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Optimization of red pigment produced by *Serratia rubidaea* KAP(LC201792)

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Abstract

Prodigiosin, a red bio-pigment produced by the *Serratia* species, has garnered significant interest due to its multifaceted applications in various industries, including food, cosmetics, textiles, and medicine. This study focuses on the optimization of prodigiosin production by *Serratia rubidaea* KAP (LC201792), isolated from oil-contaminated soil. Considering the pigment's potential pharmacological, antibacterial, antifungal, anticancer, and antiviral qualities, the study aims to increase its yield by adjusting important growth factors carefully. To ascertain their effect on pigment production, a number of variables were thoroughly investigated, including growth medium composition, optical density, incubation condition , temperature, pH, and the availability of carbon and nitrogen sources. Findings showed that *Serratia rubidaea* KAP (LC201792) can be cultured using nutrient broth at pH 7.0 and incubating at 28ºC under static conditions for the optimal production of prodigiosin. The results of this study provide valuable insights into the efficient production of prodigiosin, and reflect the potential capability of using *Serratia rubidaea* KAP (LC201792) for better production of prodigiosin thereby indicating its significance in sustainability

Keywords:

Prodigiosin, *Serratia rubidaea*, optimization, antimicrobial, pigment production, biotechnology

Introduction:

Colorants used in food and textile industries originate either from natural or synthetic sources. Synthetic pigments have gained a lot of importance over natural colorants due to their high stability and ease of abundance, variety, and availability Zollinger, (1991). However, synthetic pigments pose health and toxicity risks Downham and Collins, (2000). These chemically derived colorants are often hyperallergic and toxic, complicating environmental degradation and harming ecosystems Francis *et. al.*, (1987). They can also cause skin irritations, such as inflammation from aniline-based dyes Whitney Bauck, (2022).

The use of pigments originating from natural origin can shift industries towards an environmentally friendly and sustainable green approach Cserháti, T. (2006). Due to consumer demands for the use of safer products,

upscaling these naturally derived pigments is of more concern. These natural pigments not only help in increasing the market size but also help in meeting the consumer's requirements. Damant (2011); Scotter, (2011) These naturally derived colorants are non-toxic, and non-carcinogenic in nature, as well as easily degradable. Various sources, like plants, flowers, insects, and bacteria, are available for the extraction of biological pigments of natural origin Kiumarsi *et. al.*,(2017); Galaffu *et. al.*,(2015). These pigments are categorized under green chemistry, as they are eco-friendly and renewable to use.

Microbial derived pigments such as prodigiosin, pyocyanin, and riboflavin are valuable natural color sources found in diverse ecological environments. Bacterial pigments are more reliable than those from fungi and plants, requiring less production time and offering the possibility of genetic modification to alter production traits Ramesh *et. al.*,(2019). These pigments are preferred by industries because of their low production cost and scalability and their rapid growth. Galaffu *et. al.*,(2015). Prodigiosin is of a particular advantage as is a secondary metabolite produced in the later stages of microbial growth known for its red hue. The ability to produce prodigiosin effectively with enhanced color intensity was shown to depend on the nutritional components of the growth media and physical and biochemical conditions Zhao *et. al.*,(2020). Its bioactive properties have been widely investigated in the last decade, as prodigiosin is inside the focus of the present study, investigating the growth conditions of the isolated *Serratia Rubidea* KAP (LC201792). it was optimized to generate maximum pigment production. Also, the effect of various nutrient sources such as Nitrogen, carbon and amino acids was also studied. Furthermore, it is a low-cost substrate as far as possible used in pigment production. The findings of the research could help us understand industries' economic techniques for pigment production.

I.Materials and Methods

1.Growth Condition of Microorganisms:

Previously isolated from oil contaminated soil, the bacterial culture used in the study is *Serratia rubideae* KAP. The organisms were maintained on sterile nutrient agar slants with 1% glycerol at 4°C. The stock culture slants were and so were maintained their viability by subculturing them each week.

2. Optimization for maximal production of pigment from *Serratia rubideae* KAP.

Fresh 2% v/v inoculum of the organism was obtained to determine the optimal time for maximum pigment production. Culture was inoculated in 100 ml Sterile Nutrient Broth with an initial O.D. 0.5 at 540 nm. The flasks were incubated under static conditions at room temperature for 24, 48, 72, 96, 120, and 144 hours. The formula stated in seciton 4.3 was used to extract and quantify pigment. L. H. Pryce; F. W. Terry (2000); Gondil et.al. (2017). An uninoculated nutrient broth control flask was also maintained. A control flask of uninoculated nutrient broth was also maintained.

2.1 Optimization of Culture medium for maximum production of red pigment.

To identify the optimal medium for red pigment production, *Serratia rubidaea* KAP was grown in various media: Nutrient Broth, Peptone Glycerol Broth, Tryptic Soya Broth, Luria Bertani Broth, Nutrient Broth with 1% glycerol, and MSM with 1% glucose. Media were prepared and after autoclaving, 50 ml of each medium was inoculated with 2% v/v of an 18-hour fresh pigmented culture suspension (OD: 0.5 at 540 nm). Flasks were incubated at room temperature under static and shaker conditions for 4 days until pigmentation was visible. Pradeep *et. al.*,(2013), Haddix and Werner (2000), Venil and Lakshmanaperumalsamy (2009). Control flasks for each medium were also maintained.

2.2 Optimization of Incubation condition:

The medium supporting maximum pigment production was kept under static and shaker conditions (150rpm) to determine the optimal condition. It was inoculated with 2% v/v of 18h old culture suspension of *Serratia rubidaea* KAP with an O.D 0.5 at 540 nm and observed for 4 days. The pigment was extracted, and yield was calculated using the formula given below in Section 4.3 Elkenawy *et. al.*,(2017); Metwally *et. al.*,(2017); Shaikh, (2016). A control flask with uninoculated nutrient broth was also maintained.

2.3 Optimization of optical density:

The bacterial culture *Serratia rubideae* was adjusted to different cell densities ranging from 0.1 to 0.6 O.D. at 540 nm. A 2% v/v inoculum of 18h old hour old culture was inoculated in 100ml Sterile Nutrient Broth. The flasks with culture of different optical densities were incubated at room temperature under static conditions for 4 days. The pigment was then extracted, quantified using the formula given below in section 4.3. Along with the test flasks, a control flask of uninoculated nutrient broth was also maintained.

2.4 Optimization of Time (H):

Fresh 2% v/v inoculum of the culture was obtained to determine the optimal time for maximum pigment production. Culture was inoculated in 100 ml Sterile Nutrient Broth with an initial O.D. 0.5 at 540 nm. The flasks were incubated under static conditions at room temperature for 24, 48, 72, 96, 120, and 144 hours. The formula stated in seciton 4.3 was used to extract and quantify pigment. L. H. Pryce; F. W. Terry (2000); Gondil *et. al.*,(2017). An uninoculated nutrient broth control flask was also maintained.

2.5 Optimization of Temperature:

A 2% v/v bacterial culture suspension with an optical density (O.D.) of 0.5 at 540 nm was added to 100 ml of sterile nutrient broth and incubated under static conditions at temperatures of 4ºC, 28ºC, 37ºC, 45ºC, and 50ºC for five days to identify the optimal temperature for maximum pigment production Khanafari *et. al.*,(2006); Sundaramoorthy *et. al.*,(2009); Siva *et. al.*,(2012). A control flask containing uninoculated nutrient broth was also maintained.

2.6 Optimization of pH

According to the existing literature, Hardijito et.al. the pigmented culture suspension was cultivated in Sterile nutrient broth of pH levels of 4.5, 5, 6, 7, 8, 9, and 10 was adjusted with 0.1 N HCl and 0.1 N NaOH to a final

volume of 100 mL. NaOH and autoclaved. After, this the broth was incubated at room temperature, under static, for 5 days. Venil and Lakshmanaperumalsamy (2009); Sumathi et.al. (2014); Sundaramoorthy et.al.,(2019). A control of uninoculated nutrient broth flask was also maintained.

2.7 Impact of Different Nutrient Sources on Pigment Production:

- **Carbon Source**

100ml of nutrient broth was used to determine the effect of carbon sources on pigment production. It was supplemented with 1% Dextrose, Lactose, Fructose, Starch, Sucrose and Glycerol as carbon sources. The medium 2% v/v culture suspension was inoculated to amended media, incubated at room temperature for 5 days under static conditions. Then pigment yield was estimated. R. Suryawanshi, (2014), Gondil, V. S, (2017). Harned, R. L. (1954). Uninoculated nutrient broth control flasks with and without the respective, carbon source was also maintained.

- **Nitrogen Source**

Ammonium nitrate, ammonium chloride, ammonium sulfate, sodium nitrate, tryptone, and casein were used to experimentally investigate the effect of different nitrogen sources on pigment production. Each nitrogen source was supplemented into 100 ml of the nutrient broth at 1% concentration. Inoculum for the broth was a pigmented culture suspension of 0.5 OD, which was inoculated and incubated at room temperature for five days under static conditions into the broth . The amount of pigment produced was measured after incubation Robert, P. W. (1973); Song et. al., (2006); Sumathi et. al., (2014); Gulani et. al., (2012). Incubations of corresponding control flasks containing uninoculated nutrient broth with each of the respective nitrogen source were also carried out.

2.8 Effect of amino acid on pigment production:

100ml of nutrient broth with different peptides was used to study the effect of amino acids on pigment production. Peptides used in the study were Tryptophan, Serine and Methionine and Proline at 1% concentration each. The flask was inoculated with *Serratia rubidaea* KAP. After incubation, maximum pigment yield was estimated at the optimal amino acid precursor Willams (1971); Wei, Y.H. (2005); Xia *et. al.*,(2018). Control flasks of uninoculated nutrient broth with the respective amino acid and a nutrient broth inoculated with culture without the amino acid were also maintained.

3.Additional low-cost substrates for pigment production

To check the effect of fatty acid on pigment production, Peanut Seed Broth, Peanut Broth, Sesame Seed Broth, and Sesame Broth were utilized. The peanut and sesame seed in the media were used as fatty acid substrates, that is reported to enhance the levels of prodigiosin. The above fatty acid-containing mediums (100ml) were inoculated with 2% v/v 18h old culture suspension of 0.5 OD 540nm and were incubated at room temperature under static and shaker conditions for 5 days. After incubation, the pigment yield was calculated using the formula given in section 4.3. Harris A.K. *et. al.*,(2004); Shahitha *et. al.*,(2012).

4. Extraction of pigment using a standardized procedure:

4.1. Selection of optimum solvent for extraction of pigment:

To select an optimum solvent for maximum pigment extraction, solvents such as methanol, absolute ethanol, acetic acid, acetone, ethyl acetate, petroleum ether, chloroform, and n-hexane were tested. The solvent with the highest pigment yield was chosen for subsequent experiments.

4.2 Procedure for Extraction of Pigment:

Serratia rubidaea KAP was inoculated into a sterile nutrient broth and incubated at room temperature under static conditions for 5 days. After incubation, 25 ml of the culture broth was centrifuged at 10,000 rpm for 10 minutes, and the pellet was collected after discarding the supernatant. The pellet was resuspended in the selected solvents and vortexed for 1 minute to ensure thorough mixing, followed by centrifugation at 10,000 rpm for 10 minutes.

Spectroscopic analysis for the supernatant was conducted on precipitated particles containing the pigment from two samples, and absorbance was measured at 535nm Sundaramoorthy et.al. (2009); Palacio-Castañeda et.al. (2019).

4.3 Measurement of Red Pigment Quantity:

To quantify the pigment produced by *Serratia rubidaea* KAP, extraction was performed according to the method described in Section 4.3. The amount of pigment produced was then estimated using the formula provided by Ivanchenko, D. A. (2020), Balasubramaniam, B. (2019), and Mekhael and Yousif (2009).The absorbance was measured at 534 nm and the bacterial cell optical density at 620 nm.

$$\text{Prodigiosin (unit/cell)} = \frac{(OD534 - (1.381 \times OD620)) \times 1000}{OD620}$$

OD534 = Refers to the absorbance of pigment at 534nm.
OD620 = Refers to the optical density of the culture broth.
1.381 = Constant

Ethanol served as a blank for pigment absorbance at 534nm, and an uninoculated Nutrient broth was used as a control and a blank for recording the optical density of the culture broth.III. Results and Discussion

The present study investigates the different variables useful in improving the production of prodigiosin from *Serratia rubidaea* KAP, renowned for its significant antimicrobial, antioxidant, and dyeing properties Islan GA, et.al.(2022).

1. 1 Growth Condition and colony characteristic of Bacterial strain *Serratia rubidaea* KAP

Serratia rubidaea KAP was isolated from oil contamination and subculturing was done on Nutrient agar slant supplemented with 1% glycerol which retained the pigment-producing capability of *Serratia rubidaea* KAP.

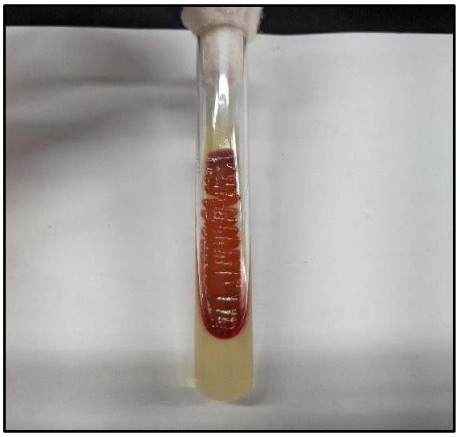

Figure 1.1 *Serratia rubidaea* KAP (LC201792) on Nutrient Agar Slant.

Macroscopic and Microscopic colony characteristics were studied to obtain large ,dark red pigmented colonies which were found to be gram negative coccobacilli in clusters and chains when observed under 100X.

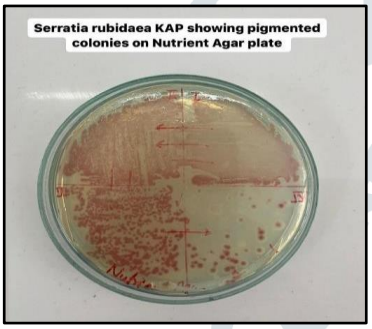

Figure 1.1.a Colony on plate

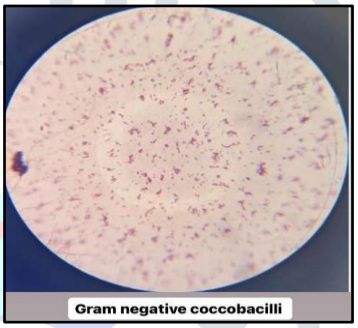

Figure 1.1.b Under the Micrscope

1.2 Optimization of physicochemical parameters for maximum pigment production.

The amount of prodigiosin produced under each parameter was assessed using the formula provided by Mekhael and Yousif (2009), which they used for optimizing prodigiosin production. The pigment absorbance was measured at 534 nm using a UV-Visible spectrophotometer, and the bacterial optical density at 620 nm was recorded using a colorimeter.

1.3 Optimization of Culture Medium for maximum production of red pigment.

The growth medium's components play a vital role in pigment production by supplying the necessary nutrients, precursors, and optimal conditions for pigment synthesis. To optimize the culture medium for maximum pigment production, the microbial strain *Serratia rubidaea* KAP was inoculated in the different culture media. Production of prodigiosin pigment across various culture media was studied. The highest amount of prodigiosin, 2522.6 units per cell, was observed in Nutrient Broth under static conditions. Nutrient Broth with 1% glycerol produced 1626.0 units per cell, while Tryptic Soy Broth and Peptone Glycerol Broth yielded 737.5 and 434.1 units per cell, respectively. In contrast, Luria Bertani Broth produced a significantly lower amount of prodigiosin, at just 10.66 units per cell. Notably, no prodigiosin production was detected in MSM with 1% glucose. Given these results, Nutrient Broth was selected for further studies due to its superior performance in pigment production. mentioned below.

The current results are consistent with the optimization studies conducted by Giri *et. al.*,(2004), Balasubramaniam *et. al.*,(2019), Srimathi *et. al.*,(2017), and Samrot *et. al.*,(2011). Previous research by Thomas *et. al.*,(2002) highlighted the importance of amino acids in the biosynthesis of red pigment by Serratia spp. However, the presence of glycerol in Nutrient Broth appears to hinder pigment production, as demonstrated by the results. Samrot *et. al.*,(2011) also observed strong pigmentation in Nutrient Broth for S. marcescens, which is consistent with our findings.

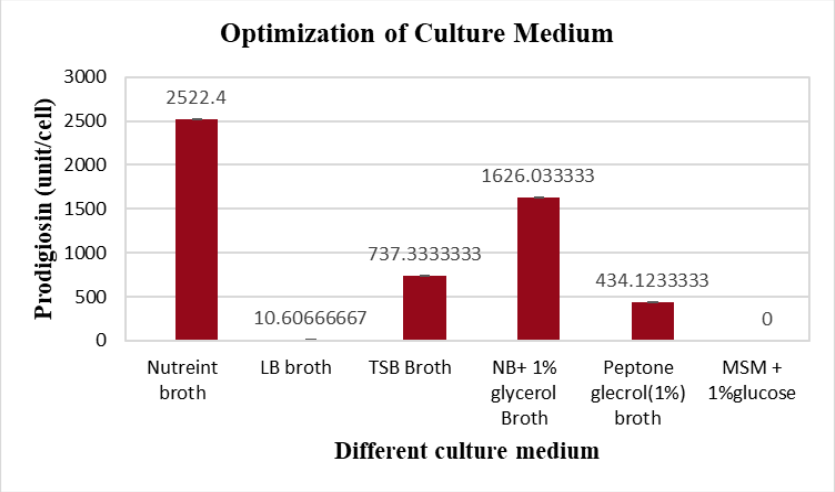

Figure 1.3 a) Optimization of Culture Medium for maximum production of red pigment.

Figure 1.3 b) Pigment production by *Serratia rubidaea* KAP under static conditions in different media.

While findings indicate that Nutrient Broth is the optimal medium for prodigiosin production by *Serratia rubidaea* KAP, other studies have reported varying outcomes depending on the strain and conditions used.

For instance, Pereira *et. al.*,(2023) reported that Marine based media like F2 (MB) Broth, supplemented with a specific carbon source, significantly enhanced prodigiosin production in *Serratia rubidaea* compared to other conventional media . This contradicts our findings where Nutrient broth is primarily used. These differences highlight the importance of strain specificity and culture conditions in optimizing prodigiosin production. While Nutrient Broth proved most effective for *Serratia rubidaea* KAP in our study, alternative media might yield better results for other strains or under different experimental conditions.

1.4 Optimization of Incubation conditions.

The bacterial culture *Serratia rubidaea* KAP was introduced into all the previously mentioned broths and

incubated under both static and shaker conditions, with all other parameters kept constant. The highest pigment production was observed in the flask incubated under static conditions (Figure 1.4.a), while no pigment production occurred in the flask under shaker conditions (Figure 1.4.b). Consequently, all subsequent experiments were conducted under static conditions. These findings align with studies by Aruldass *et. al.*,(2014) and Metwally *et. al.*,(2017). Metwally *et. al.*,(2017) reported that shear stress caused by constant shaking inhibited prodigiosin pigment production, confirming through their research that *Serratia rubidaea* RAM_Alex produced high levels of prodigiosin pigment under static conditions.

However, a contrasting study on Improving Bioprocess Conditions for the Production of Prodigiosin using a Marine *Serratia rubidaea* Strain, reports that shaking conditions can actually promote prodigiosin production under specific circumstances. The researchers found that agitation at certain speeds (200-300 rpm) led to an increase in biomass and pigment yield compared to static conditions. This suggests that while static conditions may be beneficial in some contexts, controlled shaking can also be advantageous for maximizing pigment production in others.

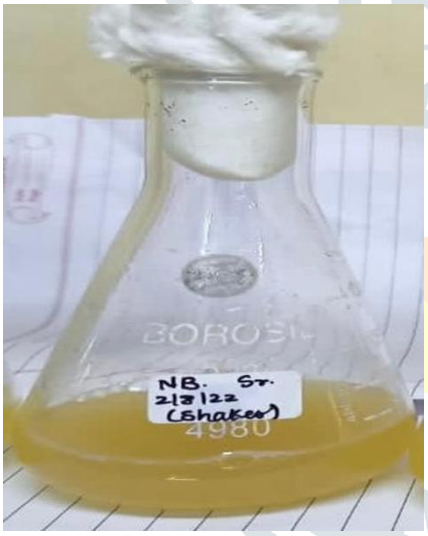

Figure 1.4 a) Pigment Production by *Serratia rubidaea* KAP under shaker conditions

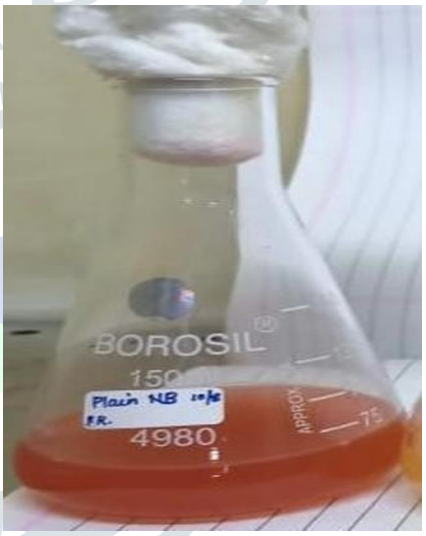

Figure 1.4 b) Pigment production by *Serratia rubidaea* KAP under static conditions

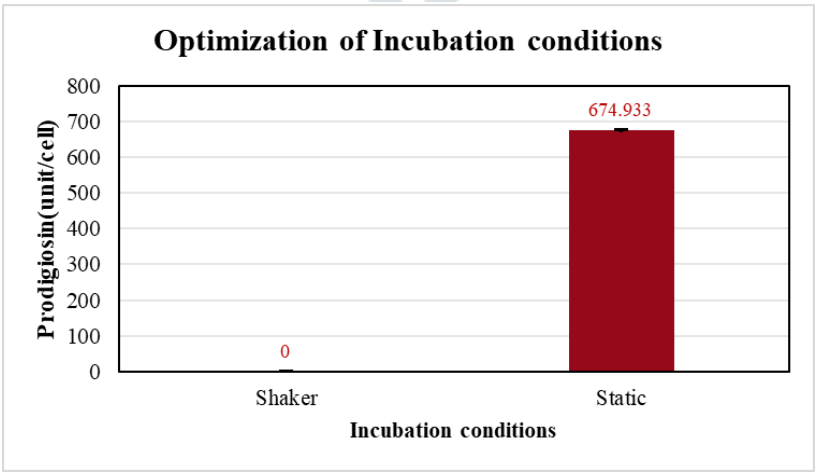

Figure 1.4. c) Optimization of incubation conditions.

1.5 Optimization of Time (hr) for production of prodigiosin.

The bacterial strain *Serratia rubidaea* KAP was inoculated in sterile Nutrient broth and the flasks were incubated under static conditions for 24hr, 48hr, 72hr, 96hr, 120hr, and 144hr respectively to determine at which hour pigment production occurs. All other physical characteristics were maintained at constant levels. The bacterial isolate grew rapidly until 48hr. Since prodigiosin is a secondary metabolite and is produced at the lateral stages of growth so no pigmentation was observed until 48hr but after 72 hr there was an increase in pigment production. When compared to the literature the studies reported by Samrot *et. al.*,(2011) their results were in accordance with the present studies. At 120 hr. maximum pigment production of 3063 units/ cell of prodigiosin was observed. Tran *et. al.*,(2021) reported that prodigiosin production starts between 48hr-72hr. As a secondary metabolite prodigiosin is often produced following the cell's log phase so, in the stationary phase the fastest increase in pigment production was observed. Additionally, the production curve's properties resemble the secondary metabolite production pattern reported by Kurbanoglu *et. al.*,(2015).

However differing results are observed in the case of reports on other Serratia species on Prodigiosin Production Choi *et. al.*,(2021). In this study, the authors found that prodigiosin production could be initiated as early as 24 hours under specific nutrient conditions and agitation levels, which contrasts with the findings that production typically starts between 48-72 hours

This indicates that the prodigiosin production timing depends on growth conditions, and that the onset may be influenced by factors like nutrient availability and environmental stressors.

In different strains or under different experimental set up, secondary metabolite production.

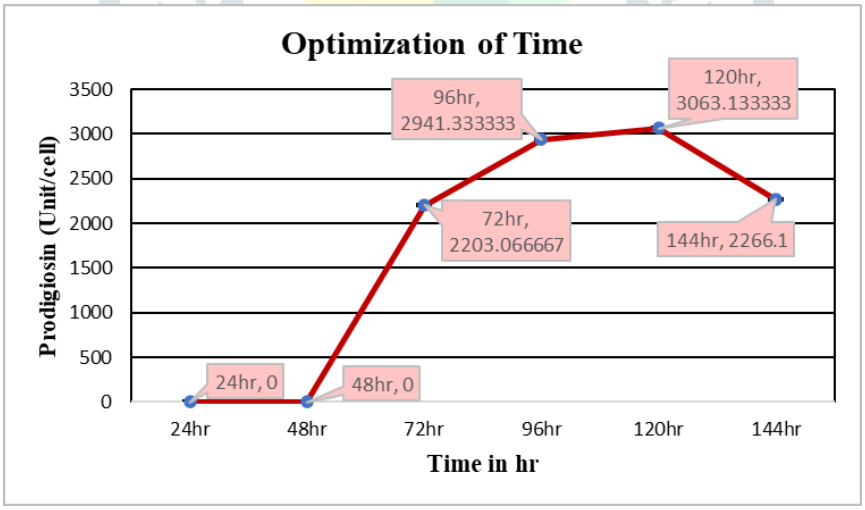

Figure 1.5 a) Optimisation of Time

1.6 Improvement of optical density

Optical density (OD) is a measure of how much light is scattered by a substance. and is correlated with the number of bacterial cells in a culture suspension Linthornze *et. al.*,(2015). By using measurement of optical density researchers can at different time points design growth curves and analyze the growth kinetics of a

bacterial culture, Mytilinaeios *et. al.*,(2012). In the optimization of the optical density , the bacterial culture for *Serratia rubidaea* KAP was adjusted to OD values of 0.1, 0.2, 0. 3, 0.4, and 0.5 at 540 nm and inoculated into sterile nutrient broth. For varying optical flasks, Incubation was done under room temperature for four days in static conditions. The results were indicated that the flask with an optical density of 0.5 yielded the highest pigment production in terms of units per cell figure 1.6.a

Several studies have documented the use of 0.5 OD at 540 nm as the initial optical density Gondil *et. al.*,(2017); Chadni *et. al.*,(2017).

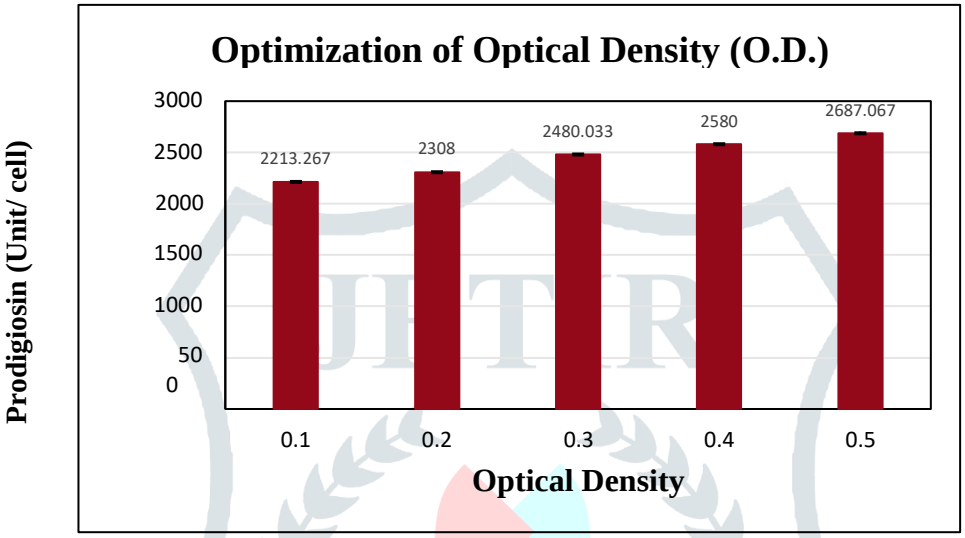

Figure 1.6. a) Amount of pigment produced at different optical densities.

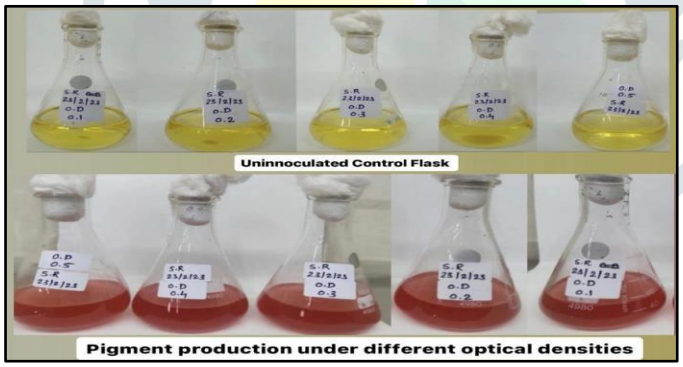

Figure 1.6. b) Optimization of optical density

However, different studies have reported varying optimal OD values for pigment production which also depends on pH . For instance, Rakh *et. al.*, (2017) found that *Serratia rubidaea* strain JCM 1240T achieved maximum prodigiosin production in the range of 470 nm to 530 nm, suggesting that optimal OD for prodigiosin production can vary between strains, underscoring the need for strain-specific optimization in experimental setups.

1.7 Optimization of Temperature.

The bacterial strain *Serratia rubidaea* KAP was inoculated into sterile Nutrient broth and incubated under static conditions at temperatures of 4ºC, 28ºC, 37ºC, 45ºC, and 50ºC for five days to determine the optimal temperature for pigment production while maintaining constant physical parameters. Temperature significantly influences the biosynthesis of the prodigiosin pigment. For *Serratia marcescens*, the optimal temperature for prodigiosin production is generally around 25-30ºC, where the genes responsible for pigment synthesis are upregulated, enhancing production. Extreme temperatures, both high and low, can inhibit this process Suryawanshi *et. al.*,(2014). In this study, complete inhibition of pigment production occurred at 45ºC, while the optimal temperature for *Serratia rubidaea* KAP was determined to be 28ºC. A slight pigment production was noted at 37ºC, indicating a decrease in production with increasing temperature. The results, illustrated in Figure 1.7.a, show pigment production at various temperatures, with similar findings reported by Khanafari *et. al.,* (2006) and Giri *et. al.,*(2004), who identified maximum pigment production at optimal temperatures of 28ºC and 30ºC, respectively.

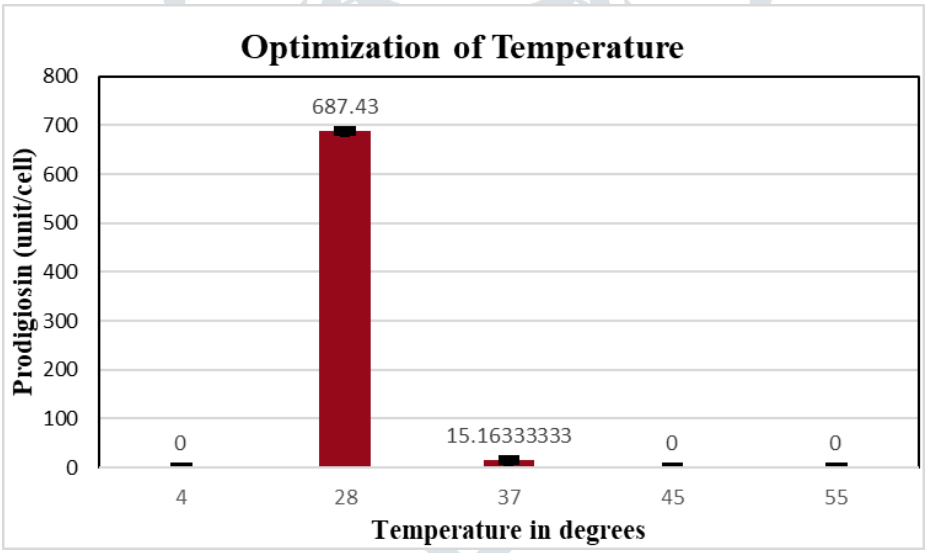

Figure 1.7. a) Optimized temperature for pigment production.

However, different findings have been reported in other studies. For example, Pereira *et. al.*,(2023) found that a strain of *Serratia rubidaea* produced maximum pigment at 37ºC, with a sharp decline in production below this temperature, contrasting with our results where optimal production occurred at 28ºC.

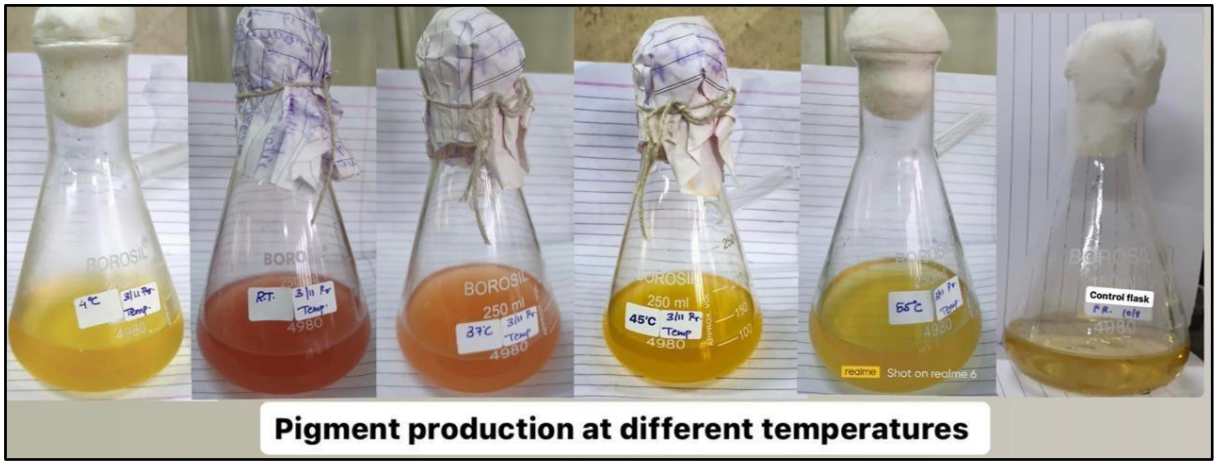

Figure 1.7. b) Pigment production by *Serratia rubidaea* KAP under different temperatures.

1.8 Optimization of pH for maximum pigment production.

In natural systems, pH is a key factor affecting the rate of substrate degradation. The production of prodigiosin per cell at different pH levels, ranging from 4 to 10, significantly influences both growth and pigment production, as shown in Figure 5.3.6. Initially, at lower pH levels, pigment production was low. However, as the pH increased beyond 6, pigment production gradually rose. The highest prodigiosin production occurred within the pH range of 7 to 9, with a peak of 2066 units per cell observed at pH 8. These findings align with previous studies by Giri *et. al.*,(2004), which noted optimal pigmentation by Serratia spp. between pH 7 and 9. Similarly, research by Sumathi *et. al.*,(2014) and Wei *et. al.*,(2005) supports these results. Additionally, Pore *et. al.*,(2016) and Solé *et. al.*,(1994) reported that maximum prodigiosin production typically occurs in alkaline pH environments. Therefore, pH 8 was chosen as the optimal pH for the biosynthesis of prodigiosin pigment.

However, some studies have reported different findings. like Bhagwat *et. al.*, reported maximum optimization at pH ranging from 6.2 to 7. These differences underscore the importance of strain-specific optimization and suggest that environmental factors such as pH must be carefully controlled to achieve maximum pigment production.

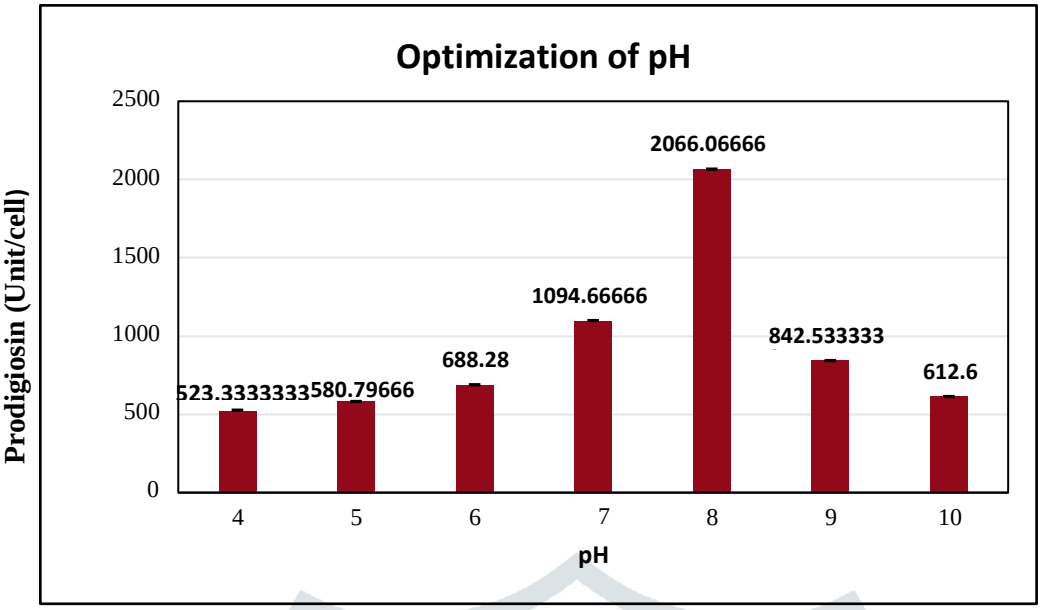

Figure 1.8. Optimization of pH

1.9 Effect of various nutrient sources for pigment production.

1.9.1 Effect of carbon source.

Utilizing alternative carbon sources is essential for enhancing pigment synthesis, as it can significantly increase pigment production. Different carbon sources such as glucose, lactose, fructose, sucrose, starch, and glycerol were assessed for their impact on pigment production, while maintaining the same physical conditions. The results indicated that lactose was the most effective, yielding the highest pigment level at 1434.133 units per cell. Starch and sucrose also showed positive effects, producing 1302.2 and 1181 units per cell, respectively. These findings align with the studies by Gulani et. al., (2012), Xu et. al., (2011), and Zhang et. al., (2014). In contrast, Su *et. al.*,(2011) also reported decreased prodigiosin levels that occurred in glucose and fructose.

Because of their inhibitory effects on pigment production, Bunting *et. al.*,(1949). Glucose, in particular, allows that increased cellular cAMP levels to cause catabolite repression and reduce pigment synthesis and pH lowering, which is harmful to prodigiosin production Clements *et. al.*,(1996); Kalivoda et. al., Fineran *et. al.*,(2005); (2014). Indeed, it is well known that prodigiosin is usually produced in alkaline conditions. Especially for easily metabolizable sugars like glucose and fructose, lowering conditions Espeso *et. al.*,(1993). Scalar interactions with the medium pH, thus inhibiting pigment synthesis further Sole *et. al.*,(1994).

However, as per the findings by Wang *et. al.*,(2024), it has found that glucose, contrary to the inhibitory effects reported by Su *et. al.*,(2011) and others, could enhance prodigiosin production under specific conditions. The researchers observed that certain concentrations of glucose, when combined with other nutrients, led to increased pigment synthesis, suggesting that the effects of glucose can vary based on the overall nutrient composition of the medium and specific fermentation conditions.

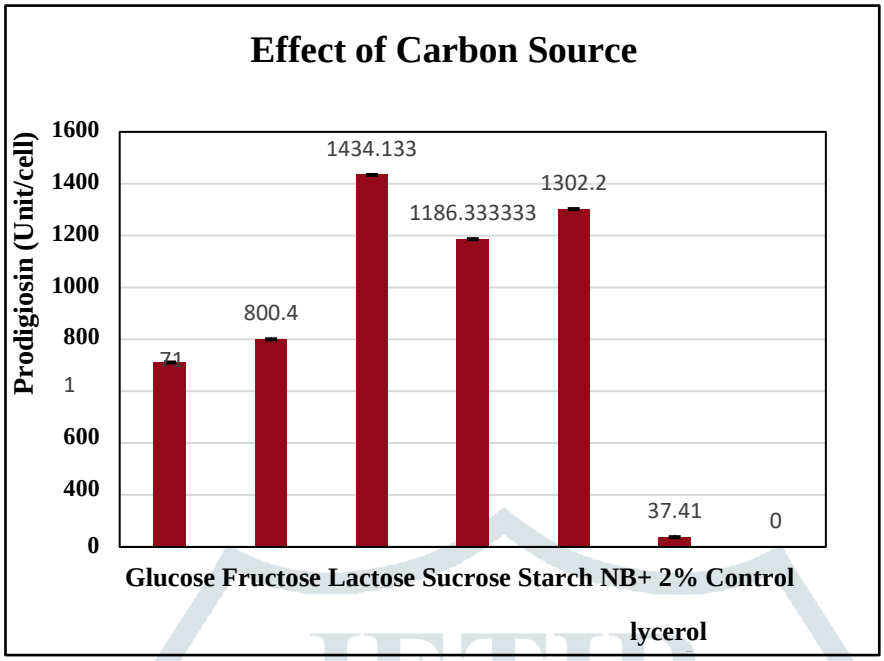

Figure 1.9.1. a) Effect of carbon source on pigment production

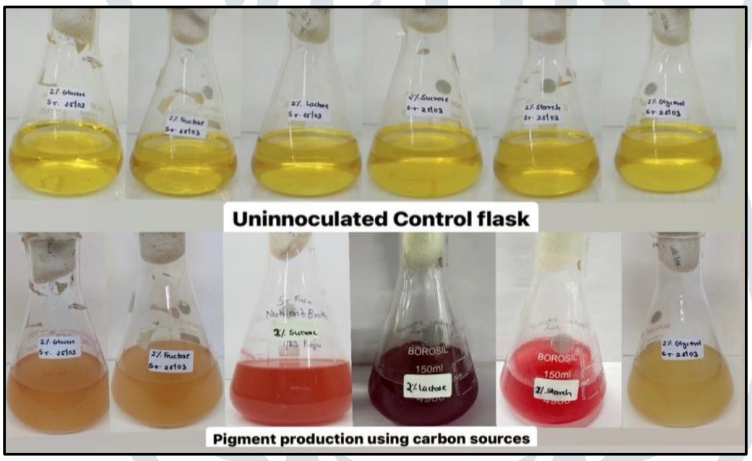

Figure 1.9.2. b) Pigment production by *Serratia rubidaea* KAP under different carbon sources

1.9.2. Effect of Nitrogen Source

To examine the impact of different nitrogen sources on pigment production, a variety of nitrogen sources, including ammonium nitrate, ammonium sulfate, ammonium chloride, sodium nitrate, tryptone, and casein, were tested. Each nitrogen source was added to 100 ml of Nutrient Broth at a 1% concentration, and the flasks were incubated under static conditions for 5 days, with all other physical parameters kept constant.

When casein was used as the nitrogen source, the bacterial strain achieved maximum pigment production, recording 2036.27 units per cell. This result aligns with the findings of Song *et. al.*,(2006). Earlier studies by Kim *et. al.*,(2007) and Song M.J *et.al.*, (2001) also reported that casein plays a significant role in prodigiosin biosynthesis, serving as both a carbon and nitrogen source for the KH-95 strain. While Wei *et. al.*,(2005) reported that increased levels of tryptone in the medium inhibited pigment synthesis, our study found that tryptone resulted in a high yield of 1244.07 units per cell of red pigment. Conversely, when inorganic nitrogen sources such as ammonium salts and sodium nitrate were used, the yield of red pigment by *Serratia rubidaea* KAP decreased significantly. Xu *et. al.*,(2011) noted in their study that these inorganic sources are detrimental to prodigiosin synthesis.

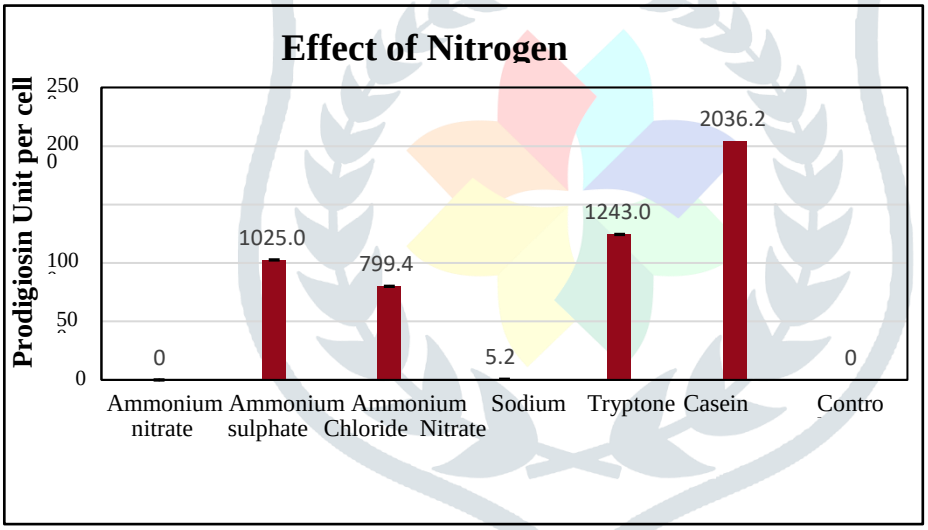

Figure 1.9.2. a) Effect of different nitrogen sources on pigment production.

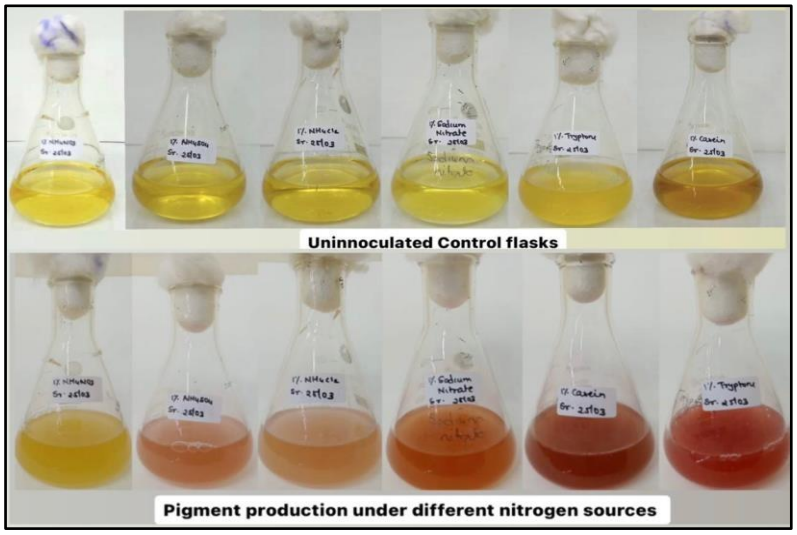

Figure 1.10. b) Pigment production by *Serratia rubidaea* KAP using different nitrogen sources.

Several reports corroborate the significance of selection of nitrogen source in accordance to the specific strains and experimental setups as perquisite in optimum prodigiosin production. Demain and Fang (2000) have reported that ammonium sulfate as a favorable nitrogen source in which pigment could be produced in *Serratia marcescens*. A like studies by Jasek *et. al.,* (2003) demonstrates presence of ammonium nitrate increased pigment. Unlike, these reports the present studies observed non-significant association of nitrogen sources and its impact in prodigiosin production. Thus, indicating nitrogen sources can have a very different effectiveness, depending on the bacterial strain and experimental conditions

1.9.3. Effect of amino acid on pigment production.

To evaluate the effect of amino acids on pigment production, 100 mL of nutrient broth was supplemented with 1% concentrations of amino acids, specifically tryptophan, serine, methionine, and proline. The amino acid-enriched medium was inoculated with the bacterial culture *Serratia rubidaea* KAP and incubated while keeping all other physical parameters constant. The study compared the impact of amino acids on pigment production against that of the nutrient broth. The results indicated that the presence of peptone in the nutrient broth significantly enhanced pigment production, yielding 2522.4 units/cell.

While the addition of amino acids supported pigment production, it was not as effective as the Nutrient broth alone. Methionine was found to promote pigment production the most, with a yield of 1958.707 units/cell, followed by proline at 1441.133 units/cell, serine at 1009.1 unit/cell, and the lowest yield from tryptophan at 37.44 unit/cell. Although Mohammed *et. al.*,2012 reported that methionine positively influences prodigiosin production, this finding contradicts the current results. During prodigiosin synthesis, the structural methyl group at the C6 position is derived from methionine, as noted by Hussain *et. al.*,(1973). Additionally, several studies highlight proline's role as a precursor in the synthesis of prodigiosin metabolites. However, tryptophan and serine resulted in relatively lower pigment production when included in the medium Siva *et. al.*,(2012); Williamson *et. al.*,(2005); Williams *et. al.*,(1971).

For instance, according to Tejada et. al., (2024), tryptophan improved pigment production in *Serratia marcescens*, potentially due to role as precursor to indole pathway biosynthesis. As Nanda *et. al.,* (2011) also

found, serine was a great enhancer of prodigiosin. perhaps due to involvement in facilitating production of precursors for the biosynthetic pathway. These products show how different strains of Serratia differ. Amino acid supplementation is responded to by Serratia spp. and thus emphasizes strain specific optimization.

The fact that these findings are contrasting implies either that methionine or proline might prove beneficial to Serratia. other strains of Serratia might respond differently. This highlights the need for careful selection and customization of amino acid supplementation to optimize prodigiosin synthesis for each specific strain.

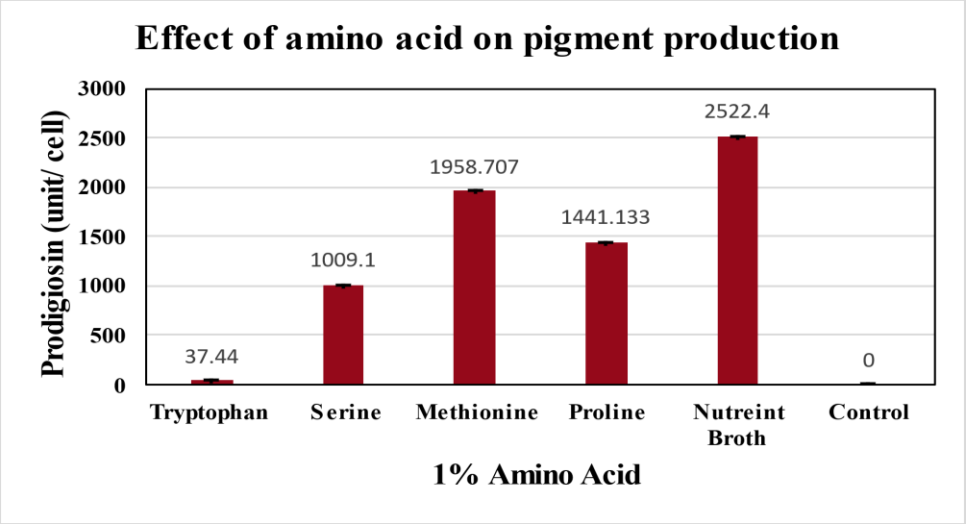

Figure 1.9.3. a) Effect of amino acid on pigment production

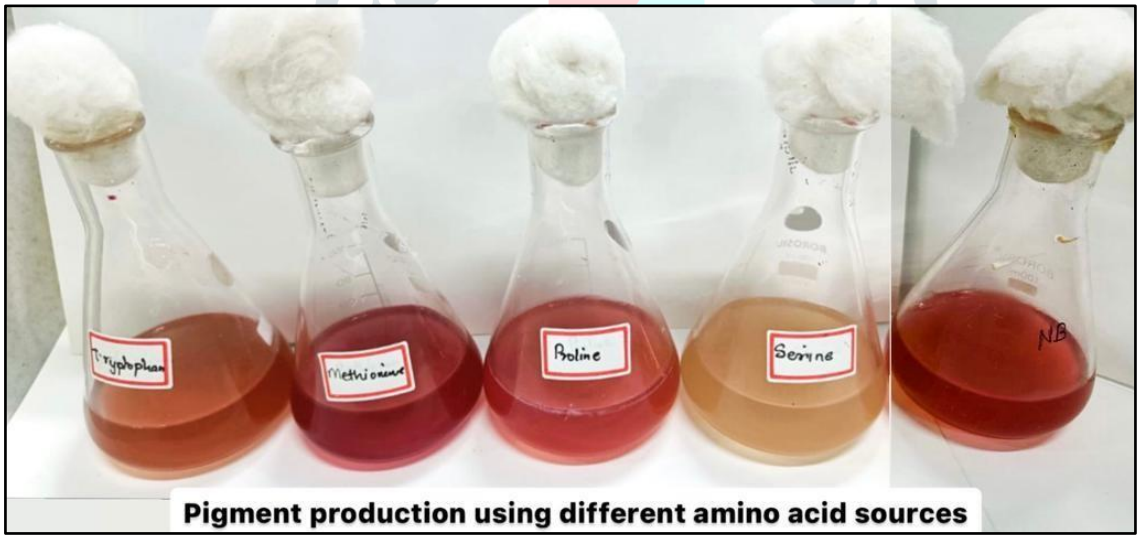

Figure 1.9.3. b) Pigment production by *Serratia rubidaea* KAP using different amino acid sources.

1.9.4 Low-cost Substrate used for pigment production.

The bacterial isolate *Serratia rubidaea* KAP had a pigment production preference. Currently, fatty acids are used as a low-cost substrate for pigment production. Peanut seed or other fatty acid sources are used as sources. In this study, sesame seeds were evaluated. Crushed produced the highest pigment productivity. The results were peanut seed broth, 1006.2 units per cell; peanut broth, 635.3 units per cell. In By contrast, crushed sesame seed broth did not produce any pigment; sesame broth, however, yielded 65.75 units. per cell. Giri *et. al.*,(2004), reported similar results, which involve the use of fatty acids as.

Prodigiosin synthesis was increased when a carbon source was used compared to traditional media. Peanuts are cheaper than conventional high-cost media and a typical peanut seed contains all the essential nutrients necessary for pigment production. 8% peanut oil was used by Naik *et. al.*,(2012) In their study they used cake as a fatty acid substrate and found that prodigiosin yield increased to 4000 mg/L. Additionally, Han *et. al.*,(1998) observed that peanut oil cake has a high crude protein and fiber contents and contains all vitamins and minerals.

These contrasting findings appear to suggest that fatty acids can serve as substrates for prodigiosin production may. Specific strain of Serratia and composition of fatty acid sources used determines its dependence. The results emphasize the need for proper selection of fatty acids sources to optimize pigment. for different bacterial isolates production in various sectors, including agriculture and pharmaceuticals.

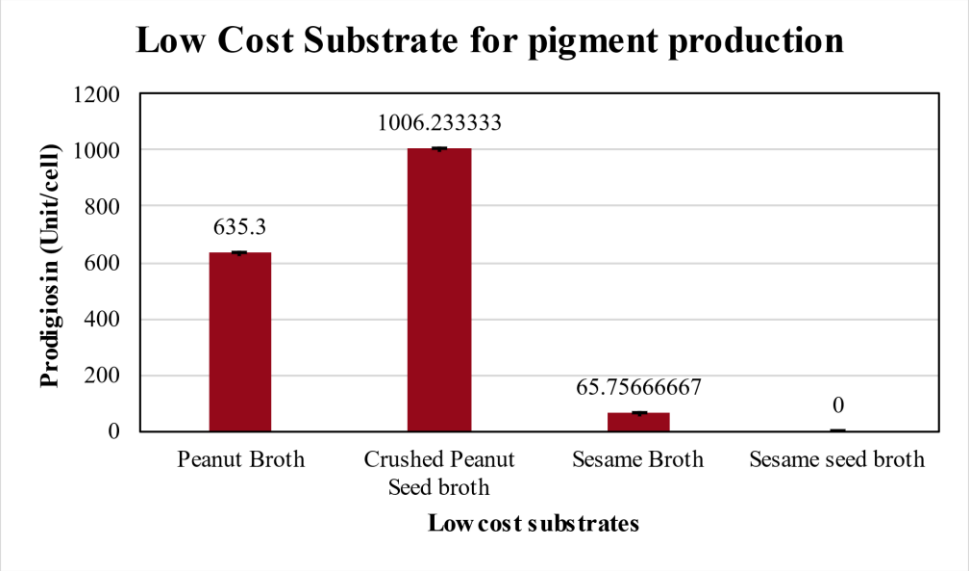

Figure 1.9.4. a) Low-cost Substrate used for pigment production

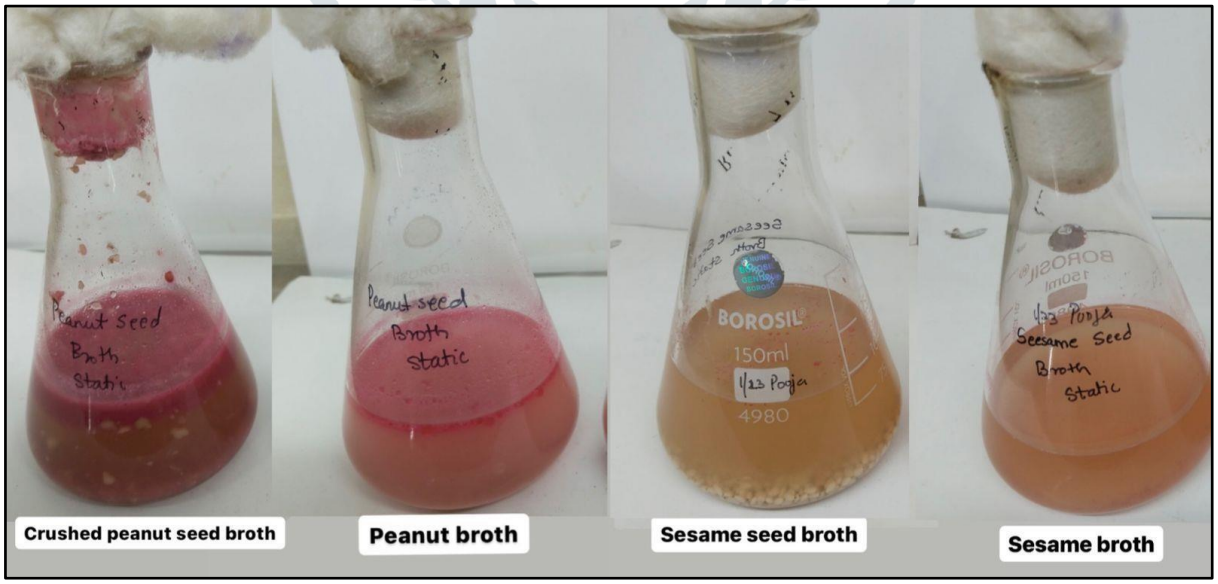

Figure 1.9.4. b) Pigment production using low-cost substrates

IV. Conclusion

One factor at a time optimization of prodigiosin production by *Serratia rubidaea* KAP was performed in this study. OFAT method. Nutrient broth supplemented gave the best results. We obtained 2522.4 units/cell with peptone and got a peak of 3063.1 units/cell after 129 hours. of static incubation. On pH 8 and 28°C, lactose was the most effective carbon source for the isolate and it thrived. The best nitrogen source of our two cases were source (1434.1 units/cell) and casein (2360.7 units/cell). Methionine, although beneficial, not as effective as nutrient broth alone. Instead, these findings highlight the critical optimizing culture conditions to increase the yield of pigment to improve the bioprocess supporting sustainable and economically viable production methods of high efficiency. Further research is However, prodigiosin is essential to address the challenges in commercializing it, particularly in advanced techniques to enhance yields and improve production costs.

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