Optimization of nutritional and physicochemical parameters for prodiogiosin production by *Serratia nematodiphila*

Environmental and
Experimental
Biology

UNIVERSITY OF LATVIA

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Abstract

The increasing interest in a sustainable approach to biotechnological applications has tremendously invigorated the scope of natural pigments. In the present study, bacterial cultures were screened from environmental samples, and *Serratia nematodiphila* was identified as a potential prodigiosin producer. The pigment was characterized as prodigiosin on the basis of a presumptive acid test and observation of absorption maxima of acidified pigment at 535 nm. In order to increase prodigiosin production from the test isolate, various nutritional and physicochemical parameters were optimized. Experiments revealed that nutrient broth medium supplemented with 0.2% mannitol and 0.2% inorganic phosphate supported high pigment production. Optimum prodigiosin production was achieved when the nutrient broth media (with 1% NaCl) was adjusted to pH 8 and incubated at 25 °C for 48 h. The optimized media showed over 64.78 and 73.23% increase in prodigiosin production as compared to the nutrient broth and Luria Bertani broth, respectively. The present study indicated a great potential of *S. nematodiphila* to produce prodigiosin by minimum optimization of culture medium.

Key words: optimization, pigment, prodigiosin, *Serratia nematodiphila*, sustainable production.
Abbreviations: NA, nutrient agar; NB, nutrient broth.

Introduction

Colour and texture significantly contribute to the visual appeal of finished goods. Although the texture of products is primarily dependent on critical manufacturing processes, it is ultimately the colour that determines the acceptance of a product in the market. Therefore, many artificial synthetic colourants find extensive applications in food, pharmaceutical, textile and cosmetic industries (Kumar et al. 2017). Many of these synthetic pigments, however, pose a serious threat to the environment and can cause severe damage to living beings (Duran et al. 2002). Most commonly, these artificial pigments cause allergies, respiratory distress and cancer in humans (Kobylewski, Jacobson 2012). As a result, many synthetic dyes are banned from production and use based on their toxicity profile. As a result, the need for a sustainable production approach of environmentaly-safe pigments has increased considerably to meet the industrial demand (Babu, Shenolikar 1995).

Natural pigments are obtained mainly from plants or microorganisms (Yusuf et al. 2017). Although plant sources of pigments are viable options, they suffer from numerous drawbacks like instability to heat, light and pH, and low solubility as compared to microbial pigments (Kim et al. 1999). Moreover, in order to meet the industrial demand, valuable resources and land will be required for its cultivation. This can negatively affect the food and agriculture sector (Purnama et al. 2017). In contrast, the microbial sources of pigments offer several advantages like fast growth rate, use of economical raw material and availability throughout the year (Narsing Rao et al. 2017). Furthermore, there is an abundance of microorganisms in nature (fungi, yeasts, and bacteria) producing pigments like carotenoids, melanins, flavins, quinones and prodigiosins (Duffose 2009). Biosynthesized pigments can also serve as major chromophores, which, on further chemical modification, provide a broader spectrum of colours (Hobson et al. 1998). Thus, diverse shades of colour can be obtained from pigments produced by microorganisms, which are beneficial in the food, textile and cosmetic industries.

Common environmental isolates of bacteria of the genus *Serratia* are known to produce various secondary metabolites including a tripyrrole pigment prodigiosin (Duffose 2009). Pigments of the prodigiosin family are particularly in demand by various industries due to their remarkable and diverse range of biological effects (Venil, Lakshmanaperumalsamy 2009). They have been widely studied for cytotoxic activity (Furstner 2003; Darshan, Manonmani 2015). In addition, these pigments also act as potential antioxidants and antimicrobial agents (Lewis, Corpe 1964).

Colour stability under extremes of pH, temperature and other processing conditions is a prerequisite for the production of a natural pigment for commercial use. Relevant literature is available on pigment production and optimization. However, more in-depth studies are required to further enhance our understanding to support a sustainable pigment industry. In respect to prodigiosin production, both strain as well as species dependent variation in prodigiosin biosynthesis gene cluster was demonstrated (Harris et al. 2004). Moreover, every strain of bacteria is unique in its nutritional requirement for production of specific secondary metabolites, including pigments. For instance, a strain of *Serratia nematodiphila* RL2 utilized 1% lactose and 1% yeast extract as carbon and nitrogen sources, respectively, and metal-containing chemical factors such as uranyl acetate for optimum pigment production (Gondil et al. 2017). Moreover, certain metabolites require a complex media that is rich in a variety of nutrients, whereas others require nutrient-limiting conditions for their production. For example, a *Serratia marcescens* strain was observed to utilize fatty acids to produce prodigiosin pigment, whereas a soft rot fungus *Chlorociboria aeruginascens* required nitrogen-limiting conditions for production of a blue-green pigment xylindein (Stange et al. 2019). For these reasons, the optimization of pigment production is a crucial process to identify the factors that specifically promote or limit its production. These factors include media composition, carbon and nitrogen sources, as well as physicochemical parameters like pH, temperature, incubation conditions, initial culture density and aeration (Wei et al. 2005). The presence of specific micronutrients like iron and sea salts (Silverman, Munoz 1973) and glycerol (Williams, Green 1956) also affects pigment production, and hence should be evaluated with respect to efficient productivity from bacteria.

The aim of the present study was to optimize various growth parameters for efficient production of prodigiosin by a particular soil isolate of *S. nematodiphila*.

Materials and methods

Sample collection
Environmental samples like garden soil and leaf swabs were collected from K.B.P College, Vashi, India. Air sampling in the microbiology laboratory in K.B.P College was also carried out. Other soil samples were collected from the textile industrial area, petrol pumps and dumping grounds in Kalyan, Ulhasnagar, Shahad and Titwala. In addition, a mangrove sample was collected from Mahim creek, and another soil sample was obtained from West Bengal.

Isolation and screening of pigment producers
The isolation and screening of pigment producing organisms from the above samples were carried out on nutrient agar (NA) medium (pH 7). The obtained pigment producers were re-isolated on NA plates to confirm their

purity and single colonies were streaked on NA slants and stored at 4 °C until further use.

Presumptive color tests for prodigiosin
The presumptive test for characterization of prodigiosin was carried out as indicated by Gerber and Lechevalier (1976). The pigmented colonies obtained on NA plates were suspended in saline and re-isolated on sterile plates to confirm the purity of the isolate. The growth obtained after 48 h was scraped from the agar plates and suspended overnight in 95% ethanol at room temperature. The suspension was centrifuged at 5000 g_n for 15 min to remove the cell debris and the clear solution was acidified with a drop of concentrated hydrochloric acid. A red or pink color in the acidified solution indicated a positive, presumptive test for prodigiosin.

Identification of the isolate
Preliminary identification of the prodigiosin producing isolate was performed based on the observed colony characteristics that defined the cultural and morphological properties. The biochemical characteristics were studied further and classified by comparison of the obtained results with standard tests (Holt et al. 1994). The results of preliminary identification were confirmed with 16s rRNA characterization that was carried out at Chromous Biotech, Hyderabad.

Characterization of prodigiosin based on UV-visible spectrophotometry
Prodigiosin displays a characteristic absorption spectrum in acidified methanol, with a strong absorption peak at λ_{max} 535 nm. Hence, in order to confirm prodigiosin production the pigment producing cultures showing a positive presumptive test were grown in nutrient broth (NB) for 24 h at 30 °C. A 1 mL volume of sample was harvested and centrifuged at 13 000 rpm for 5 min. The supernatant was discarded and the pellet was re-suspended in acidified ethanol (4% 1M HCl in ethanol) to extract prodigiosin from the cells. Cell debris was removed by a second centrifugation step, and the supernatant was transferred to a cuvette (Hardjito et al. 2002). The absorption spectrum was measured using a UV-visible spectrophotometer Systronics 2203.

The quantification of prodigiosin production
The pigment producing cells were grown and harvested as described above. The cell free supernatant was subjected to spectral scanning in the range of 300 to 700 nm. The prodigiosin production was quantified using the following equation, and expressed as relative concentration of prodigiosin produced per cell (Slater et al. 2003):

$$\text{Prodigiosin unit} = [OD_{499} - (1.381 \times OD_{620})] / OD_{620} \times 1000,$$

where OD_{499} is pigment absorbance at 499 nm, OD_{620} is bacterial cell absorbance at 620 nm and 1.381 is a constant.

Optimization of nutritional and physicochemical parameters for prodigiosin production

Different environmental parameters have significant effect on prodigiosin production in the culture medium. Considering the complexity of mineral medium, its use was avoided in our study. Instead, basic NB medium that supported good growth of isolates was selected for optimization studies. The optimization of parameters was performed by using one factor at a time approach. In this technique, one parameter was varied at a time while keeping the others constant. To study different parameters, 0.5 mL of culture suspension was inoculated in NB and incubated at room temperature (30 °C) for 48 h. The physicochemical parameters studied were pH (5, 6, 7, 8), temperature (25, 30, 37, 45, 55 °C), NaCl concentration (0.5, 1, 2, 2.5%) and aeration (static or shaker) condition (Heinemann et al. 1970; Qadri, Williams 1972; Rjazantseva et al. 1994; Sole et al. 1994).

The nutritional parameters were studied using different carbon (0.2% lactose, xylose, mannitol and glucose) and nitrogen (yeast extract, peptone, tryptophan, beef extract and ammonium sulphate) sources (Wei, Chen 2005). In addition, the effect of various amino acids (0.1% threonine, lysine, leucine, tryptophan, tyrosine, glutamate, glutamine, aspartate, asparagine, proline, phenylalanine, cysteine, isoleucine, arginine, methionine, alanine, histidine, serine, valine, and glycine) and phosphate sources ($CaSO_4$, NaH_2PO_4, $Co_3(PO_4)_2$ and K_2HPO_4) was also studied (Qadri, Williams 1973; Lawanson, Sholeye 1976; Cang et al. 2000; Slater et al. 2003).

The effect of amino acids was first checked qualitatively by disk diffusion method. Shortly, the sterilized filter paper discs were dipped in the above mentioned amino acid solutions (0.1%) and placed on M9 minimal salt agar medium (with 0.5% glucose) swabbed with test isolate. The amino acids supporting pigmentation were further studied to estimate the exact concentration required for optimized pigment production.

To determine efficiency of prodigiosin production in optimized media, it was compared with two standard media, i.e., NB and Luria Bertani broth.

Statistical analysis

All the readings were carried out in sets of triplicates and the results were reported as mean ± standard deviation.

Table 1. Pigment-producing bacteria isolated in the present study

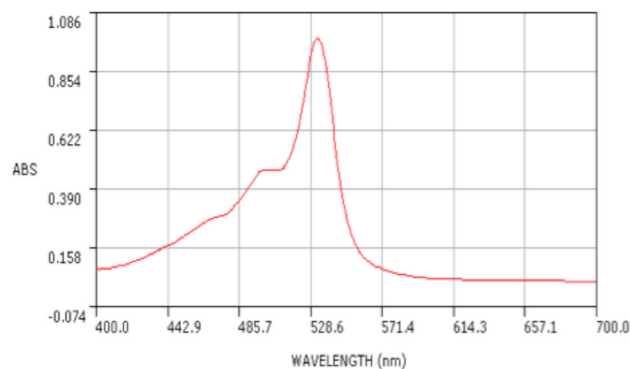| Sample No.	Color	Sample source
S01A	Bright orange	Air
S02G	Dull orange	Garden soil
S03U	Pinkish red	Ulhas river
S04K	Magenta	Kalyan soil
S05W	Orange	West Bengal soil
S06M	Orange	Mangrove sample

Fig. 1. Absorption of prodigiosin showing a peak at 535 nm.

The distribution of variables was evaluated by one-way ANOVA to assess the significance of differences between mean values at 95% confidence interval ($p < 0.05$).

Results

Six pigment-producing bacterial isolates were obtained in our study. The colour and source of these isolates are shown in Table 1. Distinct shades of orange to magenta red colonies were observed on NA plates. Among these, the presumptive test for prodigiosin was positive only for the pigment produced by isolate S04K. The pigment produced by this isolate also showed absorption maxima at 535 nm (Fig. 1) further indicating it to be prodigiosin. Hence further studies were carried out using this isolate.

Table 2 summarizes the colony morphology and biochemical characteristics of prodigiosin producing

Table 2. Morphological and biochemical characteristics of prodigiosin-producing bacterial isolate S04Ks

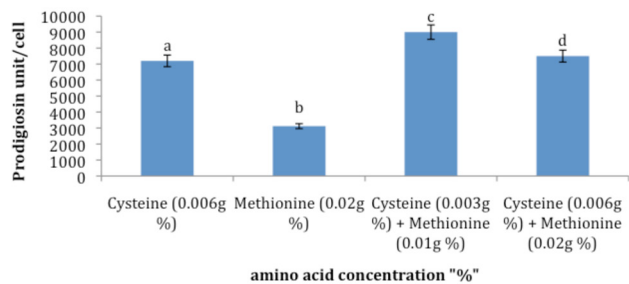| Parameter	Characteristic
Gram nature, motility and general morphology	Gram negative motile short rods
Size and color	1 mm, magenta red
Shape and consistency	Circular and smooth
Elevation	Raised
Opacity	Translucent
Margin	Irregular
Oxidase	Negative
Catalase	Positive
Sugar fermentation with acid prodction	Maltose, sorbitol, gkucose, sucrose, inulin, galactose, dextrin, mannitol
No fermentation	Glycerine, xylose, starch
Positive biochemical tests	Voges Prausker, lysine decarboxylase, gelatin hydrolysis, nitrite reductase
Negative biochemical tests	Indole, methyl red, phenylanaline deaminase

Fig. 2. Effect of pH on prodigiosin production by *S. nematodiphila* in nutrient broth. Different letters indicate statistically significant differences between groups (mean ± SD, *n* = 3, one way ANOVA, *p* < 0.05).
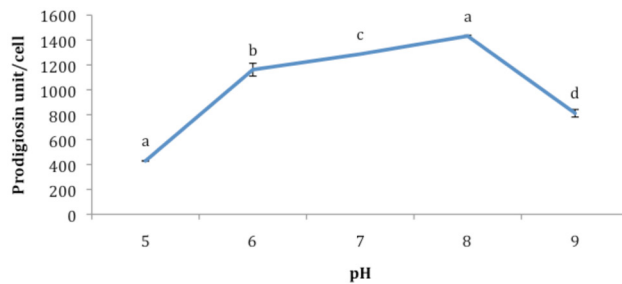
Fig. 3. Effect of temperature on prodigiosin production by *S. nematodiphila* in nutrient broth. Different letters indicate statistically significant differences between groups (mean ± SD, *n* = 3, one way ANOVA, *p* < 0.05).

isolate S04K. This isolate showed 98% homology to the *S. nematodiphila* strain, as identified by 16s rRNA technique.

Figs. 2 to 5 show the optimized physicochemical parameters for prodigiosin production by *S. nematodiphila*. isolate S04K. The differences in mean values represented in these graphs were found to be statistically significant at *p* < 0.05. It was observed that pH 8, temperature 25 °C, 1% NaCl concentration and static conditions were optimal for prodigiosin production when *S. nematodiphila* was cultivated in NB.

Results from optimization of carbon and phosphate sources for prodigiosin production by *S. nematodiphila* are shown in Figs. 6 and 7, respectively. The differences in mean values represented in both graphs were found to

be statistically significant at *p* < 0.05. It was observed that addition of 0.2% mannitol and 0.2% cobalt phosphate to previously optimized NB medium further improved prodigiosin production by *S. nematodiphila*.

The effect of various amino acids on prodigiosin production was observed qualitatively on NA plates using the disc method. Among the amino acids used, methionine and cysteine did not inhibit prodigiosin production at 0.1% concentration. Hence optimized concentrations of these amino acids were further determined in our study. Figs. 8 and 9 represent the results for cysteine and methionine concentrations, respectively, required for optimum prodigiosin production. Effect of incorporation of both these amino acids simultaneously in the production
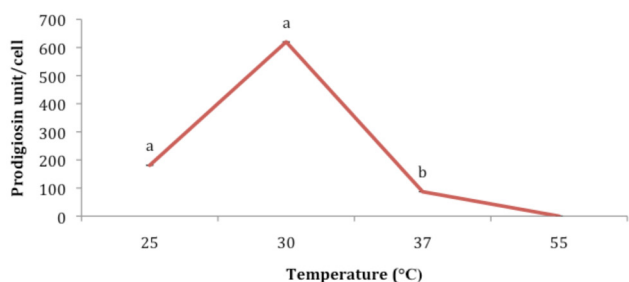

Fig. 4. Effect of NaCl concentration on prodigiosin production by *S. nematodiphila* in nutrient broth. Different letters indicate statistically significant differences between groups (mean ± SD, *n* = 3, one way ANOVA, *p* < 0.05).
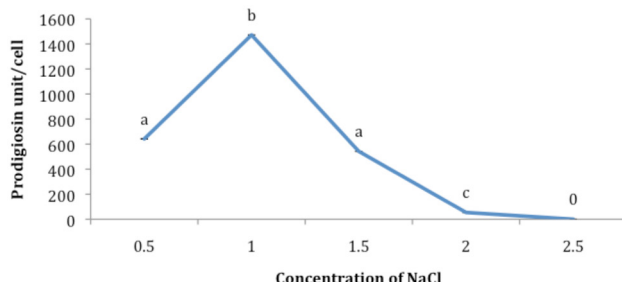
Fig. 5. Effect of aeration condition on prodigiosin production by *S. nematodiphila* in nutrient broth. Different letters indicate statistically significant differences between groups (mean ± SD, *n* = 3, one way ANOVA, *p* < 0.05).
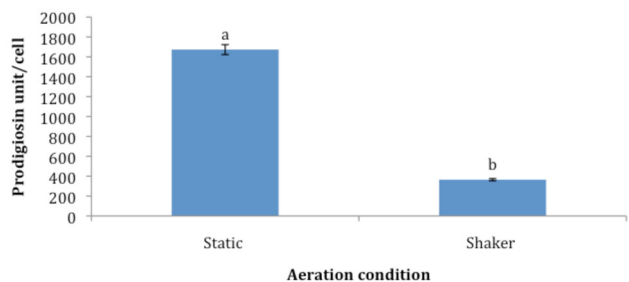

Fig. 6. Effect of carbon sources on prodigiosin production by *S. nematodiphila* in nutrient broth. Different letters indicate statistically significant differences between groups (mean ± SD, *n* = 3, one way ANOVA, *p* < 0.05).
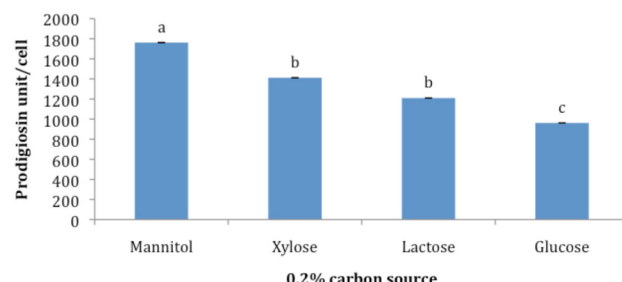
Fig. 7. Effect of phosphate sources on prodigiosin production by *S. nematodiphila* in nutrient broth containing 0.2% mannitol. Different letters indicate statistically significant differences between groups (mean ± SD, *n* = 3, one way ANOVA, *p* < 0.05).
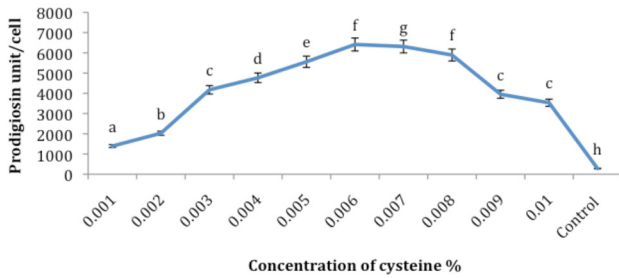
Fig. 8. Effect of concentration of cysteine on prodigiosin production by *S. nematodiphila* in nutrient broth containing 0.2% mannitol and 0.2% cobalt phosphate. Different letters indicate statistically significant differences between groups (mean ± SD, n = 3, one way ANOVA, $p < 0.05$). .
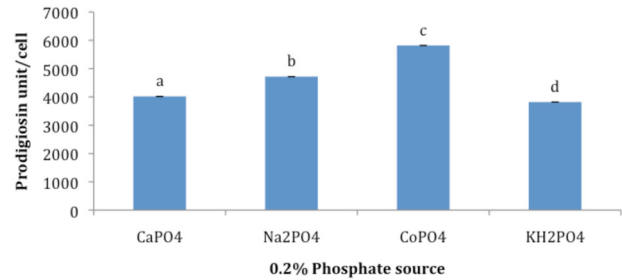

Fig. 9. Effect of concentration of methionine on prodigiosin production by *S. nematodiphila* in nutrient broth containing 0.2% mannitol and 0.2% cobalt phosphate. Different letters indicate statistically significant differences between groups (mean ± SD, n = 3, one way ANOVA, $p < 0.05$).

medium is shown in Fig. 10. The differences in mean values represented in all three graphs were found to be statistically significant at $p < 0.05$. It was observed that 0.006% cysteine and 0.02% methionine considerably improved prodigiosin production. However, when added together in optimum concentrations, it showed a decrease in prodigiosin production. Better production was obtained when the concentration of both amino acids was reduced by half.

Results from comparison of prodigiosin production in NB, LB and optimized medium is shown in Fig. 11. The differences in mean values represented in this graph were found to be statistically significant at $p < 0.05$. The pigment production in optimized media showed 64.78 and 73.23% increase as compared to standard NB and LB medium, respectively.

Discussion

The production of prodigiosin is mainly associated with, but not limited to, *S. marcescens* (Furnster 2003). The cultural and biochemical characterization of cultures remains the gold standard for preliminary identification. Also, these findings are best confirmed with the help of gene sequencing techniques, including 16s rRNA. Although the API system has become increasingly common and widely used for identification of bacteria (Janda, Abbott 2002), it does not

show absolute sensitivity in bacterial identification. A study reported correct identification of 85% of *S. marcescens* isolates using the API 20E system (Hejazi et al. 1997). This was mainly due to the limited sugar fermentation codes available in this system. For instance, the differentiating characteristic in the identification of *S. marcescens* and *Serratia liquefaciens* strains is the fermentation of arabinose. The former is unable to ferment arabinose in peptone water, whereas the latter shows an arabinose positive test. Hence, sugar fermentation tests, including arabinose and raffinose, should ideally be carried out for identification of *Serratia* sp., irrespective of the API results (Hejazi et al. 1997).

Prodigiosin is a secondary metabolite and typically appears in the later stages of bacterial growth (Harris et al. 2004). Various physicochemical factors critically influence the production of prodigiosin (Williamson et al. 2005). Characteristically, prodigiosin is produced in slightly alkaline culture conditions and low temperatures. In fact, temperatures higher than 37 °C considerably inhibit prodigiosin production (as observed in the current study). This is one of the main reasons why most of the pathogenic strains of *S. marcescens* are non-pigmented (Roy et al. 2014).

Previously, a differential and selective media i.e., capryllate thallous agar medium, has been used for isolation of *Serratia* sp. This medium contains caprylate as a carbon source, which is readily utilized by *Serratia* sp., and thallous
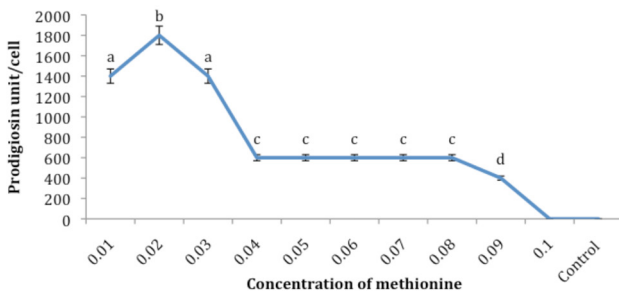

Fig. 10. Effect of concentration of amino acids on prodigiosin production by *S. nematodiphila* in nutrient broth containing 0.2% mannitol and 0.2% cobalt phosphate. Different letters indicate statistically significant differences between groups (mean ± SD, n = 3, one way ANOVA, $p < 0.05$).
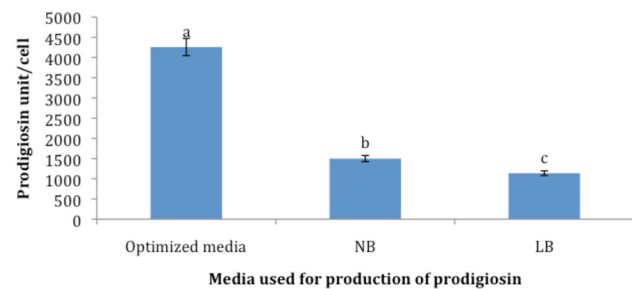

Fig. 11. Comparison of media for prodigiosin production by *S. nematodiphila*. Different letters indicate statistically significant differences between groups (mean ± SD, n = 3, one way ANOVA, $p < 0.05$).

salts that inhibit other organisms (Staunton, Wilkinson 1997). However, prodigiosin production has found to be ideal in a standard or slightly modified nutrient medium, as compared to other media (Pryce, Terry 2000; Samrot et al. 2011; Pradeep et al. 2013). Hence, NB was used for the optimization of prodigiosin in the present study.

The current study indicated optimum prodigiosin production by *S. nematodiphila* in NB (pH 8) containing 1% NaCl, on incubation at 25 °C for 48 h under static conditions. Similar to our findings, the optimum pH for prodigiosin production was reported to be between 8 and 8.5 by *S. marcescens* (Sole et al. 1994). They further reported that the prodigiosin production was independent of initial pH in un-buffered medium. The variations in prodigiosin production were noted only when buffers were used in cultures of non-proliferating cells. A recent study on optimization of parameters for production of prodigiosin by *S. nematodiphila* RL2 reported maximum production at 35 °C in 46 to 48 h in nutrient broth medium (pH 6 to 7) supplemented with 1% lactose, yeast extract and uranyl acetate (Gondil et al. 2017). However, pH 7, inoculum concentration 5%, temperature 30 °C and incubation period of 36 h were reported to be optimum for prodigiosin production by *S. marcescens* MBB05 (Pradeep et al. 2013). Similarly, the optimal condition for pigment production by another *S. marcescens* strain was found to be in NB (pH 7) at 28 °C after 72 h (Samrot et al. 2011).

In contrast to our observation, another study reported optimum production of prodigiosin under aeration conditions (Heinemann et al. 1970). Similarly, shaker conditions (150 rpm) and a lower concentration of NaCl (0.4%) were found to be ideal for prodigiosin production by another strain of *S. marcescens* (Vijayalakshmi, Jagathy 2016). The optimum temperature (25 °C) and incubation time (30 h), however, were similar to those observed in our study. A halophilic *Serratia* sp. BTWJ8 has been also reported to grow and produce prodigiosin at up to 5.8% NaCl concentration (Krishna 2008).

Apart from physicochemical and growth conditions, nutritional parameters including carbon and nitrogen sources, micronutrients and amino acids are also important for production of secondary metabolites in bacteria. In the current study, 0.2% mannitol, 0.2% cobalt phosphate, 0.006% cysteine and 0.02% methionine considerably improved prodigiosin production.

In a previous study, 2% sodium oleate resulted in optimum prodigiosin production by *S. marcescens* (Khanafari et al. 2006). Another study reported 1.5% dextrose, 1% yeast extract to be ideal carbon and nitrogen sources. However, maximum pigment production was observed using 5% peanut oil cake (Vijayalakshmi, Jagathy 2016). An interesting observation was reported by Giri et al (2004). In NB, the maximum prodigiosin production was obtained at 28 and 30 °C, and it was completely inhibited at 37 °C. However, when powdered peanut broth was used as a

carbon source for prodigiosin production by *S. marcescens*, pigment production was observed even at 37 °C. Moreover, it was equal to the amount of pigment produced at 30 °C in NB. In another study, powdered peanut seed broth at 37.6 mg mL^{-1} and powdered sesame seed broth at 16.5 mg mL^{-1} showed optimized pigment production by *S. marcescens*, as compared to nutrient broth with concentration 0.51 mg mL^{-1} (Shahitha, Poornima 2012).

In our study, the presence of nitrogen sources supported growth of culture but pigment production was very low as compared to non-optimised NB media. This may be because NB contains peptone as a nitrogen source, and further addition of other nitrogen sources may have a negative effect on pigment production. Hence optimization of nitrogen sources was not carried out in our study. In another study, 0.4% ram horn peptone along with mannitol showed maximum production of prodigiosin by *S. marcescens* after 48 h (Kurbanoglu et al. 2015). *S. marcescens* MN5 also showed enhanced production of prodigiosin at 22 °C and pH 9 on the addition of 1% (w/v) peptone and crude glycerol as nitrogen and carbon sources, respectively (Elkenawy et al. 2017). The presence of inorganic phosphates effectively increased the synthesis of prodigiosin by non-proliferating cells of *S. marcescens* at a concentration of 0.3 mM (Whitney et al. 1977). Other studies also reported positive effects of diammonium phosphate (Sumathi et al. 2014) and mono- and di-potassium phosphate (Domrose et al. 2015) on prodgiosin production. In addition, phosphates have also been reported to play an important role in biosynthesis of prodigiosin in *Serratia* sp. (Slater et al. 2003).

Although low concentrations of amino acids positively influence prodigiosin production, higher concentrations are associated with inhibition of pigment production. This is probably due to the diversion of excess amino acids to act as repressors in prodigiosin biosynthesis pathways (Qadri et al. 1973). This was further supported by a study carried out by incorporating both amino acids in growth media, and studying their effect on prodigiosin production (Fig. 10). Interestingly, increased production of prodigiosin was obtained only when the concentrations of both amino acids were reduced by half. This indicates that prodigiosin production is dependent on specific amino acids, as well as the total concentration of different amino acids. Similar to our findings, another study also reported methionine and cysteine to effectively increase prodigiosin production at 0.01 and 0.03% concentration, respectively, in presence of 0.4% mannose and 0.1% NaCl in M9 medium (pH 8) at 28 °C in 24 h (Bharmal et al. 2012). Key roles of other amino acids i.e. thiamine, alanine, histidine and proline in the formation of the monopyrrole moiety of prodigiosin were also suggested (Goldschmitt, Williams 1968; Lim et al. 1976). The incorporation of proline in the biosynthetic pathway of prodigiosin was suggested (Scott et al. 1976).

The basic aim of optimization studies is to improve the

metabolite yield by bacteria. In the current study, 64.78 and 73.23% increase in prodigiosin production was observed as compared to standard NB and LB medium, respectively. Similarly, a 65% increase in prodigiosin production by *S. nematodiphila* RL2 in optimized medium as compared to nutrient broth was reported (Gondil et al. 2017). Considerable increase (over three times) in prodigiosin production by *S. marcescens* strain MSK1 has also been reported in optimized M9 medium (Bharmal et al. 2012). Another study reported over 1.5 to 3 times increase in prodigiosin production in optimized media containing casein rich broth and vegetable oils, as compared to nutrient broth and glycerol yeast broth (Parani, Saha 2008).

Conclusions

Prodigiosin production in *Serratia* sp. is dependent on nutritional as well as physicochemical parameters that are unique to every strain isolated from the environment. For instance, methionine and cysteine do not necessarily induce or improve pigment production in other strains of *S. nematodiphila*. Also, the amino acids selected in our study, on the basis of literature survey, did not support prodigiosin production by *S. nematodiphila*. This suggests that either a novel derivative of prodigiosin may be produced by the test strain, or that it follows an unique biosynthesis pathway for prodigiosin production. Further studies are hence required to study the biosynthetic pathway of prodigiosin by *S. nematodiphila* to obtain more insights into the intermediates of the pathway that will help us in biochemical characterization of the pigment. The optimized media showed over 60% increase in prodigiosin production in our study, indicating good potential of the test isolate for application in industries that use a sustainable approach for production of raw materials.

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