

Optimization of Growth Conditions of *Serratia marcescens* for Prodigiosin production

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Abstract

This study examined the optimization of growth conditions of *Serratia marcescens* isolated from a loamy soil of the Federal University Technology Minna Nigeria, for prodigiosin production. The different optimized conditions examined were extraction solvents, incubation time, pH, temperature, carbon sources, organic nitrogen sources, inorganic nitrogen sources and agitation levels. The result reveals that methanol is an ideal solvent recording absorbance of 0.69nm, which is slightly followed by ethanol and acetone with absorbance of 0.4nm and 0.3nm respectively. The bacterium produced maximum level of pigment after 36 hours of incubation (22.20µg/L), although pigment production was observed from 12 hours of incubation onwards (7.40µg/L). The pigment production declined after 36 hours of incubation. The results also revealed that pigment production by *S. marcescens* was maximum at 25°C (25.10µg/L) followed by 30°C (22.50µg/L). The optimal production was obtained at pH 7 (25.00mg/ml) with dextrose as optimal source of carbon (22.40mg/ml). There was no pigment production at static condition but maximum pigment production was recorded at 150 rpm (22.50µg/L). All the inorganic nitrogen sources caused a reduced biomass production. Among the organic nitrogen sources tested, yeast extract supported maximum pigment (26.75µg/L) but peptone led to a decreased pigment production (9.15µg/L) compared to other organic sources. Addition of urea did not support pigment as well as biomass production. Statistical analysis shows significant differences ($p < 0.05$) in prodigiosin productions with different substrates, temperature and agitation levels. The study revealed that the production of prodigiosin was significantly influenced by the extraction solvents, incubation time, pH, temperature, carbon sources, organic nitrogen sources, inorganic nitrogen sources and agitation levels.

Keywords: Prodigiosin, solvents, substrates, agitation, incubation conditions, and *Serratia marcescens*.

INTRODUCTION

Prodigiosin is a tripyrrole metabolite first characterized from *Serratia marcescens*, which forms beautiful pillar box red colonies. Its name is derived from “prodigious”- something marvelous. They have a common pyrrolydipyrrolylmethene skeleton (Azuma *et al.*, 2000; Bennett and Bentley, 2000; Moraes *et al.*, 2009). These pigments are emerging as a novel group of compounds having distinct antibacterial, antifungal, antimycotic, immunomodulating, anti-tumor, antibacterial, antimalarial, and nuclease activities (Azuma *et al.*, 2000; Bennett and Bentley, 2000; Moraes *et al.*, 2009). A wide variety of bacterial taxa, including *Serratia rubidaea*, *S. marcescens*, *Vibrio gazogenes*, *Alteromonas rubra*,

Rugamonas rubra, *Streptoverticillium rubrireticuli* and *Streptomyces longisporus ruber* produce prodigiosin and/or derivatives of this molecule (Chidambaram and Perumalsamy, 2009).

Numerous types of differential and selective media have been used for prodigiosin production however, regular prodigiosin production has been carried out in nutrient broth containing sesame seeds, peanut seed broth, maltose broth, peptone glycerol broth (Giri *et al.*, 2004), and a medium designed by Nakamura and Kitamura (1981) containing 2% sodium oleate and also oleic acid substitution instead of sodium oleate and triolein alone as substrate and produced a yield of 0.69mg/mL prodigiosin.

Report by Giri *et al.* (2004) indicated that the initial comparative work was carried out using powdered sesame seed in water, nutrient broth and peptone glycerol broth as a growth medium for *S. marcescens*. After having observed that sesame seed gives a better yield in term of prodigiosin biosynthesis further comparison was done with readily available cheaper and cost effective sources like peanut and coconut. The aim of this study therefore was to optimize conditions for optimum production of prodigiosin using *Serratia marcescens* obtained from the Microbiology Laboratory, Federal University of Technology Minna, Nigeria.

MATERIALS AND METHODS

Composition and Preparation of Media used for Growth and Prodigiosin Production

Nutrient broth [peptone 10 g/l, sodium chloride 5 g/l, yeast extract 3 g/l], nutrient broth with 0.5% maltose, nutrient broth with 0.5% glucose, peptone glycerol broth [meat extract 10 g/l, peptone 10 g/l, glycerol 10%], 2% powdered sesame seed in distilled water, 2% powdered sesame seed with 0.5% maltose in distilled water, 2% powdered sesame seed with 0.5% glucose in distilled water, 2% powdered peanut seed in distilled water, and 2% peanut seed broth in distilled water, were the different media used for the production of pigment from the *Serratia marcescens* isolated. Fifty millilitre (50 ml) of each of the broth was prepared in 250 ml glass conical flasks. The pH of all the above media were maintained at 7.0. The various media were autoclaved at 120°C for 15 minutes. Maltose and glucose was filter sterilised and added to the respective media. Peanut and sesame obtained from Minna Market were crushed in a mixer and then sieved to fine particles before preparing the broth. The experiment was conducted in triplicates.

Optimization of Bioprocess Variables for Pigment Production by *Serratia marcescens*

Various physico-chemical and bioprocess variables that influence pigment production by bacterium under submerged fermentation (SmF) were optimized towards achieving maximal pigment production using Peanut broth medium. Strategy adopted for the optimization was to evaluate individually the effect of different parameters, through "one-variable-at-a-time" approach, on pigment production under SMF, and perform a time course experiment under optimized conditions. The parameters optimized included incubation time, incubation temperature, initial pH of the medium, agitation, effect of different carbon and

nitrogen sources, concentration of calcium chloride and inoculum.

Selection of Suitable Solvent for Extracting the Bacterial Pigment

The solvent that could support maximal yield of pigment on extraction of culture broth was standardized, using different solvents viz; ethanol, acetone, methanol, petroleum ether, ethyl acetate, chloroform, hexane, diethyl ether and distilled water.

Effect of Varying Incubation Time on Pigment Production

Optimal incubation time for maximal pigment production was determined by incubating the inoculated media for a total period of 24, 48, and 72 hours at room temperature ($28 \pm 2^\circ\text{C}$) and analyzing the samples after every 24 hours for pigment production.

Effect of Varying Incubation Temperature on Pigment Production

Optimal incubation temperature for maximal pigment production was evaluated by incubating the inoculated media at various temperatures (15°C , 20°C , 25°C , 30°C , 40°C and 50°C), and determining the pigment production after the optimum incubation time.

Effect of Varying pH of the Medium on Pigment Production

Initial pH of the medium that could support maximal pigment production was determined by adjusting the pH of the medium to various levels (i.e., pH 5, 6, 7, 8,9,10, 11) using 1 N NaOH and determining the pigment production after the optimum incubation time.

Effect of Varying Agitation on Pigment Production

Effect of agitation on pigment production was studied by incubating the inoculated media taken in conical flasks on an orbital shaker at different agitation levels (50, 100, 150, 200 rpm) and the pigment production was determined after 48 hour of incubation. Control experiment was done by incubating the inoculated media at static condition.

Effect of Varying Carbon Sources on Pigment Production

Effect of carbon sources on pigment production was studied by the addition of dextrose, fructose, galactose, xylose, mannose, glycerol, starch, mannitol and maltose at 2 % (w/v) each.

Control experiment was set up with sucrose as carbon source 2 % (w/v). Pigment production was determined after the optimum incubation time.

Effect of Varying Organic Nitrogen Sources on Pigment Production

Effect of organic nitrogen sources on pigment production was studied using 1 % (w/v) yeast extract, beef extract, peptone, malt extract, and urea, and tryptone. Medium without any nitrogen source was used as control. Pigment production was determined after the optimum incubation time.

RESULTS

Suitable Solvent for Extraction of Pigment from *Serratia marcescens*

The result presented in Figure 1 shows the suitable solvent for extracting pigment from *S. marcescens*. The result reveals that methanol is an ideal solvent recording absorbance of 0.69nm which is slightly followed by ethanol, and acetone with absorbance of 0.4nm and 0.3nm respectively.

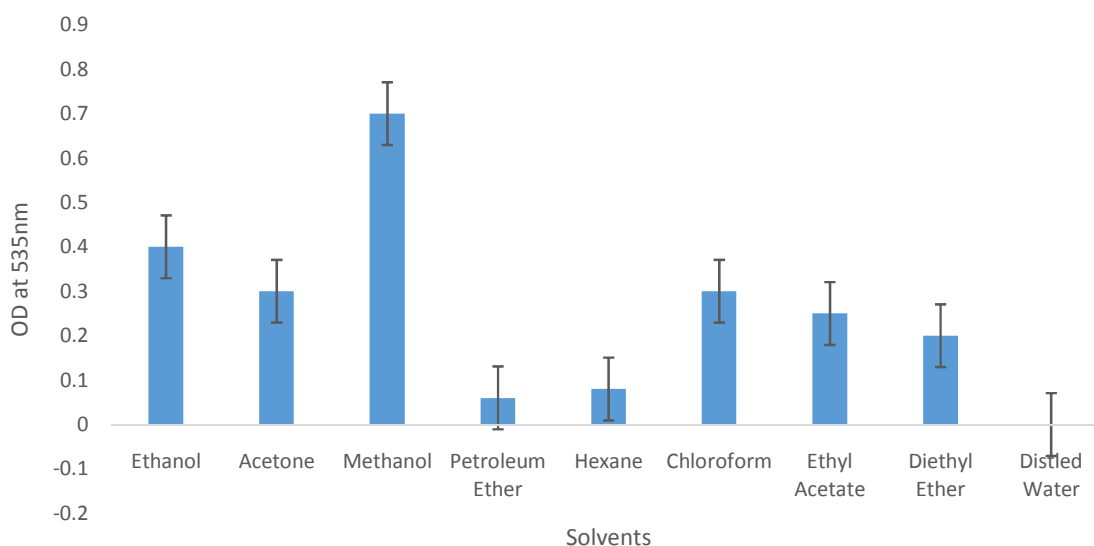


Fig. 1. Suitable solvent for extracting pigment from *Serratia marcescens*.

Effect of Incubation Time on Pigment Production by *Serratia marcescens*

The results in Figure 2 show that the bacterium produced maximum level of pigment after 36 hours of incubation (22.20µg/L) although pigment production was observed from 12 hours of incubation onwards (7.40µg/L). However, the pigment production declined after 36 hours

of incubation. The biomass production was also maximum at 36 hours of incubation (2.10g/L) although a gradual decrease in biomass was observed during 48 to 60 hours of incubation. Hence, 36 hours of incubation was considered as optimum for maximal production of pigment by *Serratia marcescens*.

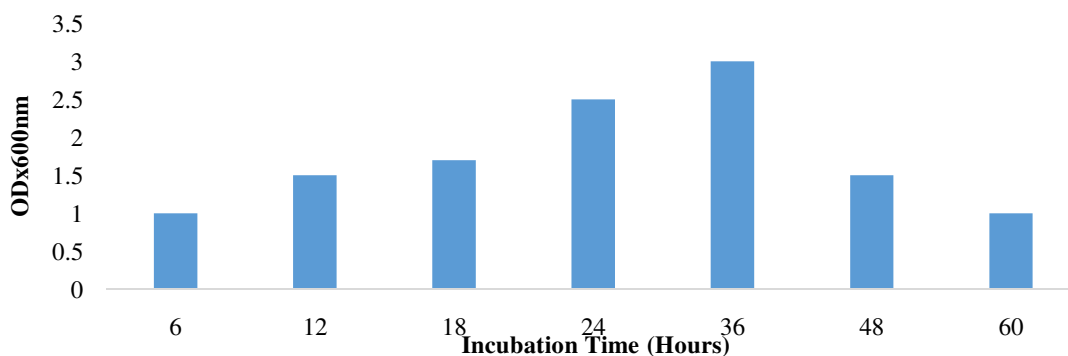


Fig.2. Effect of incubation time on pigment production by *Serratia marcescens*:

Optimization of Incubation Temperature on Pigment Production by *Serratia marcescens*

Results presented in Figure 3 show that pigment production by *Serratia marcescens* was maximum at 25°C (25.10 µg/L) followed by 30°C (22.50 µg/L). In general, it was observed that *Serratia marcescens* could produce the pigment at all incubation temperatures tested.

There were significant differences between various temperatures and also between pigment and biomass production (p value= 0.00028). Incubation temperatures above 30°C led to a rapid decline in pigment production. Biomass production was maximum at 25°C (2.5 g/L) and a gradual decrease was recorded after 72 h of incubation.

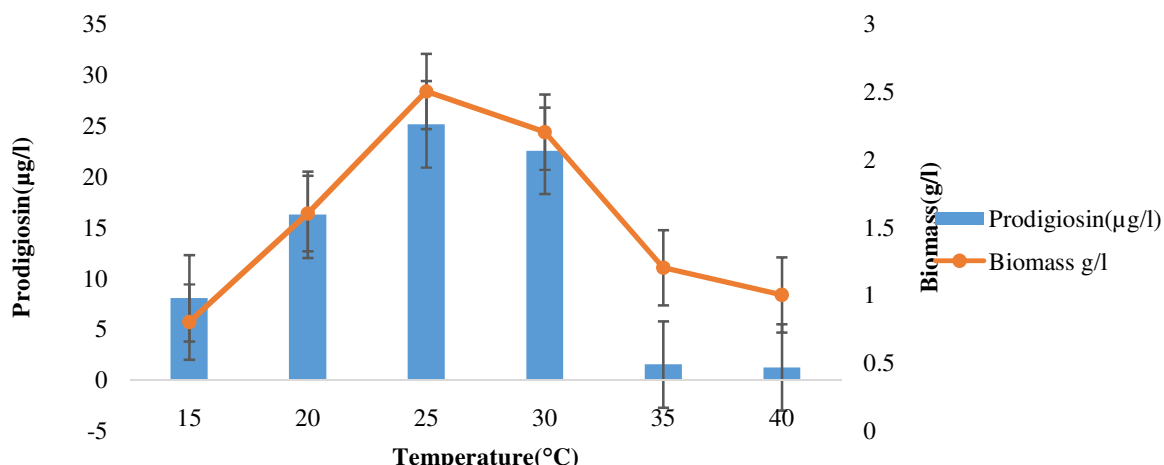


Fig. 3. Effect of incubation temperature for pigment production by *Serratia marcescens*.

The result presented in Table 1 shows production of prodigiosin at pH 6 with highest pigment production observed in peanut seed broth media at 72 h of incubation (22.32mg/mL) followed by sesame seed broth

(8.40mg/mL). The lowest production was observed with peptone glycerol broth (0.80mg/mL). Statistically there was significant difference between peanut seed broth with other medium used $p < 0.05$.

Table 1. Growth and production of prodigiosin by *Serratia marcescens* at pH 6 in different media for the period of 120 hours

Media	Time (h)				
	24	48	72	96	120
Nutrient Broth with glycerol	1.28±0.17 ^{ab}	1.36±0.17 ^b	1.50±0.24 ^a	1.48±0.50 ^a	1.42±0.09 ^{ab}
Sesame seed broth with 0.5% maltose	6.31±0.75 ^c	7.20±0.82 ^c	8.40±0.67 ^b	8.20±0.74 ^b	7.40±0.51 ^c
Peanut seed with maltose	1.33±0.34 ^{ab}	1.45±0.21 ^b	1.60±0.18 ^a	1.50±0.73 ^a	1.48±0.12 ^{ab}
Nutrient Broth with 0.5% glucose	1.31±0.50 ^{ab}	1.34±0.09 ^b	1.50±0.24 ^a	1.48±0.31 ^a	1.44±0.45 ^a
Peanut seed broth	18.30±1.58 ^d	20.42±1.75 ^d	22.32±2.04 ^c	22.10±2.11 ^c	21.00±1.59 ^d
Nutrient Broth with 0.5% maltose	1.52±0.41 ^b	1.66±0.11 ^b	1.83±0.12 ^a	1.72±0.62 ^a	1.68±0.15 ^b
Peptone glycerol broth	0.51±0.08 ^a	0.62±0.05 ^a	0.80±0.05 ^a	0.71±0.04 ^a	0.65±0.06 ^a

Values are Mean±Standard Error of Mean of triplicate determinations. Values with different alphabets are significantly different ($p < 0.05$) along the column

The result documented in Table 2 reveals production of prodigiosin at pH 7 with highest pigment production observed in peanut seed broth media at 72 h of incubation (25.10mg/mL) followed by sesame seed broth (9.45mg/mL) within the same incubation period. The least production was observed with

peptone glycerol broth (1.00mg/mL). The reason could be as a result of higher percentage of fatty acid in peanut and sesame seed broth which enhanced more pigment formation than sugar. There was significant difference between peanut seed broth with other medium used $p < 0.05$.

The result presented in Table 3 shows production of prodigiosin at pH 8 with highest pigment production observed in peanut seed broth media at 72 h of incubation (24.10mg/mL) followed by sesame seed broth (9.40mg/mL). The least production was observed with peptone glycerol broth and nutrient broth with glycerol (0.90 and 1.52mg/mL) respectively. There was significant difference between peanut seed broth with other medium used $p < 0.05$.

The result presented in Table 4 reveals production of prodigiosin at pH 9 with highest pigment production observed in peanut seed broth media at 72 h of incubation (20.10mg/mL) followed by sesame seed broth (9.20mg/mL). The lowest production was observed with peptone glycerol broth (0.88mg/mL). The result shows significant difference between peanut seed broth with other medium used $p < 0.05$.

Table 2. Growth and production of prodigiosin by *Serratia marcescens* at pH of 7 in different media for the period of 120 hours

Media	Time (h)				
	24	48	72	96	120
Nutrient Broth with glycerol	1.42±0.11 ^b	1.52±0.13 ^b	1.52±0.19 ^a	1.51±0.30 ^a	1.46±0.22 ^b
Sesame seed broth with 0.5% maltose	6.51±0.43 ^c	7.75±1.04 ^c	9.45±1.60 ^b	8.61±1.05 ^b	8.31±0.96 ^c
Peanut seed with maltose	1.45±0.55 ^b	1.45±0.21 ^b	1.60±0.18 ^a	1.50±0.73 ^a	1.60±0.12 ^b
Nutrient Broth with 0.5% glucose	1.44±0.42 ^b	1.55±0.15 ^b	1.63±0.35 ^a	1.53±0.42 ^a	1.50±0.31 ^b
Peanut seed broth	18.40±1.30 ^d	20.48±1.52 ^d	25.10±1.75 ^c	22.20±2.01 ^c	19.20±1.76 ^d
Nutrient Broth with 0.5% maltose	1.71±0.30 ^b	1.82±0.11 ^b	1.83±0.31 ^a	2.00±0.16 ^a	1.82±0.17 ^b
Peptone glycerol broth	0.56±0.04 ^a	0.72±0.08 ^a	1.00±0.07 ^a	0.90±0.05 ^a	0.81±0.06 ^a

Values are Mean±Standard Error of Mean of triplicate determinations. Values with different alphabets are significantly different ($p < 0.05$) along the column

Table 3. Growth and production of prodigiosin by *Serratia marcescens* at pH of 8 in different media for the period of 120 hours.

Media	Time (h)				
	24	48	72	96	120
Nutrient Broth with glycerol	1.38±0.25 ^b	1.46±0.22 ^b	1.52±0.19 ^a	1.51±0.30 ^a	1.46±0.22 ^b
Sesame seed broth with 0.5% maltose	6.41±0.63 ^c	7.70±1.14 ^c	9.40±1.20 ^b	8.50±1.11 ^b	8.20±1.06 ^c
Peanut seed with maltose	1.43±0.48 ^b	1.55±0.28 ^b	1.72±0.23 ^a	1.60±0.51 ^a	1.51±0.43 ^b
Nutrient Broth with 0.5% glucose	1.41±0.50 ^b	1.51±0.18 ^b	1.60±0.22 ^a	1.52±0.47 ^a	1.50±0.62 ^b
Peanut seed broth	18.30±1.09 ^d	20.46±1.44 ^d	24.10±2.01 ^c	22.10±2.11 ^c	19.00±1.59 ^d
Nutrient Broth with 0.5% maltose	1.62±0.60 ^b	1.72±0.31 ^b	1.92±0.45 ^a	1.80±0.11 ^a	1.70±0.15 ^b
Peptone glycerol broth	0.54±0.05 ^a	0.67±0.06 ^a	0.90±0.03 ^a	0.81±0.06 ^a	0.71±0.02 ^a

Values are Mean±Standard Error of Mean of triplicate determinations. Values with different alphabets are significantly different ($p < 0.05$) along the column

Table 4. Growth and production of prodigiosin by *Serratia marcescens* at pH of 9 in different media for the period of 120 hours.

Media	Time (h)				
	24	48	72	96	120
Nutrient Broth with glycerol	1.36±0.20 ^b	1.44±0.22 ^b	1.53±0.11 ^a	1.53±0.21 ^a	1.34±0.36 ^b
Sesame seed broth with 0.5% maltose	6.40±0.54 ^c	7.66±1.20 ^c	9.20±1.42 ^b	8.40±1.07 ^b	8.10±1.14 ^c
Peanut seed with maltose	1.40±0.40 ^b	1.51±0.31 ^b	1.70±0.25 ^a	1.58±0.55 ^a	1.49±0.61 ^b
Nutrient Broth with 0.5% glucose	1.38±0.34 ^b	1.48±0.13 ^b	1.59±0.27 ^a	1.48±0.39 ^a	1.45±0.38 ^b
Peanut seed broth	17.20±1.15 ^d	18.20±1.06 ^d	20.10±1.70 ^c	19.10±2.11 ^c	18.90±1.33 ^d
Nutrient Broth with 0.5% maltose	1.58±0.57 ^b	1.68±0.29 ^b	1.82±0.33 ^a	1.78±0.31 ^a	1.66±0.0.45 ^b
Peptone glycerol broth	0.50±0.03 ^a	0.62±0.04 ^a	0.88±0.05 ^a	0.78±0.05 ^a	0.64±0.05 ^a

Values are Mean±Standard Error of Mean of triplicate determinations. Values with different alphabets are significantly different (p<0.05) along the column

Effect of initial pH of the fermentation media for pigment production

Studies conducted for optimization of initial pH (Figure 4) indicated that the bacterium could produce pigment over a pH range of pH 5.0 to 9.0 although maximal pigment production was recorded at pH 7.0 (25.00µg/L). Media with high acidic (pH 2.0 - 4.0) and high alkaline (pH

10.0 - 13.0) did not support pigment production. Biomass was maximum at pH 7.0 (2.4 g/L). Both pigment production and biomass showed a direct relationship, both in their increase as well as decrease, in response to variations in pH. There was no significant difference between various pH tested, and pigment and biomass production (p =0.083833).

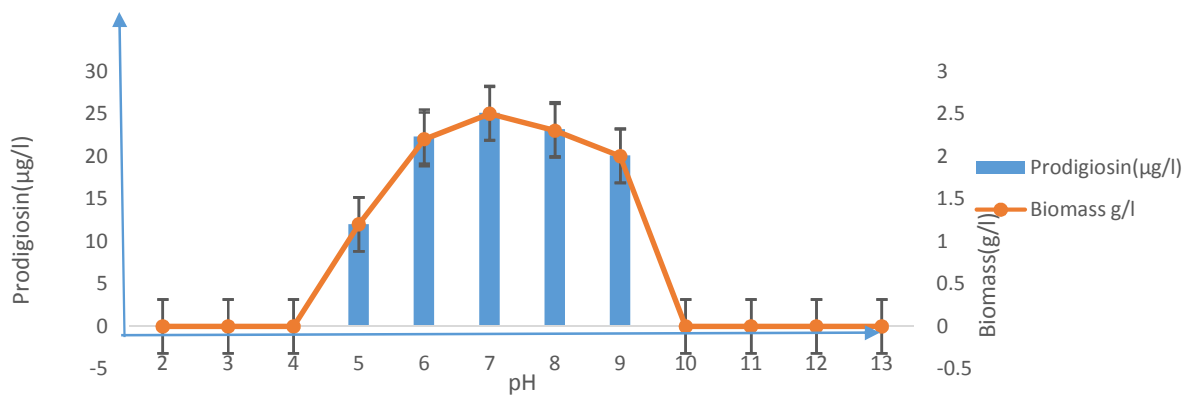


Fig. 4.Effect of initial pH for pigment production by *Serratia marcescens*:

Effect of Agitation on Production of Pigment by *S. marcescens*

Agitation of the culture medium was required for pigment production by *Serratia marcescens* since there was no pigment production at static condition (Figure 5). Maximum pigment production was recorded at 150 rpm (22.50µg/L) while agitation above and below 150 rpm led to a decrease in pigment production. Biomass production also increased

gradually during incubation from static condition to 150 rpm (2.5 g/L), after which it decreased at 200 rpm (Figure 5). Hence, agitation of 150 rpm was required for maximal pigment production and biomass by this bacterium. There was significant difference between agitation rate of 150rpm with other rates, since calculated value is greater than critical value that is (p value is 0.002159).

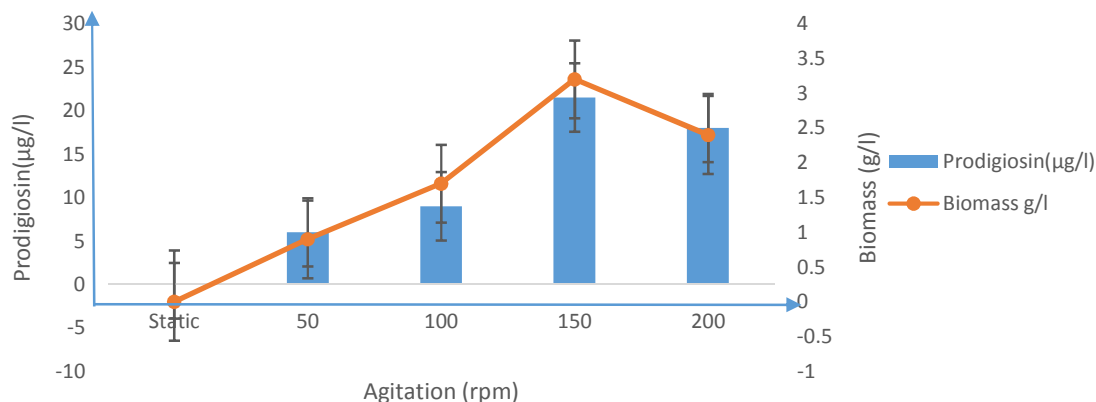


Fig.5. Effect of agitation on pigment production by *Serratia marcescens*:

Effect of Carbon Sources on Pigment Production by *S. marcescens*

Data documented for the studies conducted on the effect of carbon sources on pigment production showed that among the various carbon sources tested only dextrose enhanced pigment production (22.40µg/L). All the other sugars led to a reduction in the level of pigment production when compared with that of the control, (Figure 6). With respect to biomass, dextrose supported enhanced level of

biomass production (2.60 g/L) when compared to sucrose as control, and maltose led to a decrease in biomass compared to the control. Hence, dextrose was selected as the ideal carbon source for pigment production by *Serratia marcescens*. The result statistically proves that there were significant differences ($p < 0.05$) between dextrose with other carbon sources and pigment production as well as biomass.

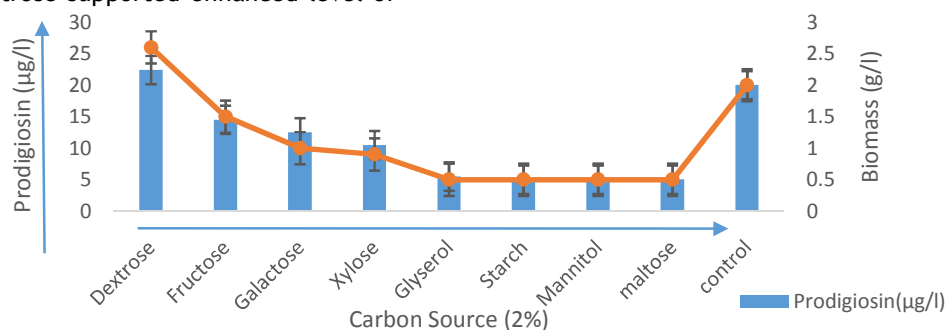


Fig. 6. Effect of carbon sources on pigment production by *Serratia marcescens*:

Effect of Inorganic Nitrogen Sources on Pigment Production by *S. marcescens*

Data obtained for the studies conducted on the effect of inorganic nitrogen sources on pigment production (Figure7) showed that some inorganic nitrogen sources can enhance pigment production by the bacterium compared to the control medium, which was devoid of nitrogen source. However, in the presence of both ammonium sulphate and sodium nitrate there was no pigment production. It was also

observed that inorganic nitrogen source provided as potassium nitrate (0.38µg/L), ammonium nitrate (0.25µg/L) and ammonium chloride (0.2µg/L) could support very low level of pigment production by *S. marcescens* (Figure 7). Nevertheless, all the inorganic nitrogen sources led to a reduced biomass production (Figure 7). Maximum biomass production was observed in the medium supplemented with potassium nitrate (0.66 g/L).

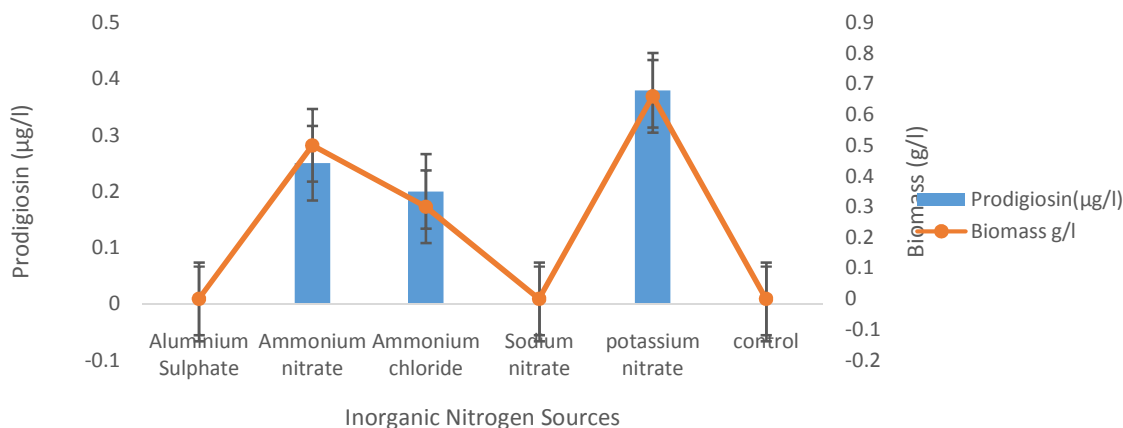


Fig. 7. Effect of inorganic nitrogen sources on pigment production by *Serratia marcescens*:

Effect of Organic Nitrogen Sources on Pigment Production by *S. marcescens*

Data presented in Figure 8 indicate that among the organic nitrogen sources tested, yeast extract supported maximum pigment (26.75µg/L) and biomass production (3.53 g/L). Similarly, the results also show that beef

extract and malt extract supported pigment production (20.23µg/L and 22.63µg/L respectively). Peptone led to a decreased pigment production (9.15µg/L) compared to other organic sources. Addition of urea did not support pigment as well as biomass production (Figure 8).

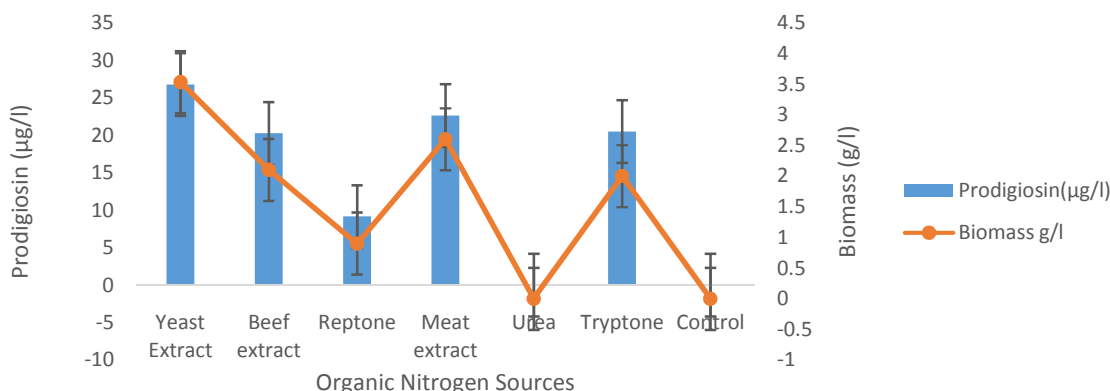


Fig. 8. Effect of organic nitrogen sources on pigment production by *Serratia marcescens*

DISCUSSION

Among the different media evaluated in the present study, peanut seed broth medium supported maximum pigment production when compared to sesame seed broth, Nutrient broth, peptone broth and Glycerol asparagine broth. Since peanut seed broth medium led to enhanced red pigment production by *Serratia marcescens*, this medium was optimized further towards maximizing pigment production.

In the present study prodigiosin at 72 h of incubation was estimated to be 25.10µg/l this is similar to observation by Mekhael and Yousif (2009) who reported that strain of *Serratia marcescens* isolated from milk produced 33.35µg/l of prodigiosin with peanut seed medium after 72 h of incubation. The production of pigment increases gradually up to

72 h but ceases after 96 h of incubation. In the present study prodigiosin exhibit absorption of 535nm using methanol as an ideal solvent for extraction. This is in agreement with report of Giri *et al.* (2004) and Pradeep *et al.* 2012) who observed absorption at 535nm with methanol but disagreed with the report of Kamble and Hiwarale (2012) who observed absorption at 499nm using ethanol.

At temperatures over 30°C a particular enzyme in the pathway to synthesize prodigiosin may lose activity. So, in the present study maximum prodigiosin production by *Serratia marcescens* was observed at 25°C followed by 20°C and the production decreased proportionately along with increase in incubation temperature from 30°C to 40°C.

This is similar to the observation made by (Giri *et al.*, 2004), who recorded maximum yield of prodigiosin from *S. marcescens* at 28°C, while at 37°C no pigment production was observed and the culture broth was white in colour. Maximal level of prodigiosin was synthesized by *S. marcescens* at 27°C while no pigment was formed at 16 or 32°C (Williams *et al.*, 1971). Further, it has been reported that the production of prodigiosin from *Serratia marcescens* was inhibited when the temperature was lower than 20°C or higher than 37°C (Furstner 2003; Giri *et al.*, 2004). The present work is in contrast to observation made by (Davis *et al.*, 2007), who reported production of prodigiosin by some strains of *S. marcescens* when incubated at 37°C.

Extremes of pH, either acidic range (below pH 4.0) or alkaline range (above pH 10.0), prevented pigmentation by *S. marcescens* (Williams *et al.*, 1971), while the optimum growth of all strains of *Serratia* has been observed at pH 9.0 (Giri *et al.*, 2004), the optimum pH for prodigiosin production by *S. marcescens* was observed at 8.0 (Wei *et al.*, 2005). In the present study, it was observed that the pH influenced the pigment production by *Serratia marcescens*. Thus there was considerable level of pigment production over a range of pH from 5.0 to 9.0 and maximum pigment was recorded at pH 7.0 in spite of a decline in pigment production with increase in pH. Highly acidic (2.0-4.0) and alkaline (10.0 - 13.0) media did not support pigment production. The present study is in accordance with the observation of Khanafari *et al.* (2006), who reported that the lower the pH, the more acidic the medium and lower the yield of the pigment. They are also similar to the report of Mekhael and Yousif (2009), who reported that the ideal pH for optimum prodigiosin production was found to be 8.

The agitation rate, which influences the mass transfer of both oxygen gas and medium components in the medium become a crucial factor in prodigiosin synthesis by *S. marcescens* which recorded maximum pigment production at 200 rpm (Wei *et al.*, 2005). Prodigiosin biosynthesis by the non-proliferating cells was maximum when *S. marcescens* strain was incubated under aeration (Khanafari *et al.*, 2006). In the present study it was observed that mass transfer of medium components into the cells play an important role in the relationship between agitation rate and prodigiosin production. This observation is in agreement with the report of Wei *et al.* (2005), that higher rate of agitation led to a slight decrease in

pigment production, probably due to the problem of high shear force. In the present study also it was observed that agitation significantly led to increase in the growth and pigment production by *S. marcescens*. Maximum pigment production was recorded at 150 rpm and there was a decrease in pigment production at agitation rates above 150rpm and below 150 rpm. Biomass production also increased from static condition to 150 rpm.

Dextrose supported highest level of pigment and biomass production, when it was used as the sole source of carbon was compared to all other sugars, which led to a reduction in the level of pigment production. Hence, dextrose was selected as the ideal carbon source for pigment production by *S. marcescens*. Further, it was noted that 40 mM of dextrose supported maximum pigment production. The results indicated that dextrose supported cell growth and prodigiosin production. This finding with similar reports by Jissa *et al.* (2008) in their comparative analysis of carbon source used in prodigiosin production.

Result obtained for the studies conducted on the effect of inorganic nitrogen sources on prodigiosin production indicated that the inorganic nitrogen source gives optimum pigment production by the bacterium compared to the control medium, which was devoid of nitrogen source. However, when the inorganic nitrogen source was provided as ammonium sulphate and sodium nitrate, there was no pigment production. Interestingly both nitrate and ammonium in other combinations supported pigment production. Hence, it is possible that sodium and sulphate could have blocked the induction role of nitrogen for pigment production in their respective combination.

The maximum biomass production was observed in the medium supplemented with potassium nitrate (0.66 g/L). This result is in accordance with observation made by An *et al.* (2001); Bau and Wong (2009), who reported that trace elements are important factors that affect pigment production in several microorganisms and there is an interaction between pigment and metal ions. Zinc ions were reported to have detrimental effect on *Monascus* pigment production. Iron was responsible for decreased astaxanthin production and its composition in *Phaffia rhodozyma* (Anet *et al.* 2001). In the present study selected trace salts (FeCl₂, MnCl₂, Na₂MoO₄ and ZnSO₄) were observed to enhance the pigment production significantly (P<0.05) in the peanut medium when they were included in the medium.

A reduction in the level of pigment production was observed in the medium prepared without the trace salts and when one of the four salts was left out. Interestingly, even in the absence of all four salts the bacterium could produce pigment. Of the four salts, ZnSO₄ had relatively a greater influence on pigment production compared to other salts, whereas biomass production by the bacterium was observed more in media without ZnSO₄. This means that this bacterium preferred zinc sulphate for pigment production and not for biomass yield. In the present study, it was observed that pigment production was greatly influenced by the varying concentrations of sodium chloride. There was no pigment production in media lacking sodium chloride, indicating that the organism required NaCl for pigment production. Both pigment and biomass production gradually increased with increase in concentration of NaCl from 10mM to 60mM and maximal pigment and biomass was recorded with 100mM sodium chloride. *Serratia marcescens* was considered as slightly halophilic since it can tolerate NaCl concentration from 10mM to 700mM (0.05-5.5%). This agrees with the report of Allen et al. (2003), who studied the effect of sodium ions on growth and prodigiosin production by *V. gazogenes* ATCC29988 at varying concentrations of NaCl in defined medium from 10 to 600mM. The investigator observed that optimal cell growth occurred in the presence of 100mM NaCl and decreased at higher sodium ion levels whereas, prodigiosin synthesis was affected by

increased levels of sodium ions in the medium, Yamazaki *et al.*, (2006).

The present result is similar to the observation by Yamazaki *et al.* (2006), who reported higher level of pigment production by *S. rubidaea* N-1 at 0.6-1.0M NaCl when the cells were incubated for 48 h in LB media with 0-2.0 M NaCl, indicating that the organism was a salt-tolerant bacterium of which prodigiosin production depended on the salt concentration. Statistical analysis reveals the relationship between the salts with pigment and biomass production, (Abdel-Fattaha *et al.*, 2005). Among the twelve factors namely incubation period, inoculum, yeast extract, dextrose, NaCl, CaCl₂, K₂HPO₄, KH₂PO₄, trace salts, pH, incubation temperature, rate of agitation evaluated with this package for pigment production. Inoculum (X₁), Calcium chloride (X₂), Sodium chloride (X₃), and Temperature (X₄) were found to be the most significant variables.

CONCLUSIONS

This study revealed that methanol is an ideal solvent for prodigiosin extraction recording absorbance of 0.69nm. Pigment production by *S. marcescens* was maximum at 25°C (25.10 µg/L), pH 7.0 (25.00µg/L), 150 rpm (22.50µg/L) and 36 hours (2.10g/L) incubation period. The maximum biomass production was observed in the medium supplemented with potassium nitrate (0.66 g/L). Yeast extract(26.75µg/L) supported maximum pigment production and dextrose gave optimal carbon source (22.40mg/ml) for prodigiosin production.

REFERENCES

- Abdel-Fattaha, Y. R., Saeedb, H. M., Goharc, Y. M. and El-Baz, M. A. (2005). Improved production of *Pseudomonas aeruginosa* uricase by optimization of process parameters through statistical experimental designs. *ProcessBiochemistry*.40, 1707-1714.
- Allen, G.R., Reichelt, J.L. and Grayv, P.P. (2003). Influence of environmental factors and medium composition on *Vibro gazogenes* growth and prodigiosin production. *Applied Environmental Microbiology*, 1727-1732.
- An, G.H., Jang, B.G., Suh, O.S., Kim, C.I. and Song, K.B.(2001). Iron (II) decreases astaxanthin production in *Phaffia rhodozyma* (*Xanthophyllomyces dendrorhous*). *Food Science and Biotechnology*, 10, 204-207.
- Austin, D.A. and Moss, M.D. (1986). Numerical taxonomy of red-pigmented bacteria isolated from a lowland river, with the description of a new taxon, *Rugamonas rubra*, new genus, and new species. *Journal of General Microbiology*,132, 1899-1910.
- Azuma, T., Watanabe, N., Yagisawa, H., Hirata, H., Iwamura, M. and Kobayashi, Y, (2000) Induction of apoptosis of activated murine splenic T cells by cycloprodigiosin hydrochloride, a novel immunosuppressant, *Immunopharmacology*, 46, 29-37.
- Bau, Y. S. and Wong, H. C. (1979): Zinc effect on growth, pigmentation and antibacterial activity of *Monascus purpureus*. *Physiol. Plant*, 46, 63-67.
- Bennett, J.W. and Bentley, R.(2000). Seeing Red: The Story of Prodigiosin. *Advance Applied Microbiology*, 47, 1-32.
- Cheesbrough, M. (2006). *District laboratory practice in tropical Countries*, part 2, low price Edition, Cambridge University, Press, London.

- Chidambaram, K.V. and Perumalsamy, L. (2009). An Insightful Overview on Microbial Pigment, Prodigiosin, *Electronic Journal of Biology*, 5(3), 49-61
- Furstner, A. (2003). Chemistry and biology of roseophilin and the prodigiosin alkaloids: a survey of the last 2500 years. *Angew. Chemistry International Edition England*, 42, 3582-603.
- Giri, A.V., Anandkumar, N., Muthukumar, G. and Pennathur, G. (2004). A novel medium for the enhanced cell growth and production of prodigiosin from *Serratia marcescens* isolated from soil. *BMC Microbiology*, 4, 11-18.
- Jissa, G. K., Soorej, M., Beena, P. S, Ansu, J., Elyas, K. K and Chandrasekaran, M. (2008). Prodigiosin-like pigment from marine *Serratia* sp. BTWJ8. Purification, Characterization and application as a dye. *Applied Microbial Biotechnology*, 8, 71-78.
- Kamble, K.D. and Hiwarale, V.D. (2012) Prodigiosin production from *Serratia marcescens* strains obtained from farm soil. *International Journal of Environmental Sciences*, 3(1), 631-638.
- Khanafari, A., Assadi, M.M. and Fakhri, F.A. (2006). Review of Prodigiosin, Pigmentation in *Serratia marcescens*. *Online Journal of Biological Sciences*, 1, 1-13.
- Mekhael, H, Yousif, H. (2009). The role of red pigment produced by *Serratia marcescens* as antibacterial plasmid curing agent. *Journal of Duhok University*, 12(1), 268-274.
- Moraes, C.S., Seabra, S.H., Albuquerque-Cunha, I. M., Castro, D.P., Genta, F.A., De Souza, W., Brazil, R.P., Garcia, E.S. and Azambuja, P. (2009). Prodigiosin is not a determinant factor in lysis of *Leishmania (Viannia) braziliensis* after interaction with *Serratia marcescens* d-mannose sensitive fimbriae. *Experimental Parasitology*, 122, 84-90.
- Nakamura, K. and Kitamura, K. (1981). Process for preparation of prodigiosin. U.S. Patent 4,266.
- Pandey, R., Chander, R. and Sainis, K.B. (2009). Prodigiosin as anti-cancer agents: Living up to their name. *Curr Pharm. Des.*, 15, 732-741.
- Wei, Y.H. and Chen, W.C. (2005). Enhanced production of prodigiosin-like pigment from *Serratia marcescens* SMAR by medium improvement and oil-supplementation strategies. *Journal Biosciences and Bioengineering*, 99, 616-622.
- Williams, R.P., Gott, C.L., Hussain, S., Qadri, M. and Scott, R.H. (1971). Influence of temperature on incubation and type of growth medium on pigmentation in *Serratia marcescens*. *Journal of Bacteriology*, 106, 438-443.
- Yamazaki, G., Nishimura, S., Ishida, A., Kanagasabhapathy, M., Zhou, X., Nagata, S., Morohoshi, T., and Ikeda, T. (2006). Effect of salt stress on pigment production of *Serratia rubidaea* N -1: a potential indicator strain for screening quorum sensing inhibitors from marine microbe. *Journal of General and Applied Microbiology*, 52, 113-117.
- Zobell, C.E. and Feltham, C.B. (1934). Preliminary studies on the distribution and characteristics of marine bacteria. *Bulletin Scripps Institute of Oceanology Technology*, 3, 279-296.