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STUDY ON EXIGUOBACTERIUM ACETYLICUM KI3 STRAIN PIGMENT OPTIMIZATION AND ANTIBACTERIAL ACTIVITY

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ABSTRACT

This study delves into the pigment extraction process from the Exiguobacterium acetylicum KI3 strain, known for its prolific pigment production and versatile applications. By fine-tuning physical and chemical parameters, the research achieved optimal pigment yield. The findings underscore the strain's potential for industrial-scale pigment production under specific conditions. Additionally, the study evaluated the antimicrobial properties of the extracted pigments against Escherichia coli, Staphylococcus aureus, Proteus vulgaris, and Pseudomonas aeruginosa, revealing noteworthy inhibitory effects. These results not only shed light on the extraction techniques and pigment synthesis but also highlight the strain's promising antimicrobial activity. Overall, this research contributes valuable insights into the biotechnological aspects of Exiguobacterium acetylicum KI3, paving the way for further exploration in various industrial and medical applications.

INTRODUCTION:

Natural colors, traditionally sourced from plants, can indeed be influenced by seasonal and geographical factors. However, modern trends are emphasizing microbial sources for pigments. Microbial sources offer benefits such as high biodiversity, availability throughout the year, and high production capacity (Deepti *et al.,* 2020).

Being gram-positive, facultative anaerobes, Exiguobacterium, thriving in diverse environments, exhibits remarkable adaptability to environmental fluctuations. Noted for its biotechnological promise, select species produce enzymes, biofuels, and pigments: orange-to-yellow. Recent attention has shifted towards their pigment synthesis capabilities, presenting potential industrial applications. This has prompted extensive scientific investigation into harnessing Exiguobacterium's versatility for various biotechnological endeavors, particularly in pigment production (Cavanaugh *et al.,* 2021).

The study investigated the specific conditions that foster maximum production of the cultural orange-to-yellow pigment by the Exiguobacterium *acetylicum* KI3 strain. Additionally, assessed the optimal conditions for extracting the pigment using solvents to obtain the highest concentration possible. Furthermore, explored the potential antimicrobial activity of this pigment.

MATERIALS AND METHODS:

1. Optimization of pigment production:

1.1.Physical parameters of bacterial pigment production :

The pigmented colony underwent incubation at temperatures (25-40°C) and pH (4-14) for 24 hours, yielding maximum pigment production, assessed via OD at 425nm. UV exposure (30sec-5min) followed by 37°C incubation for 24 hours induced mutations, enhancing pigment production, measured similarly (Fatima *et al.,* 2022) (Fatima Shatila *et al.,* 2013).

1.2.Chemical parameters of bacterial pigment production:

- **Carbon Source:** Carbon sources (Glucose, Sucrose, Fructose, Mannitol, Lactose, and Maltose) were assessed in *Exiguobacterium acetylicum* KI3 strain nutrient broth for 24 hours, measuring OD (425nm) for pigment production. Similarly, different nitrogen sources and NaCl concentrations were tested, monitoring OD at 425nm for pigment coloration evaluation in sterile nutrient broth incubation.
- **Nitrogen Source:** A range of nitrogen sources including Tryptone, Ammonium chloride, Ammonium sulphate, Urea, Ammonium oxalate, Ammonium nitrate, Ammonium acetate, Yeast extract, and Peptone were assessed, with OD measured at 425nm. Each source underwent similar testing conditions to evaluate its impact on pigment production.
- **NaCl Concentration**: Different concentrations of NaCl, FeCl3, and K2HPO4 were examined. *Exiguobacterium acetylicum* KI3 was cultured in sterile nutrient broth for 24 hours, and pigment colour was assessed by measuring OD at 425nm. Varied levels of each compound were tested to determine their influence on pigment production in the strain (Ratnakaran *et al.,* 2020).

2. Biomass and pigment production:

The isolate was cultured in a 500 ml Erlenmeyer flask with 250 ml sterile Nutrient broth and a 2.5 ml inoculum. Incubated on a rotary shaker at optimal conditions for 48-72 hours, resulting in dark orange-to-yellow medium, indicating pigment production. Harvested cells underwent centrifugation, washing, drying, and weighing to remove moisture (Sandanakirouchenane *et al.,* 2022).

3. Pigment extraction, purification and drying:

Different solvents (methanol, ethanol, acetone, and ethyl acetate) were tested for optimal pigment extraction. Orange-to-yellow dried cells mixed with each solvent were centrifuged at 6000-10000 rpm for 20 minutes until cell pellets lost colour. The resulting supernatant was dried into powder for subsequent analysis (*Sabbarapu et al.,* 2016) (Sandanakirouchenane *et al.,* 2022) (Sasidharan P *et al.,* 2013).

4. Antibacterial activity of pigment:

Mueller-Hinton agar was autoclaved and poured into Petri plates. Extracted pigment's antimicrobial activity was assessed via well diffusion against significant pathogenic bacteria: Staphylococcus aureus, E. coli, Klebsiella spp., and Pseudomonas aeruginosa. Test organisms were sourced from Hindustan College of Arts and Science, Chennai. Ethanolic pigment extracts were placed in wells, with streptomycin discs as positive controls. Zones of inhibition were measured (K.B. Haripriya *et al.,* 2022) (Shashi *et al.,* 2023).

RESULTS:

1. Pigment Production Optimization:

Optimal conditions for pigment production include a temperature of 30°C, basic pH, Fructose as the preferred carbon source, Peptone extract as the nitrogen source, and

NaCl as the preferred inorganic salt. UV-induced mutation occurs after 2.5 mins exposure.

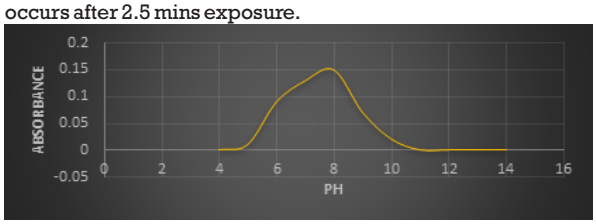


Fig.1: Effect of different temperatures on growth of Exiguobacterium acetylicum Ki3strain

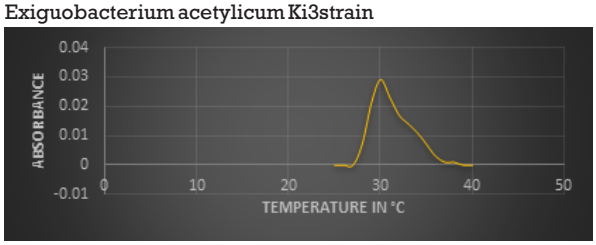


Fig.2: Effect of different pH on growth of Exiguobacterium acetylicum Ki3strain

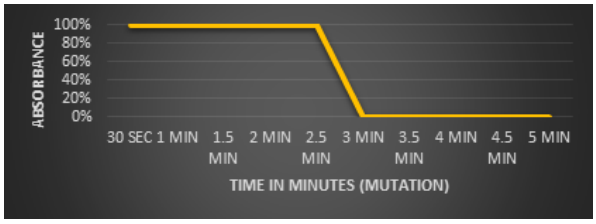


Fig.3: Effect of different time duration for mutation on growth of Exiguobacterium acetylicum Ki3strain

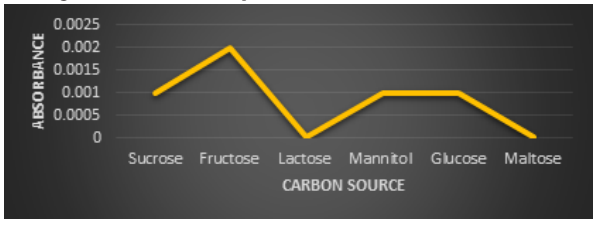


Fig.4: Effect of different carbon sources on growth of Exiguobacterium acetylicum Ki3strain

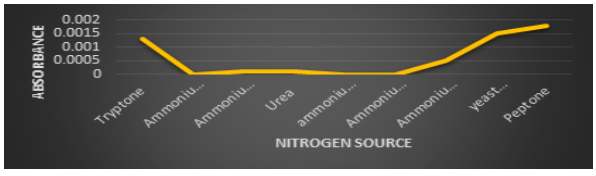
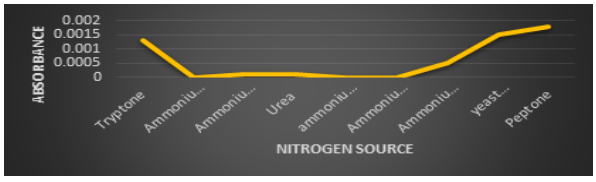
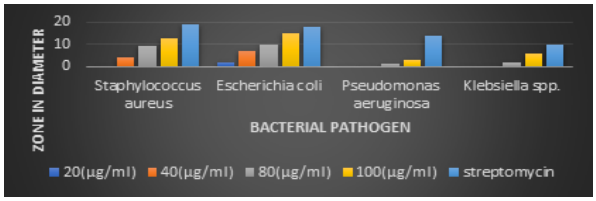


Fig.5: Effect of different nitrogen sources on growth of Exiguobacterium acetylicum Ki3strain



Pigment Extraction: Ethanol is the most efficient solvent for extracting bacterial pigments. After extraction, solvent removal by centrifugation yields a concentrated pigment solution, which is then dried for further analysis or application.



Antibacterial Activity: The extracted pigment demonstrates significant inhibition against Staphylococcus aureus and E. coli, with lesser effects on Klebsiella spp. and minimal inhibition against Pseudomonas aeruginosa. This suggests variable susceptibility among pathogens, possibly due to differences in cell wall composition.

SUMMARY AND CONCLUSION:

These discoveries offer crucial guidance for enhancing pigment production, refining extraction techniques, and exploring Exiguobacterium acetylicum KI3 pigment applications in industry and medicine. Future studies might delve into further optimization methods, uncover antibacterial activity mechanisms, and assess pigment safety and efficacy for real-world use.

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