BMC Microbiology

Research article

A novel medium for the enhanced cell growth and production of prodigiosin from *Serratia marcescens* isolated from soil

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Published: 18 March 2004

BMC Microbiology 2004, **4**:11

Received: 19 November 2003
Accepted: 18 March 2004

This article is available from: http://www.biomedcentral.com/1471-2180/4/11

Abstract

Background: Prodigiosin produced by *Serratia marcescens* is a promising drug owing to its reported characteristics of having antifungal, immunosuppressive and antiproliferative activity. From an industrial point of view the necessity to obtain a suitable medium to simultaneously enhance the growth of *Serratia marcescens* and the pigment production was the aim of this work. The usage of individual fatty acid as substrate in industries would be cost-effective in the long run and this paved the way for us to try the effect of different fatty acid-containing seeds and oils of peanut, sesame and coconut as source of substrate.

Results: The addition of sugars only showed slight enhancement of prodigiosin production in nutrient broth but not in fatty acid containing seed medium. The powdered peanut broth had supported better growth of *Serratia marcescens* and higher yield of prodigiosin when compared with the existing nutrient broth and peptone glycerol broth. A block in prodigiosin production was seen above 30°C in nutrient broth, but the fatty acid seed medium used by us supported prodigiosin production upto 42°C though the yields were lower than what was obtained at 28°C. From the results, the fatty acid form of carbon source has a role to play in enhanced cell growth and prodigiosin production.

Conclusion: We conclude by reporting that the powdered and sieved peanut seed of different quality grades were consistent in yielding a fourty fold increase in prodigiosin production over the existing media. A literature survey on the composition of the different media components in nutrient broth, peptone glycerol broth and the fatty acid containing seeds and oils enabled us to propose that the saturated form of fatty acid has a role to play in enhanced cell growth and prodigiosin production. This work has also enabled us to report that the temperature related block of prodigiosin biosynthesis varies with different media and the powdered peanut broth supports prodigiosin production at higher temperatures. The medium suggested in this work is best suitable from an industrial point of view in being economically feasible, in terms of the higher prodigiosin yield and the extraction of prodigiosin described in this paper is simple with minimal wastage.

Background

Serratia sp are gram negative bacteria, classified in the large family of *Enterobacteriaceae*. *Serratia* can be distinguished from other genera by its production of three special enzymes DNAase, lipase and gelatinase. Another characteristic feature of the *Serratia* among the *Klebsiellaea* is the production of cell associated red color pigment. *Serratia*, like other *Enterobacteriaceae*, grow well on ordinary media under anaerobic and aerobic conditions. They grow well on synthetic media using various compounds as a single carbon source. Optimum growth of all strains of *Serratia* has been observed at pH 9 and at temperatures from 20–37°C.

Secondary metabolites of bacterial origin include various enzymes, pigments, antibiotics etc which could be of importance to mankind in many ways. Prodigiosin is a multifaceted secondary metabolite. It is produced by *Serratia marcescens*, *Pseudomonas magneslorubra*, *Vibrio psychroerythrous* and other bacteria [4,14]. The prodigiosin group of natural products are a family of tripyrrole red pigments that contain a common 4-methoxy, 2-2 bipyrrole ring system. The biosynthesis of the pigment is a bifurcated process in which mono and bipyrrole precursors are synthesized separately and then assembled to form prodigiosin [1]. However, pigmentation is only present in a small percentage of isolated cultures. Pigment production is highly variable among species and is dependent on many factors such as species type and incubation time. Prodigiosin have been shown to be associated in extracellular vesicles, cell associated or present in intracellular granules [10,8]. It has been proved from studies that the non pigmented strains are clinically more significant in causing infections [2]. A synergistic inhibitory activity of prodigiosin and chitinolytic enzymes was observed against spore germination of *Botrytis cinerea* [13], selective activity against cancer cell lines [9], enhanced lethal and inhibitory activity of Cry1C BT toxin along with prodigiosin [16] and the lipase of *Serratia* used for the manufacture of an intermediate of diltiazem a vasodilator [5] is well studied. Pryce & Terry [15] in their unpublished observation have reported on the possibility of a membrane permeable positive prodigiosin regulator synthesized by *Serratia marcescens*. Many types of differential and selective media have been developed for the isolation and presumptive testing of *Serratia*. Capryllate Thallous [CT] agar contains caprylate as a carbon source for *Serratia* and thallous salts as inhibitors for other organisms [17] and CT is the best at selecting for *Serratia*. The regular liquid media currently being used for prodigiosin biosynthesis is nutrient broth [15], peptone glycerol broth [11], and production medium [6] etc. According to the medium patented by Nakamura [12] the author has used sodium oleate 2% and has also studied oleic acid substitution instead of sodium oleate and has used only triolein as substrate and reported a yield of 0.69 mg/ml.

Having an insight on the composition of already published media the idea of designing a new, nutritious and economically cheap medium was thought of for the prodigiosin biosynthesis. Initial comparative work was done using powdered sesame seed powder in water, nutrient broth and peptone glycerol broth as a growth medium for *Serratia marcescens*. After having observed sesame seed to give a better yield in terms of prodigiosin biosynthesis further comparision was done with readily available cheaper sources like peanut and coconut. Sesame oil, peanut oil and coconut oil were also compared with the rest of the media. The media were also compared for growth at three different temperatures in terms of prodigiosin production. This work lead to the observation that fatty acids as the substrate supported enhanced prodigiosin production The various components in the seeds as substrate could have stimulated cell density which in turn could have resulted in higher accumulation of the positive regulator inside the cell there by triggering excessive pigment production. The powdered peanut seed medium supported the prodigiosin biosynthesis even at 37°C which was not so in the case of nutrient or peptone glycerol broth with and with out sugars.

Results

Isolation and characterization of the soil isolate

The culture isolated from soil taken from a site in Anna University, Chennai was characterized as belonging to the *Serratia* by various biochemical tests, the major identifier being the culture morphology, Gram negative, production of cell associated prodigiosin, lipase positive and non lactose fermentation on MacConkey plates. The PCR product for the 16s rRNA based primers gave a sharp band on agarose gel corresponding to a 417 base pair product when compared to the molecular ladder, thus identifying the isolate as *Serratia marcescens* and the genomic DNA of *Micrococcus* sp did not show the PCR product as shown in Figure 1. The negative reaction having no genomic DNA also showed the absence of the PCR product.

Purification of pigment for mass spectrometry analysis

The pure pigment extracted from the culture broth analysed by mass spectrophotometry showed a molecular weight of 324 and wavelength scanning using UV spectrophotometer gave an absorbance peak at 535 nm.

Growth of serratia marcescens in different media at 28°C, 30°C and 37°C

As indicated from Table 1 the crushed sesame seed broth gave the maximum yield of prodigiosin at 28°C, 30°C, and 37°C when compared to nutrient broth and peptone glycerol broth. Amongst the three temperatures, the

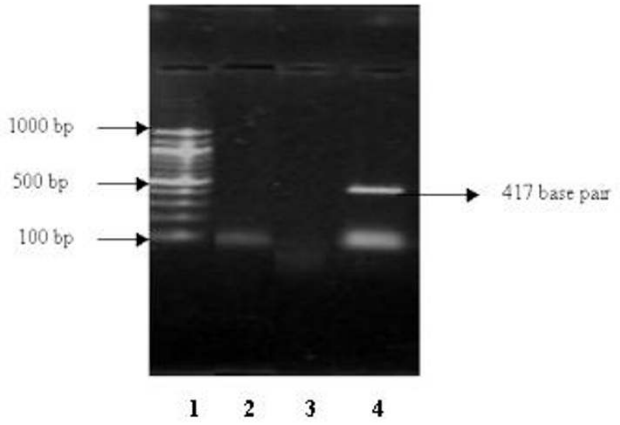


Figure 1
Denotes the 417 base pair product amplified of the 16s rRNA sequence of the soil isolate indicating the culture to be *Serratia marcescens* Lane 1: Molecular Ladder [100 to 1000 base pair ladder], Lane 2: Negative PCR reaction [no genomic DNA] Lane 3: Genomic DNA of *Micrococcus* sp Lane 4: 417 Base Pair PCR product of 16s rRNA sequence of *Serratia marcescens*

Table 1: Comparative analysis of prodigiosin production by *Serratia marcescens* in different media at 28°C, 30°C and 37°C temperatures

S.No:	Media used	28°C mg/ml	30°C mg/ml	37°C mg/ml
1	Nutrient broth	0.52	0.354	0.111
2	Peptone glycerol broth	0.302	0.569	0.111
3	Sesame seed broth	16.68	9.3	0.319

Table 2: Comparative analysis of extracellular protein production by *Serratia marcescens* in different media at 28°C, 30°C and 37°C temperatures

S.No:	Media used	28°C mg/ml	30°C mg/ml	37°C mg/ml
1	Nutrient broth	1.66	2.50	2.11
2	Peptone glycerol broth	2.58	2.32	3.04
3	Sesame seed broth	1.32	1.72	1.17

maximum yield of prodigiosin was observed at 28°C for nutrient broth and sesame broth. In the case of peptone glycerol broth maximum pigment production was seen only at 30°C. The yield of pigment in sesame broth was greater at 37°C, when compared to nutrient broth and peptone glycerol broth at 30°C. The maximum yield of pigment in sesame medium was ~17 mg/ml and in nutrient broth was ~0.52 mg/ml at 28°C. The maximum extracellular protein following 36 hours of incubation was found in peptone glycerol broth at 28°C and in the case of nutrient broth and sesame broth maximum extracellular protein was seen at 30°C as indicated in Table 2.

Effect of sugars on the growth of Serratia marcescens *in nutrient broth and powdered sesame seed medium at 28°C, 30°C and 37°C*

Addition of maltose to nutrient broth enhanced pigment production only by 2 fold as at 28°C and 30°C as shown in Table 3. Nutrient broth with glucose showed a two fold increase at 28°C. The pigment production was more in sesame seed broth even without the addition of any sugars, when compared to sesame seed broth with glucose or maltose. The pigment production was reduced in sesame seed medium with maltose at 28°C when compared to only powdered sesame seed broth. Glucose in powdered sesame seed medium showed a complete decrease of prodigiosin production at both 28°C and 30°C. Amongst the two sugars substituted, maltose acts as a better source of substrate in enhancing pigment production in nutrient broth. This clearly showed that in sesame medium the addition of maltose or glucose does not significantly enhance the pigment production. In fact the addition of glucose or maltose caused a reduction in prodigiosin production which could be due to catabolite repression. The pigment production in nutrient broth with sugars was not more than what was observed in sesame seed medium.

Comparative study of different fatty acid containing seeds as source of substrate for serratia marcescens

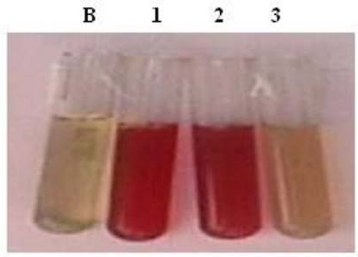
Following incubation of the different fatty acid containing seed media at different temperatures of 28°C, 30°C, and 37°C at 180 rpm for 36 hours, the samples analysed for extracellular protein concentration and prodigiosin production were tabulated in Table 4. As indicated from Table 4 the medium containing powdered peanut seed gave maximum yield of prodigiosin at 28°C, 30°C, and 37°C. Amongst the three temperatures maximum yield was observed at 28°C in all the three fatty acid containing seed medium. When compared to powdered peanut seed medium, sesame medium gave a half fold decrease in the pigment production at all the three temperatures. The descending order of pigment production in powdered seed media was peanut seed medium, sesame seed medium, and coconut medium, nutrient broth and peptone glycerol broth. The yield of pigment in peanut and sesame media is greater at 37°C when compared to nutrient broth and peptone glycerol broth at 30°C. The maximum yield of pigment in case of peanut medium is ~39 mg/ml and in the case of sesame medium is ~17 mg / ml.

Table 3: Comparative analysis of prodigiosin production in nutrient broth and powdered sesame broth in maltose and glucose at 28°C, 30°C and 37°C

S.No:	Media used	28°C mg/ml	30°C mg/ml	37°C mg/ml
1	Nutrient broth	0.52	0.354	0.111
2	Nutrient broth with 0.5% maltose	1.836	0.79	0.104
3	Nutrient broth with 0.5% glucose	1.689	0.29	0.104
4	Sesame seed broth	16.68	9.3	0.319
5	Sesame seed broth with 0.5% maltose	9.43	8.56	1.63
6	Sesame seed broth with 0.5% glucose	1.47	1.16	0.42

Table 4: Comparative analysis of prodigiosin production in powdered sesame broth, sesame oil broth, powdered peanut seed broth, peanut oil broth, coconut seed broth and coconut oil broth

S.No:	Media used	28°C mg/ml	30°C mg/ml	37°C mg/ml
1	Sesame seed broth	16.68	9.3	0.319
2	Sesame oil broth	0.767	1.006	0.107
3	Peanut seed broth	38.75	25.98	1.49
4	Peanut oil broth	2.89	0.559	0.111
5	Copra seed broth	1.94	1.39	0.1736
6	Coconut oil broth	1.42	0.05	0.177



Pigment Production in Nutrient broth
B- blank, 1- 28°C, 2- 30°C, 3- 37°C

Pigment Production in Powdered Peanut broth

B- blank, 1- 28°C; 2- 37°C; 3- 42°C I generation; 4- 42°C II generation; 5- revert of inoculum from 42°C II generation in powdered peanut broth at 28°C

Figure 2
Growth of *Serratia marcescens* and prodigiosin production at different temperature in different media

Comparative study of different fatty acid containing seed oils as source of substrate for Serratia marcescens

A comparative tabulation of the pigment production by *Serratia marcescens* in different seeds and the respective seed oil broth was tabulated in Table 4. The maximum yield of pigment was found with peanut oil at all the three temperatures. The utility of peanut oil as substrate was very low when compared to powdered peanut seed as substrate. All the three oils as substrate was more efficient in inducing pigment production when compared to the use of nutrient broth or peptone glycerol broth. The yield was more or less similar when compared to nutrient broth with maltose or glucose.

Comparative study on effect of different temperatures on the growth of Serratia marcescens *and prodigiosin production in nutrient broth and powdered peanut seed broth*

From a comparative study of Table 3 &4, the maximum prodigiosin production was seen at 28°C and 30°C in nutrient broth. At 37°C *Serratia marcescens* did not show any pigment production in nutrient broth and the culture broth was white in color. In case of the powdered peanut broth, even at 37°C, pigment production was observed and in fact it was equal to the amount of pigment production seen in nutrient broth at 30°C. In case of peanut broth only after second generation growth of *Serratia marcescens* at 42°C, there was complete block of pigment production as shown in Figure 2. Reversion of the 42°C grown white culture of *Serratia marcescens* which had shown pigment block in powdered peanut broth, showed the re-synthesis of pigment production when incubated at 28°C. Reversion experiment was done to confirm that at 42°C the culture was still viable and only the pigment production was blocked.

Pigment production and extracellular protein secretion pattern of Serratia marcescens *in three different media*

Due to the interference of the powdered peanut seed and sesame seed in broth the optical density could not be measured to depict the growth pattern of *Serratia marcescens*. Thus the growth of the culture was monitored in terms of the extracellular protein and the pigment produced for every two hours from the time of inoculation to

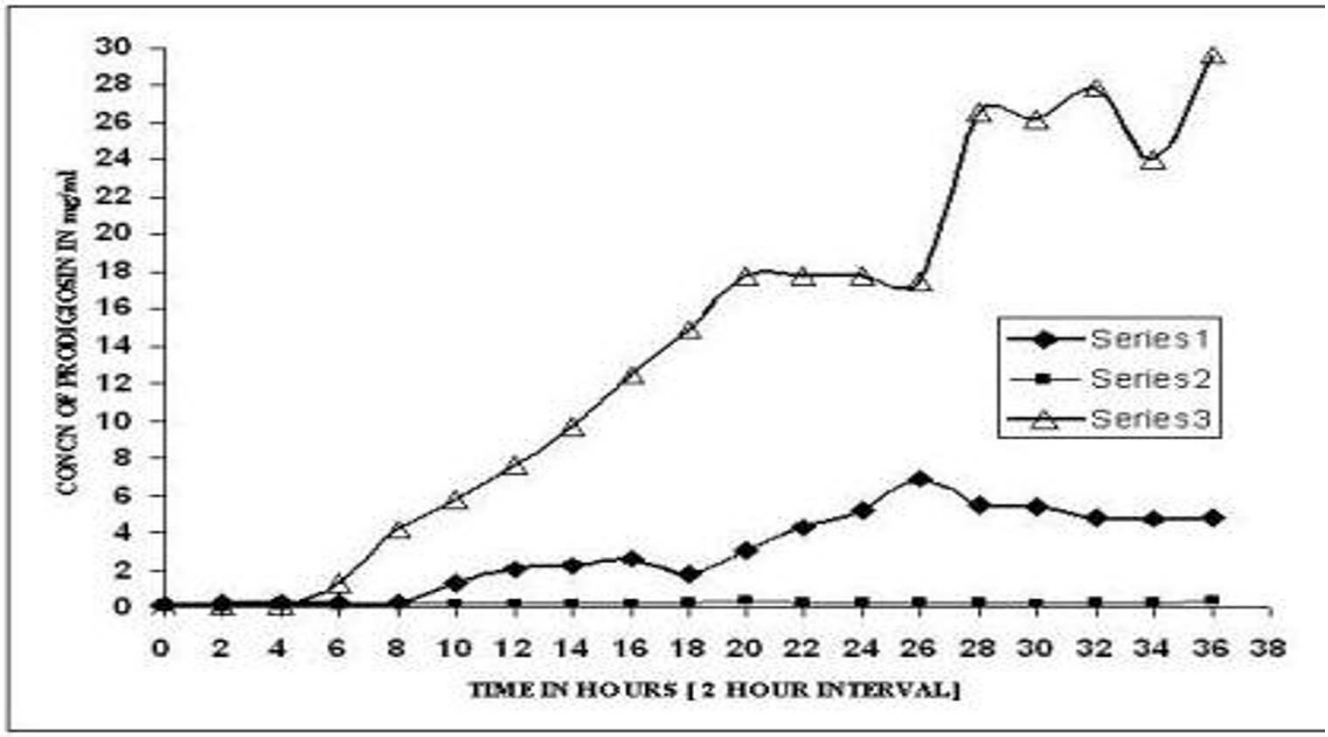


Figure 3
Consolidated view of the pigment produced for every two hours by *Serratia marcescens* in nutrient broth [series 2], powdered sesame seed broth [series 1], and powdered peanut seed broth [series 3]

a period of 36 hours. Initial inoculum was taken as 2% to avoid initial introduction of pigment along with the starter culture. In powdered peanut seed medium induction of pigment was seen from the 6th hour of growth. In powdered sesame seed medium and nutrient broth medium induction of pigment formation was seen from the 8 th hour of inoculation. Figure 3 gives a comparative picture of pigment production at two hour time points. Maximum pigment formation was seen in powdered peanut seed medium which was ~30 mg at the end of the 36th hour and a linear increase in production from the 15th to the 36th hour. In case of powdered sesame seed medium maximum yield of ~7 mg/ml was seen in the 16th hour of growth. In case of the nutrient broth medium a linear range of maximum production of pigment was seen from the 15th to the 36th hour and maximum yield was ~0.4 mg/ml. Figure 4 gives a comparative extracellular protein profile of the samples taken every two hours of growth where mg/ml of protein concentration was assayed by Lowry's method. In all the three cases the protein already present in the medium was utilized till the stationary growth of the culture and then extracellular protein was secreted out into the medium. The initial protein concentration present in nutrient broth at the time of inoculation was higher, when compared to initial protein concentration in powdered sesame seed or powdered peanut seed medium, but the yield of prodigiosin was higher in powdered peanut broth.

Discussion

Biopigments synthesized by bacteria possess enormous efficiency as medicinally important products. In order to increase the potentiality of the bacteria to synthesize large quantities of the pigment a comparative study of different

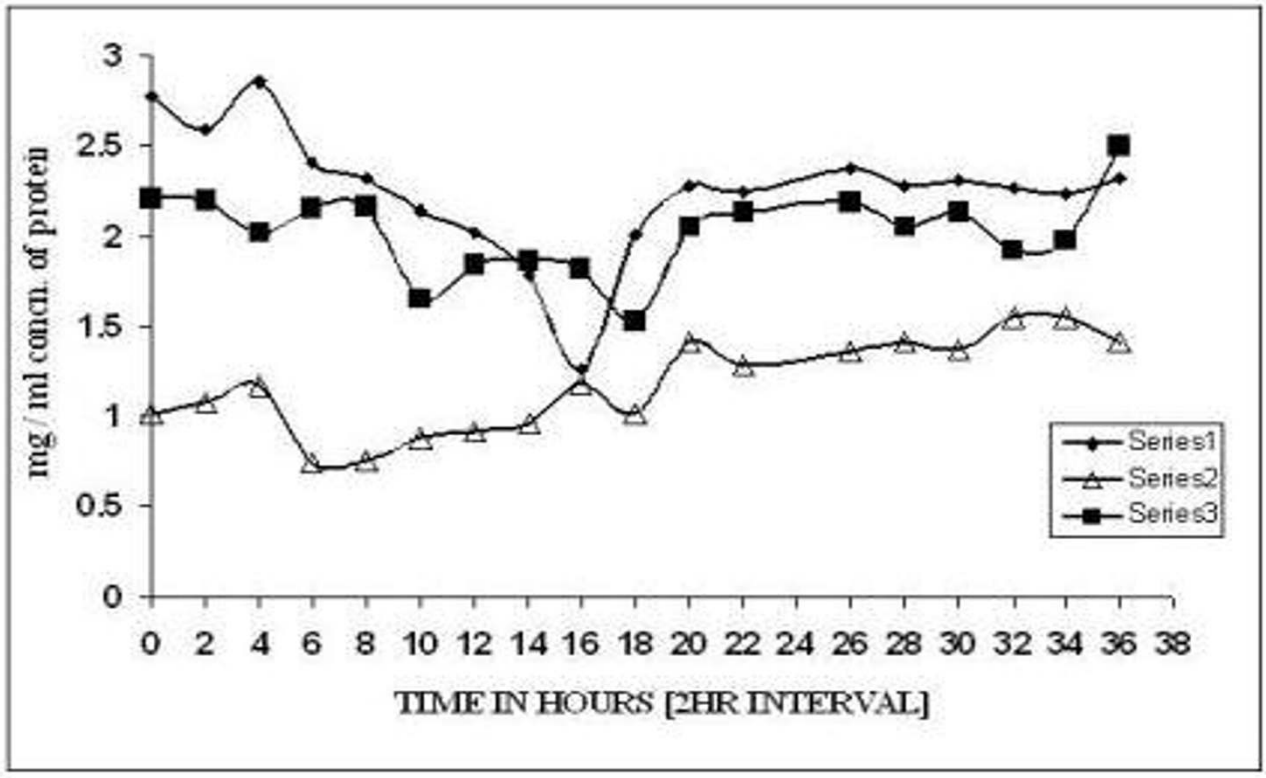


Figure 4
Consolidated graph of extracellular protein profile of *Serratia marcescens* for every two hours grown in nutrient broth [series 1], powdered sesame seed broth [series 3] and powdered peanut seed broth [series 2]

media, role of temperature, growth of the organism in the different media and pigment production must be studied. The components of the different media should be analysed and compared effectively, to deduce the most probable reason for the enhancement or the decline in pigment production. Keeping these objectives in mind, a media which would could support the growth of the bacteria and at the same time prove efficient to trigger high levels of pigment formation was designed. The powdered peanut medium gave the highest yield of ~39 mg/ml over all the medias compared in this paper and from the existing literatures. In the bioreactor study with an an internal adsorbent for prodigiosin conducted by Jungdon *et al*., [6] the final yield was 13 mg/ml and the media used had dextrose in the culture broth and casein in production medium. Chang *et al*., [3] have quoted a medium containing ethanol and carbon source but the yield was 3 mg/ml. Nakamura [12] in his patent describes the use of sodium oleate media and the substitution of sodium oleate with oleic acid. Pure saturated and unsaturated fatty acids were substituted in the medium and triolein an unsaturated fatty acid gave the maximum of 0.69 mg/ml yield of the pigment.

In this paper we have compared nutrient broth and peptone glycerol broth with powdered sesame seed, powdered peanut seed and coconut medium. The inherent concentration of protein was maximum in nutrient broth followed by powdered sesame seed broth and powdered peanut broth. Both in nutrient broth and peptone glycerol broth the major components were peptone, meat and yeast extract. Peptone is a commercially available digest of a particular plant or animal protein, made available to organisms as peptides and amino acids to help satisfy

requirements for nitrogen, sulfur, carbon and energy. Peptones also contain small amounts of various organic and inorganic compounds [19]. But they may be deficient in certain minerals and vitamins. Yeast and meat extracts contain eukaryotic tissues (yeast, beef muscle, liver, brain, heart, etc.) that are extracted by boiling and then concentrated to a paste or dried to a powder. These extracts are frequently used as a source of amino acids, vitamins and coenzymes, growth factors by fastidious organisms. Trace elements, minerals and usually some sugar are also present. In peptone glycerol broth, the glycerol was the carbon source. The seeds and oils contain metals, vitamins, saturated and unsaturated fatty acids and the concentration of these components are variable in each kind of seed or oil.

An enhanced pigment production was seen at 28°C in all the different media studied except in peptone glycerol broth. The reason could be that the viscosity of glycerol decrease at higher temperature and thereby the carbon source in the form of glycerol becomes more accessible to the bacteria. Thereby an enhanced prodigiosin production was seen at 30°C in peptone glycerol broth. The yield of prodigiosin from the powdered peanut, sesame and coconut fatty acid seed broth tested at 37°C was similar to what was seen in the nutrient broth and peptone glycerol broth at 30°C. In nutrient broth and peptone glycerol broth the prodigiosin production was completely blocked at 37°C similar to the report of Pryce and Terry [15]. In the powdered peanut seed broth which showed the maximum yield, a block in prodigiosin production was seen only with the second generation at 42°C. The impact of the physiological role of temperature in blocking prodigiosin production, thus seems to vary with medium of different substrate compositions.

Taking into consideration the basic role of carbon source in enhancing pigment production, two justifications can be made. The first point is that in nutrient broth, which is basically devoid of carbon source, the addition of maltose or glucose enhanced the pigment production but not so in the case of sesame broth which already has carbon in the form of fatty acids. The decrease in prodigiosin production seen in powdered sesame seed broth with the addition of glucose or maltose could be due to a catabolite repression. Maltose and glucose added in nutrient broth gave a two fold increase in yield over nutrient broth or peptone glycerol broth alone. The second point is that a slight enhanced pigment production was seen in the case of peptone glycerol broth at 30°C over nutrient broth at 28°C and this could be attributed to the glycerol present as carbon source. This clearly justifies the fact that carbon does support cell growth and thereby prodigiosin production.

Fatty acids as a carbon source also play a role in enhanced cell density thereby an enhanced pigment production. The role of saturated fatty acids as a better carbon source in terms of pigment yield can be discussed with the following points. The overall saturated fatty acid composition is highest in copra, followed by peanut and then sesame. In terms of yield peanut medium has given the maximum of ~39 mg/ml.

The reason for this could be that ~50% lauric and 7% capric acid known for their antibacterial activity present in coconut could have inhibited the growth of *Serratia marcescens* in the medium thereby giving a very low yield. The second point validating the role of saturated fatty acid is that as per literature peanut has a higher concentration than sesame and the yield of prodigiosin is also higher in powdered peanut broth than in powdered sesame broth.

The role of unsaturated fatty acids as a carbon source can be disproved in this discussion. The oils are known for their high levels of unsaturated fatty acid content and a very low percentage of saturated fatty acids. From the results observed the pigment yield is 15 times more in media containing fatty acid seeds than in oils. According to Kim *et al.*, [7] oil gave a better yield over the various carbon [not fatty acid containing seeds] and nitrogen sources tested. In our case also oil has given a better yield when compared to nutrient broth and peptone glycerol broth. Even this low level could be due to the presence of low concentration of saturated fatty acid present in oils. The prodigiosin yield was higher in peanut oil broth when compared to sesame oil broth, but the level of unsaturated fatty acid is higher (~47%) in sesame oil. From this it can be proposed that the bonded fatty acids as carbon source is less accessible by *Serratia marcescens*.

Conclusions

The final conclusion based on the experimental results could be that the fatty acid form of carbon source is a better substrate for the growth of *Serratia marcescens* than sugars. Based on the comparision between the composition of the different fatty acid containing seeds and oils in terms of prodigiosin yield, the saturated form of fatty acid as a carbon source could be a better choice of carbon as the maximum yield of pigment of approximately ~39 mg/ml was seen in the case of powdered peanut broth. We have been successful in designing a economically feasible medium supporting the enhanced growth of *Serratia marcescens* and simultaneously supporting a high yield of medicinally important biopigment prodigiosin. This powdered peanut broth has been also been successful to support prodigiosin production at higher temperatures.

Methods

Isolation and characterisation of soil isolate

Pigment producing strain of *Serratia* sp was isolated from soil of Anna University campus Chennai. The bacterial isolate was subjected to regular biochemical test, lipase assay, and lactose fermentation to charaterise the genus.

The isolate was further confirmed till the species level by using primers specifically designed for the identification of *Serratia marcescens* based on the 16s rRNA sequence alignment of different environmental isolates [18]. The genomic DNA of *Serratia* sp was used as positive control and *Micrococcus sp* was used as negative control for the PCR analysis. The other control for the PCR reaction was devoid of any genomic DNA. The primers for the 417 kb product of the 16s rRNA sequence comprised the following nucleotides

Smar16SV 89–108 GGGAGCTTGCTCACTGGGTG

Smar16SWR 499–471
GCGAGTAACGTCAGTTGATGAGCGTATTA

DNA isolated from pigment producing soil isolate *Serratia* and *Micrococus* sp was quantified by spectrophotometric measurement at A_{260}, and a concentration of 1.6 **μ**g/**μ**l was used for each reaction. 5 picomoles/**μ**l of forward and reverse primer was used for each reaction. The master mix for two 20 **μ**l reaction each containing 2 **μ**l each of buffer and dNTP, 3 **μ**l each of forward and reverse primer, 0.2 **μ**l of Taq and 19 **μ**l of core water was added. To one of the reaction mixture 1 **μ**l of *Serratia* genomic DNA was added and to the other 1 **μ**l of *Micrococcus* genomic DNA was added and the other reaction had no DNA in it. The final reaction mixture was made upto 20 **μ**l. The PCR cycle was set up as step 1 denaturation: 95°C for 5 minutes, step 2 annealing: 95°C for 1 minute, step 3 annealing; 69°C for 1 minute, step 4 elongation: 72°C for 1 minute, and step 5 final elongation: 72°C for 10 minutes and reaction halt at 4°C in the end. The reaction was carried out for 35 cycles.

The PCR product was then electrophoresed in 1.2% agarose. 5 **μ**l of each positive and negative reaction was mixed with 3 **μ**l of orange G and 8 **μ**l was added to the each well. 1 **μ**l of molecular marker [100 to 1000 base pair ladder] was added to the other well. The sample was electrophoresed at a current of 50 mA for 20 minutes. The agarose gel was stained in ethidium bromide and viewed under UV light for detection of the PCR product.

Purification of pigment for mass spectrometry analysis

Serratia marcescens grown in powdered peanut broth was centrifuged at 10,000 rpm for 15 minutes using Rota 4 centrifuge and the supernatant was extracted with ethyl acetate. The pigment from the cell pellet was extracted with acetone and the extraction was centrifuged at 10,000 rpm for 15 minutes and the white pellet was discarded. The pigment extracted acetone fraction was mixed with ethyl acetate fraction and dried with sodium sulphate. The extracts were evaporated and a wave length scan was done from 200 to 700 nm. 2.5:2.5:0.5 ratio of dichloromethane, chloroform and acetone was the solvent mixture for effective separation of the impurities extracted along with the pigment by thin layer chromatography. Silica column of mesh size 80–100 was used for separation of the non colored impurity from the pigment. The dried powder at different concentration was used for plotting the standard graph versus absorbance at 535 nm. The purified sample showing a single peak absorbance at 535 nm in the UV spectrophotometer [U-3210 HITACHI] was further analysed for determination of molecular weight using mass spectrophotometer.

Composition and preparation of medias used for the comparision study

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, 2% powdered coconut in distilled water, 2% sesame oil in distilled water, 2% peanut oil in distilled water, and 2% coconut oil in distilled water were the different media used for the production of pigment from the *Serratia marcescens* isolated. 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 20 minutes. Maltose and glucose were filter sterilised and added to the respective media. Peanut, sesame and coconut obtained from provisional stores was crushed in a mixer and then sieved to fine particles before preparing the broth. The experiment was conducted in triplicates.

Growth of Serratia marcescens *in different media at 28°C, 30°C and 37°C*

50 ml of nutrient broth, peptone glycerol broth and powdered sesame seed broth was prepared in 250 ml glass conical flasks. 5% of fresh pigmented inoculum was added to each of the broth and incubated at 28°C, 30°C and 37°C to study the effect of temperature on pigment production. The pH of the medium prior to inoculation was uniformly adjusted to pH 7. Following 36 hours of incubation 1 ml from each culture broth was taken for extracellular protein estimation and one ml for pigment extraction. Protein estimation was done by Lowry's

method. Pigment production was estimated as absorbance at 535 nm.

Effect of sugars on the growth of Serratia marcescens *in nutrient broth and powdered sesame seed medium at 28°C, 30°C and 37°C*

Nutrient broth with 0.5% maltose, nutrient broth with 0.5% glucose, 2% powdered sesame seed with 0.5% maltose in distilled water and 2% powdered sesame seed with 0.5% glucose in distilled water was prepared as 50 ml in 250 ml conical flasks. The pH of all the broth was maintained at 7. Maltose and glucose were filter sterilised and added to the respective media before inoculation. 5% of fresh pigmented *Serratia marcescens* was added to each of the broth. The flasks were incubated at 28°C, 30°C and 37°C to study the effect of sugar as a carbon source on growth of bacteria and pigment production. Following 36 hours of incubation 1 ml from each culture broth and the control medium was taken for extracellular protein estimation and one ml for pigment extraction.

Comparative study of different fatty acid containing seeds as source of substrate for Serratia marcescens

5% of fresh pigmented *Serratia marcescens* was added to 2% powdered sesame seed in 50 ml of distilled water, 2% powdered peanut seed in 50 ml of distilled water and 2% powdered coconut in 50 ml of distilled water. The standard size of the flask taken was 250 ml. The control flask for each medium was without the inoculation. The pH of all the above media were maintained at 7.0. The flasks were incubated at 28°C, 30°C and 37°C to study the effect of substrate on growth of bacteria and pigment production. Following 36 hours of incubation 1 ml from each culture broth and the control medium was taken for extracellular protein estimation and one ml for pigment extraction. The optical density could not be measured due to the interference of powdered broth.

Comparative study of different fatty acid containing seed oils as source of substrate for Serratia marcescens

5% of fresh pigmented *Serratia marcescens* was added to 2% sesame seed oil in 50 ml of distilled water, 2% peanut oil in 50 ml of distilled water and 2% coconut oil in 50 ml of distilled water. The standard size of the flask taken was 250 ml. The control flask for each medium was without the inoculation. The pH of all the above media were maintained at 7.0. The flasks were incubated at 28°C, 30°C and 37°C to study the effect of substrate on growth of bacteria and pigment production. Following 36 hours of incubation 1 ml from each culture broth and the control medium was taken for extracellular protein estimation and one ml for pigment extraction. The optical density could not be measured due to the interference of oil in the medium.

Comparative study on effect of different temperatures on the growth of Serratia marcescens *and prodigiosin production in nutrient broth and powdered peanut seed*

5% of *Serratia marcescens* inoculum was added to 50 ml of nutrient broth in three flasks. Each of the flask was incubated at 28°C, 30°C and 37°C at 180 rpm. Nutrient broth without the inoculum was used as the control. 5% of *Serratia marcescens* inoculum was added to 50 ml of powdered peanut broth in three flasks. Each of the flasks was incubated at 28°C, 37°C and 42°C at 180 rpm. Powdered peanut broth without the inoculum was used as the control. Following incubation at 42°C, 5% of inoculum from this flask was added to fresh 50 ml of powdered peanut broth and incubated at 42°C at 180 rpm. Following the second generation at 42°C, 5% of inoculum from this flask was added to fresh 50 ml of powdered peanut broth and incubated at 28°C at 180 rpm to show the reappearance of pigment production.

Pigment production and extracellular protein secretion pattern of Serratia marcescens *in three different media*

50 ml of nutrient broth with 0.5% maltose, 1% powdered sesame seed in 50 ml of distilled water, and 1% powdered peanut seed in 50 ml of distilled water was taken in 250 ml conical flasks as six sets each. 2% of fresh pigmented inoculum was added to each of the six set of flasks for the three different media. The control flasks had 50 ml of the respective media in 250 ml conical flask with no inoculum in it. The flasks were incubated at 30°C at 180 rpm and monitored for 36 hours. From the zero hour of inoculation one ml sample was drawn from each of the medium from the first flask numbered one, and after two hours of incubation one ml was taken from each of the medium from the second flask numbered two and so on till the sixth flask. Following twelfth hour of incubation one ml of sample from each medium was again taken from the first flask and continued till the sixth flask following 22nd hour of incubation. Following 24th hour of incubation one ml of sample from each of the medium was taken from the first flask and continued till the sixth flask for 34th hour of incubation. The 36th hour of sample for each of the medium was taken from the first flask. Thus at the end of the experiment only four ml of sample was withdrawn uniformly for each of the medium from the first flask containing 50 ml media and three ml from the rest of the five flasks for each of the medium containing 50 ml. The samples withdrawn were assayed for extracellular protein and pigment production. Protein and prodigiosin content was expressed as mg/ml as calculated from the standard graphs. The growth of the organism could not be measured due to the interference of the powdered fatty acid seed in the broth.

Authors' contributions

The idea for the comparitive study of medium for enhanced prodigiosin production was conceived by PG and AG. The work was designed and carried out by AG as a part of her Ph.D thesis work. GM has contributed with her valuable scientific suggestions in drafting the manuscript. NAK has contributed as a training student for her M.Sc training programme. All authors have read and approved the final manuscript.

Acknowledgements

Anuradha Giri would like to thank the Council of Scientific and Industrial Research for the Senior Research Fellowship. P. Gautam would like to thank UGC for the financial support.

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