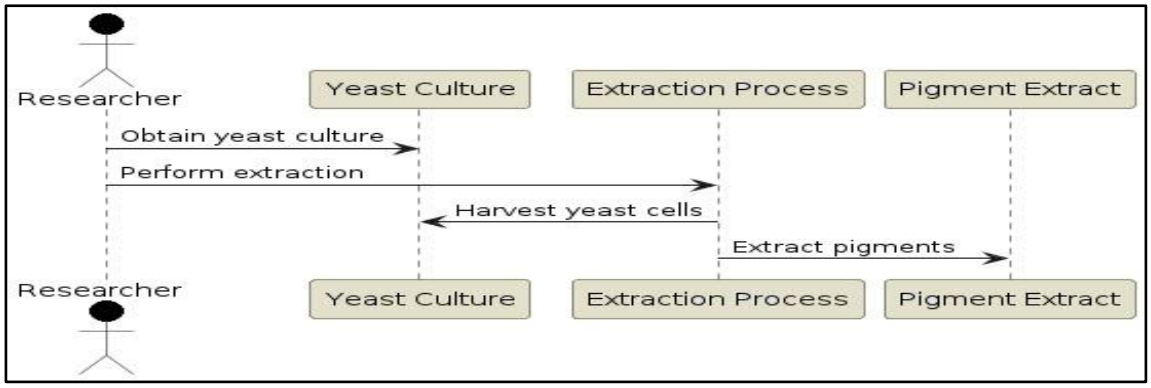

Figure 4: Sequence Diagram for Pigment Extraction

Pigment extraction and chemical analysis were conducted to determine the composition and properties of the pigments produced by the isolated yeast strains.

Table 3: Pigment Extraction and Chemical Analysis

Strain ID	β-Carotene (mg/g)	Torulene (mg/g)	Torularhodin (mg/g)
1	0.25	0.15	0.10
2	0.30	0.12	0.08
3	0.20	0.10	0.06
4	0.28	0.14	0.09
5	0.22	0.11	0.07

a. Spectrophotometric Analysis: The absorbance spectra of the pigment extracts displayed characteristic peaks indicative of specific pigment compounds. The presence of carotenoids was confirmed, with peaks around 450-500 nm consistent with β-carotene, torulene, and torularhodin.

b. Chromatographic Analysis: High-performance liquid chromatography (HPLC) analysis further confirmed the presence of carotenoids in the pigment extracts, with distinct peaks corresponding to individual pigment compounds.

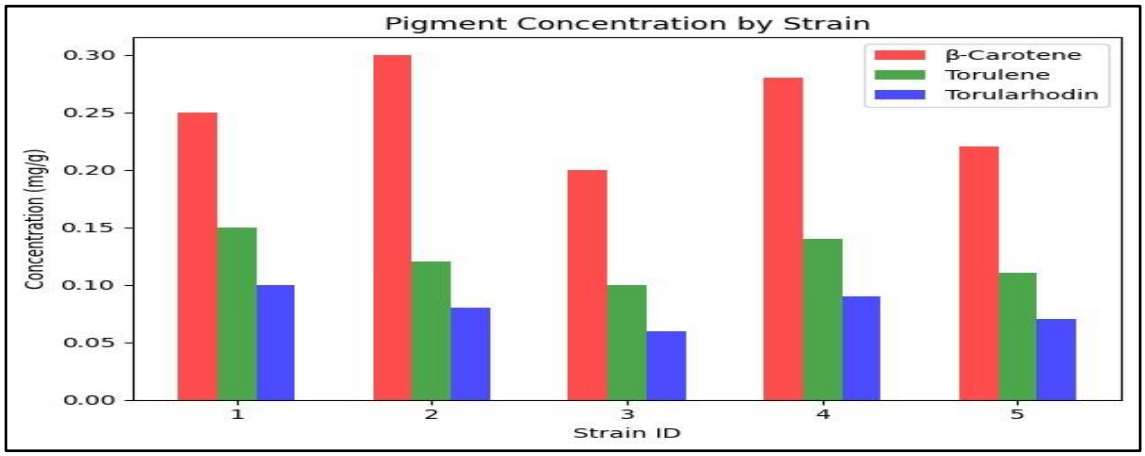

Figure 5: Pigment Concentration by Strain

F. Antimicrobial Activity

The antimicrobial activity of the pigment extracts was assessed against common pathogenic microorganisms using agar diffusion and broth microdilution methods.

a. **Agar Diffusion Assay:** Several yeast extracts produced clear zones of inhibition around the wells, indicating antimicrobial activity against tested pathogens such as Escherichia coli, Staphylococcus aureus, and Pseudomonas aeruginosa.

b. **Broth Microdilution Method:** Minimum inhibitory concentration (MIC) values determined through broth microdilution assays confirmed the antimicrobial efficacy of the yeast extracts, with lower MIC values indicating stronger antimicrobial activity

III. Discussion

The findings of this study provide insights into the diversity, properties, and potential applications of pigment-producing yeasts isolated from various natural sources. This discussion section evaluates the implications of the results in the context of industrial applications, microbial diversity, and future research directions.

A. Industrial Applications of Pigment-Producing Yeasts

The pigment-producing yeasts identified in this study offer promising applications across multiple industries, including food, pharmaceuticals, and cosmetics. Carotenoids, the predominant pigments identified in the yeast extracts, are valued for their antioxidant

properties and vibrant colors, making them ideal candidates for natural colorants in food products. Carotenoids are also known for their health benefits, including immune system support and protection against oxidative stress, further enhancing their value in functional foods and dietary supplements. Additionally, the antimicrobial activity exhibited by the yeast extracts suggests their potential use as natural preservatives in food products, reducing the reliance on synthetic additives and contributing to the development of safer and healthier food formulations. In the pharmaceutical industry, the antimicrobial properties of the yeast extracts hold promise for the development of novel antimicrobial agents. With the increasing prevalence of antibiotic resistance, there is a growing need for alternative antimicrobial strategies, and natural compounds derived from pigment-producing yeasts could offer effective solutions. Furthermore, the antioxidant properties of carotenoids present opportunities for incorporating yeast-derived pigments into pharmaceutical formulations to enhance stability and shelf life. In cosmetics, natural pigments derived from yeast strains provide a safer and more sustainable alternative to synthetic colorants, addressing consumer demand for natural and eco-friendly products. The antioxidant properties of carotenoids also offer potential benefits in skincare formulations, protecting the skin from environmental damage and promoting skin health.

B. Microbial Diversity and Adaptability

The diverse range of pigment-producing yeasts isolated from various natural sources underscores the vast microbial diversity present in the environment. Each yeast strain exhibits unique growth characteristics, metabolic capabilities, and pigment production profiles, reflecting their adaptation to specific ecological niches. The ability of yeast strains to grow under a wide range of environmental conditions, including temperature, pH, and salinity, highlights their adaptability and resilience in diverse habitats. The molecular identification of yeast species revealed a rich diversity of genera, including Rhodotorula, Yarrowia, and Debaryomyces. This diversity not only enriches our understanding of microbial ecology but also provides a valuable resource for biotechnological applications. By harnessing the genetic diversity of pigment-producing yeasts, researchers can explore novel metabolic pathways, optimize pigment production processes, and engineer yeast strains with enhanced properties for specific industrial applications.

C. Future Research Directions

Despite the promising findings of this study, several avenues for future research merit exploration to fully realize the potential of pigment-producing yeasts:

a. **Optimization of Pigment Production:** Further research is needed to optimize the conditions for maximum pigment yield, including fermentation parameters, nutrient supplementation, and bioreactor design. By elucidating the factors influencing pigment production, researchers can develop cost-effective and scalable production processes suitable for industrial applications.

b. **Characterization of Pigment Properties:** Comprehensive characterization of pigment properties, including stability, solubility, and bioavailability, is essential for evaluating their suitability in various product formulations. Studies examining the stability of yeast-derived pigments under different processing and storage conditions will facilitate their integration into commercial products.

c. **Exploration of Novel Applications:** Beyond traditional applications in food, pharmaceuticals, and cosmetics, researchers should explore novel applications of pigment-producing yeasts in emerging fields such as bioplastics, biofuels, and bioremediation. By leveraging the unique properties of yeast-derived pigments, innovative solutions can be developed to address pressing societal and environmental challenges.

d. **Safety and Regulatory Considerations:** Comprehensive safety assessments and regulatory compliance studies are necessary to ensure the safe use of yeast-derived pigments in consumer products. Toxicological studies evaluating the safety of pigments in vitro and in vivo, as well as adherence to regulatory guidelines, are essential for market acceptance and consumer confidence.

e. Exploration of Synergistic Effects: Investigating the potential synergistic effects of pigment extracts with other natural compounds or antimicrobial agents could enhance their efficacy and broaden their applications. By combining yeast-derived pigments with complementary bioactive compounds, researchers can develop multifunctional formulations with enhanced health benefits and performance.

D. Limitations and Challenges

It is essential to acknowledge the limitations and challenges encountered in this study. The isolation and characterization of pigment-producing yeasts are inherently complex processes influenced by various factors, including sample diversity, culture conditions, and analytical techniques. Additionally, while the antimicrobial activity of yeast extracts was demonstrated against common pathogens, further studies are needed to evaluate their efficacy against a broader range of microbial strains, including clinically relevant pathogens and multidrug-resistant strains.

The isolation and characterization of pigment-producing yeasts from natural sources such as soil, fruits, and leaves face several limitations and challenges. One major limitation is the difficulty in isolating pure yeast cultures due to the presence of a complex microbial community, which requires meticulous and often prolonged culturing techniques. Additionally, the characterization of pigments involves sophisticated analytical methods like chromatography and spectroscopy, which are resource-intensive and necessitate specialized equipment and expertise. Another challenge is the variability in pigment production influenced by environmental factors such as pH, temperature, and nutrient availability, making it difficult to standardize and reproduce results. Assessing antimicrobial activity further complicates the process, as it requires a series of bioassays against a wide range of pathogens, demanding rigorous controls to ensure reproducibility and reliability. The potential for bioactive compound degradation during extraction and testing phases also poses a significant hurdle. Moreover, the regulatory and safety evaluation of these pigments for potential applications in food, pharmaceuticals, or cosmetics involves comprehensive toxicological studies, adding to the complexity and time required for development. Overall, these challenges highlight the need for interdisciplinary approaches and advanced methodologies to effectively explore and utilize pigment-producing yeasts from natural sources.

IV. Conclusion

In conclusion, this study presents a comprehensive investigation into the isolation, characterization, and potential applications of pigment-producing yeasts from diverse natural sources. The findings highlight the remarkable diversity of yeast strains capable of producing a wide range of pigments, including carotenoids with valuable properties such as antioxidant activity and vibrant coloration. Through morphological, physiological, and molecular analyses, the study elucidates the growth characteristics, metabolic capabilities, and genetic diversity of these yeast isolates, underscoring their adaptability to various environmental conditions. Moreover, the identification of diverse yeast species, including representatives from genera such as Rhodotorula, Yarrowia, and Debaryomyces, underscores the rich microbial diversity present in natural habitats. The pigment extraction and chemical analysis reveal the presence of carotenoids, particularly β-carotene, torulene, and torularhodin, in the yeast extracts, highlighting their potential applications in industries such as food, pharmaceuticals, and cosmetics. The significant antimicrobial activity exhibited by the yeast extracts further expands their utility as natural preservatives and antimicrobial agents, addressing the growing demand for safer and more sustainable alternatives to synthetic additives. However, the study also acknowledges the limitations and challenges encountered, including the complexity of pigment production processes and the need for further research to optimize production methods, explore novel applications, and ensure safety and regulatory compliance. Moving forward, continued research in this field holds promise for unlocking the full potential of pigment-producing yeasts, driving innovation, and contributing to the development of eco-friendly and biologically sustainable solutions for diverse industrial applications. By harnessing the unique

properties of yeast-derived pigments, researchers can advance the fields of biotechnology and microbial ecology, paving the way for a greener and more resilient future.

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