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ORIGINAL RESEARCH PAPER

ISOLATION AND CHARACTERIZATION OF A MICROBIAL PIGMENT OBTAINED FROM *SERRATIA MARCESCENS* AS A NATURAL FOOD COLOURANT

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The use of naturally sourced pigments in the food industry is limited due to their instability when exposed to processes and parameters such as light, pH, and high temperatures. Research has moved towards exploiting pigmented microorganism as sources of potential natural colorants, with the color red representing the largest market segment in the color industry, utilized in various food applications. This study was aimed isolation and characterization of Prodigiosin obtained from *Serratia marcescens* for potential application as a food colorant. Optimization results were achieved by cultivation in lysogeny broth (Lb) at 25ºC for a duration of 120h at pH 6.5, prodigiosin showed a gradual reduction in pH stability as well as a rapid change in color upon the addition of respective buffers due to protonation and deprotonation of respective groups. Instability to light due to chemical reaction such as oxidation and metal ion loss and instability at high temperatures was due to destruction of pyrrole group which is responsible for the reddish characteristic color. Results concluded that prodigiosin would be more applicable for utilization as a colorant in food applications within a temperature range of 0 to 20ºC and pH of range 2 to 7.

Keywords: Prodigiosin, *Serratia marcescens*, pigment stability

Introduction

An existent problem in the food industry is the limitation of naturally derived red colorants that are stable when exposed to processing parameters such as heat or pH (Nachay, 2014). Color is suggested to play a crucial role in determining both the quality and acceptability of foods and beverages. Due to legislative demand and consumer concerns regarding synthetic colorings, the demand for naturally derived colorants has increased. Although red is suggested to be a complex color to produce, it occupies the majority share in the color segment of the market (Dabas and Kean, 2014).

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While there is an increased demand for natural colorants, they were found to display less stability when exposed to light, temperature and pH (Malik *et al*., 2012). The two-basic sources from which natural color can be derived are plants and microorganisms. However, previous studies utilizing bio-colorants from plant sources depict various levels of instability when exposed to different processing parameters. Anthocyanins were found to exhibit instability when exposed to pH>4.5, above the pH range the pigment changes hue from red to blue and were deemed unsuitable for acidic applications.

Betanin also commonly known as beetroot red is a root crop which is consumed globally. The red colorant obtained from betanin significantly degrades when exposed to parameters such as light, heat, and oxygen (Chandran *et al*., 2014). Alternatively, carmine derived from cochineal insects was proposed, however due to the extract possessing proteins, it was considered hazardous to humans with the potential to cause severe allergic reactions and was ruled out by regulatory bodies. Thus, the need for a natural, stable, cost-effective and clean-labelled red colorant occurred. The aim of the study was to isolate and characterize pigment isolated from *S. marcescens* strain with potential utilization in a food product as a natural colorant in order to optimise growth conditions for increased yield and pigment quality from *S. marcescens,* to determine the stability of the extracted pigment against pH; temperature; light and degradation kinetics and to apply the colorant to a food product.

Various microorganisms have the ability to produce arrays of pigments and have potential to be utilized as natural food colorants (Malik *et al*., 2012). Pigments obtained from microbes are considered advantageous in comparison to synthetic colors or pigments sourced from plants due to efficient processes of production (Kumar *et al*., 2015). As proposed by Dufossé *et al*. (2005), the cultivation and growth of microorganisms can be facilitated on relatively cheap culture media as they are independent of climatic conditions. Aside from the array of shades, microbial pigments are considered biodegradable and environment-friendly. Microbial pigments have been observed to have medical and clinical potential such as antioxidant potential, immunosuppressive properties, and utilization as treatment for diabetes mellitus and cancer (Kumar *et al*., 2015). The most common microorganisms capable of pigment production are fungi; yeast; algae and bacteria.

Since microorganisms adversely have more versatility and productivity than any other forms of life, encompassing relatively large gene strands, research such as genetic engineering enables manipulation to generate increased pigment production. Research and development of microbial pigments are relatively new, thus there are many opportunities to further source, cultivate and reproduce stable natural colorants acceptable for industrial production (Kumar *et al*., 2015).

Serratia marcescens is an opportunistic Gram-negative bacillus and is a member of the *Entrobacteriaceae* group. *S. marcescens* pigment-producing bacterium can be sourced from ecological niches such as soil, plants, water, air, and in animals (Boone and Castenholz, 2001). Previous research is indicative of *S. marcescens* extracellular enzyme efficacy; these enzymes depict abilities of the destruction of

chitin which mainly comprises in the cell walls of fungi. Chitinolytic enzymes possess potential for both industrial and agricultural application. Scientific literature present that *Serratia marcescens* produces color varying pigmentation such as red, white and pink as a function of temperature, nutrient content in media, and contact with ultraviolet light (El-Bialy and El-Nour, 2015).

Prodigiosin is a pigment that can be sourced from microorganism (Khanafari *et al*., 2006): *Serratia marcescens*, *Vibrio psychoeytrus*, *Rugamonas rubra* and *Streptoyverticillum rubrireticuli.* Prodigiosin is a secondary metabolite that exhibits a distinctive red color. It was reported to have no direct role in development or reproduction of the cell; however it was suggested to serve as a defence mechanism. Structurally, prodigiosin belongs to the chemical family of tripyrrole which was reported to contain a common 4-methoxy, 2-2 bipyrrole ring. The synthesis of the pigment is suggested to be made up of two pathways, where mono and bipyrrole precursors are separately synthesized and then combined to form prodigiosin (Boger and Patel, 1988). The production of the pigment was suggested to essentially vary depending on the species type, duration of incubation and temperature (Giri *et al*., 2004). Prodigiosin is said to have and exhibit antifungal, antibacterial, and antiprotozoal activities (Moraes *et al*., 2009). It is also considered to be antimalarial, antibiotic, immunosuppressive and anticancer (Suryawanshi *et al*., 2014). These properties make prodigiosin research appealing. Montaner *et al*. (2000) examined the effects of prodigiosin on haematopoietic cancer cells and assessed for possible cytotoxic activity. The study concluded that prodigiosin has potential in being utilized as an anticancer drug. The prodigiosin pigment was characterized and the ability to dye different fabrics tested. Therefore, the application behind the experimental design targeted the textile industry. Many theories support the use of prodigiosin in the medical field as prodigiosin depicts many therapeutic attributes. However it's potential to be utilized as a natural colorant in foods has yet to be explored. This study attempted to determine the extracted pigments stability potential and its suitability as a natural colorant when applied to a food product.

Materials and methods

Optimization of Bacterial Growth

A strain of *Serratia marcescens* was obtained from the Durban University of Technology, Microbiology Laboratory. The strain was preserved by preparing an overnight liquid culture incubated at 28°C for 18 h, thereafter 500 µL of the overnight liquid culture with an absorbance of 0.6 OD_{600} was transferred into 2 mL cryovial tube containing 500 µL of 50% glycerol. The prepared stock culture was stored at -80°C. Growth of *Serratia marcescens* was observed by transferring a full loop inoculum into a 50 mL flask containing nutrient broth (Sigma-Aldrich). The inoculum was incubated at 28°C for 24 h (exponential phase). Following incubation, 1 mL of a 0.6 OD_{600} inoculum was transferred into 100 mL nutrient broth. Growth of the bacteria was observed over a duration of 24 h to observe when pigment production becomes visible. Optimization was carried out using the

method of Ramani *et al*. (2014) with modifications. As per literature the following factors: pH, duration, temperature, and selective media play a role in influencing pigment production in *S. marcescens*. Thus, optimization was conducted capitalizing on the various mentioned factors. Media that was selected for the optimization was nutrient broth and lysogency broth (Lb) (Casullo de Araújo *et al*., 2010).

The pH of the media were adjusted to the pH values of 4, 6, 5, 7, and 9 units utilizing 1M of Hydrochloric acid (HCl) and 1M of Sodium hydroxide (NaOH) with the control being at pH 7. According to a study conducted by Das *et al*. (2018), *S. marcescens* depicted viability in pH ranging from 5 to 9, however the production of prodigiosin was only noted in pH ranging between 5 to 8. A study conducted by Elkenawy *et al*. (2017) revealed that pigment production was higher at pH 9 in comparison to pH 5. Since pH plays a crucial role in the development of prodigiosin, the study focused on utilizing a broader pH range to justify the effect of pH on pigment production. One mL of 0.6 OD_{600} inoculum was transferred aseptically into flasks containing 100 mL of nutrient broth and lysogeny broth followed by incubation at the 25 and 30°C for 120 h. According to a study conducted by Williams *et al.* (1971), the production of prodigiosin is favoured between 25-28°C, over a 7 day period. Casullo de Araújo *et al*. (2010) found that at temperatures exceeding 30°C, pigment production in *Serratia marcescens* ceased, and colonies were characteristically creamy white in color. This observation suggested that at temperatures exceeding 30°C there is a possible loss in enzyme activity, which results in no prodigiosin production.

Observations were made at the following intervals: 24, 48, 72, 96 and 120 h. Estimation of prodigiosin was obtained utilizing a formula by Mekhael and Yousif (2009) as presented in equation 1. The absorption of the bacterial cells was observed prior to extraction. The observations were carried out at the mentioned respective intervals.

$$Prodigiosin\ unit/cell = \frac{OD\ 533 - (1.381\ x\ OD\ 600)]\ x\ 1000}{OD\ 600} \quad (1)$$

OD_{533} is optical density of the prodigiosin at λ= 533 nm

OD_{600} is optical density of the bacterial cells at λ= 600 nm; 1.381 is a constant

Extraction of pigment

Cultivation and isolation was carried out as per optimization results obtained. Pigment extraction was carried out utilizing the method by Nakashima *et al*. (2005) with modifications. In this study, the type of media and period of incubation differed. *Serratia marcescens* was cultivated in 100 mL lysogeny broth (Lb) at 28°C for 96 h. An equal volume of methanol was added and the mixture was vortexed. After centrifugation at 10000×g for 30 min, the pellet was discarded and the supernatant evaporated at 45-50°C. Then, 10 mL of chloroform was added with the addition of 95% ethanol:water in the ratio of 1:4 (v/v). The resultant mixture was freeze-dried to evaporate the solvents and obtain the powered pigment. A presumptive test was conducted as per Ding and Williams (1983), by the addition

of 1M HCl or alternatively 1M of NaOH in crude pigment indicating the presence of prodigiosin upon a distinctive color change.

UV-VIS analysis was conducted on the extracted pigment by dissolving it in methanol (Vora *et al*., 2014). The scan was conducted between 200 to 800 nm, to determine the maximum absorbance spectra. In this case the blank was methanol.

Purification by thin layer chromatography (TLC)

Thin layer chromatography was preformed utilizing the method by Vora *et al*. (2014). The extracted pigment was dissolved in methanol and then applied by a capillary tube on silica gel. Chloroform:methanol (95:5 v/v), was the solvent system used. The chamber and system were kept for 20 min until equilibration. The sheet was removed and thereafter air dried. The retention factor (RF) was calculated using equation 2:

$$Rf = \frac{Distance\ travelled\ by\ the\ compound}{Distance\ travelled\ by\ the\ solvent\ front} \quad (2)$$

Pigment stability

Evaluation of pH stability

To evaluate extracted pigment stability, the extracted pigment was exposed to various pH values and was conducted in accordance to the method by Namazkar and Ahmad (2013). The crude pigment (100 mg/L in methanol) was recorded by varying the pH between 2 and 11 utilizing 1M of NaOH or 1M of HCl buffers to achieve the required pH range. pH 7 was utilized as a control and observations were conducted over a duration of 120 h. Absorbance was recorded utilizing wavelength λ= 200-800nm at intervals 0; 24; 48; 72; 96; and 120 h.

Evaluation of temperature stability

To observe the effect of temperature on the pigment, 10 mL of crude pigment (100 mg/L in methanol) was dispensed into test tubes and kept in a water bath at various temperatures ranging between 20, 40, 60, and 80°C. The absorbance was read at 20 minute intervals for a duration of 120 min.

Evaluation of light stability of pigment

Extracted pigments response to light stability was evaluated utilizing the method by Namazkar and Ahmad (2013). A quantity of 10 mL of crude pigment (100 mg/L in methanol) was dispensed into test tubes and exposed to fluorescent light at 1.5 meters away from the source. Positive control was stored in the absences of light. Absorbance was measured at 533 nm every 24 h for a duration of 120 h.

Thermal degradation kinetics

The ability of natural pigments to retain their color when exposed to thermal processes remains a major challenge in food processing. Undesirable visual color and degradation of pigment structure was found to affect sensory attributes. Natural red pigments such as anthocyanins, lycopene and betacyanins have health promoting compounds (Leong *et al*., 2018). Most natural pigments depict color stability below 50°C and were observed to begin degrading when exposed to

temperatures exceeding 50°C. A study conducted by Herbach *et al*. (2006), depicted that betalains when heated above temperatures exceeding 50°C, display rapid loss in color, which make their use as food colorants unsuitable. Thermal degradation of prodigiosin was carried out according to method by Fernández-López *et al*. (2013). Crude pigment (100 mg/L methanol) was thermally heated in a water bath at temperatures of 50, 70 and 90°C for 5 h. The absorbance was monitored at 533 nm. After heating, the samples were placed in an ice bath to cool in order to facilitate a stop in thermal degradation. Absorbance was recorded for the samples and the thermal degradation was calculated as follows (Equation 3) using a method established by Van den Broeck *et al*. (1998).

$$ln = \frac{Ai}{Ao} - kt \qquad (3)$$

Where, A denotes residual absorbance after treatment,
A_0 is initial absorbance,
t and k represent time in hours
Reaction rate constant $(h-1)$ at a specific temperature
Half -life values were determined by plotting A_0/A ratio vs the thermal period of heating.

Colorimetry Evaluation of pigment

The colorimetry assay was determined utilizing method by Fernández-López *et al*. (2013). Color was monitored using the CIELab parameters. The parameters were obtained using a colorimetric reader. The differences in $\Delta E = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2}$ between the sample and a red synthetic standard.

Results and discussions

Growth of Serratia marcescens strain

The culture was monitored over a 24 h period at a temperature of 28°C to observe when pigment production becomes visible and to establish factors influencing the production of pigment.

The pigment prodigiosin produced by *S. marcescens* can be classified as a secondary metabolite. Figure 1 reveals the multiplication dynamics of bacterial cells which correlates to the synthesis of prodigiosin. As discussed by Bu" Lock *et al*. (1965), the maximum production occurs in the stationary growth phase. Growth and synthesis of the pigment usually occur in two distinctive phases, namely the trophic phase said to be the nourishment phase which enhances cellular growth and the idiophase which is the individual phase, a period in which secondary metabolites, in this case prodigiosin, is produced. Growth can be influenced by factors such as temperature and carbon/nitrogen sources which assist in the regulation of the production of the pigment. Characteristically prodigiosin production is regulated in senescent cells during the stationary phase. *Serratia marcescens* is known to produce characteristic bright red colonies (Shalinimol, 2012).

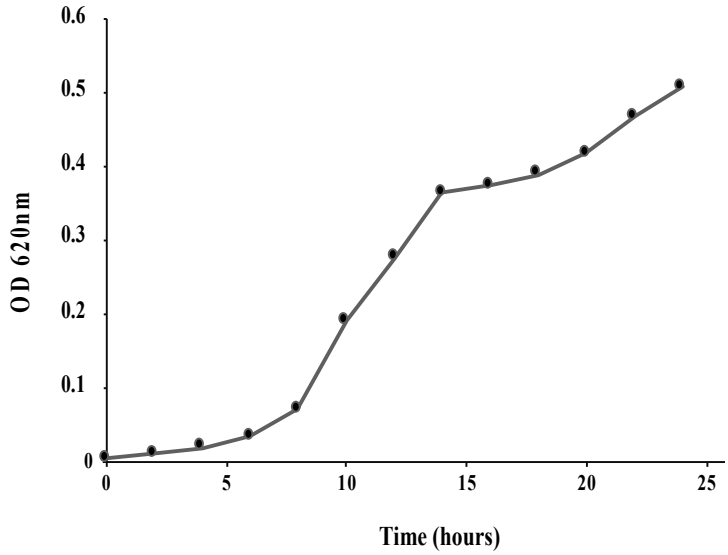


Figure 1. Growth curve of *Serratia marcescens* strain

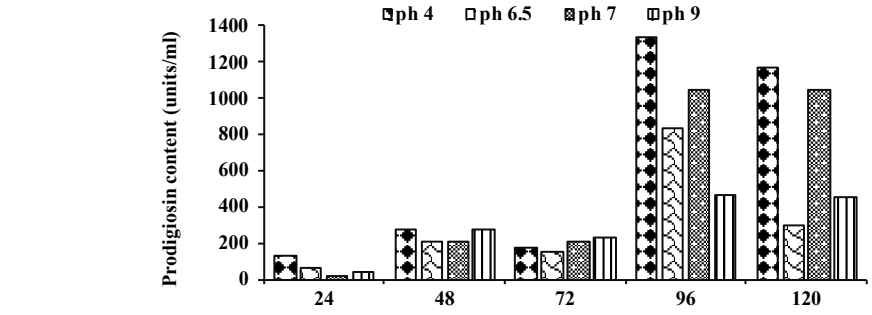
Prodigiosin pigment produced by bacteria *S. marcescens* was suggested to develop deep red color when the culture is aged (older than 8 days) and media possesses glycerol (oil) (Shalinimol, 2012). In order for *S. marcescens* to develop and yield the desired deep red pigment a low phosphate and glucose media is required (Venil and Lakshmanaperumalsamy, 2009). Although specific synthetic media was developed and utilised for the cultivation of *S. marcescens* growth and prodigiosin production, many studies conducted thus far have facilitated cultivation in media such as nutrient broth, maltose broth, lysogeny broth and peptone glycerol broth (Giri *et al*., 2004).

Optimization of prodigiosin

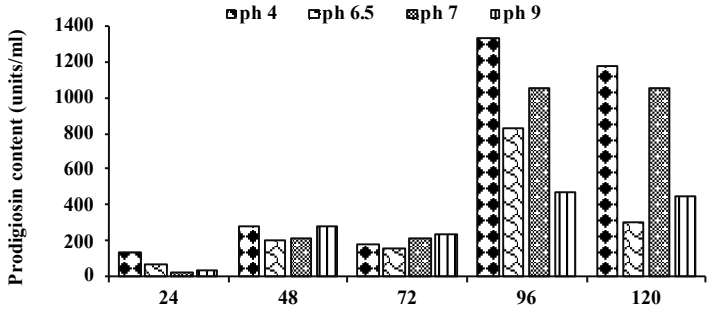
There are various factors that influence the production of prodigiosin, such factors include incubation temperature, pH, duration of incubation and selective media which were simultaneously examined (Figure 2).

Media Composition

Nutrients are required by microorganism as an energy source enhancing growth and reproducibility. This study examined prodigiosin production in nutrient broth (Nb) and lysogeny broth (Lb). As seen in Figure 2c there was a significant increase in prodigiosin production (1332.67 units/cell) in Lb broth at 25ºC, for 120 h. As seen in Figure 2, prodigiosin production occured during the stationary phase in both Lb broth and Nb broth. The results obtained in Figure 2c correspond to similar results obtained in a study conducted by Ramani *et al*. (2014). One of the proposed reasons as to why pigment production is significant higher in Lb broth rather than nutrient broth is due to the composition of nutritional ingredients.



a)



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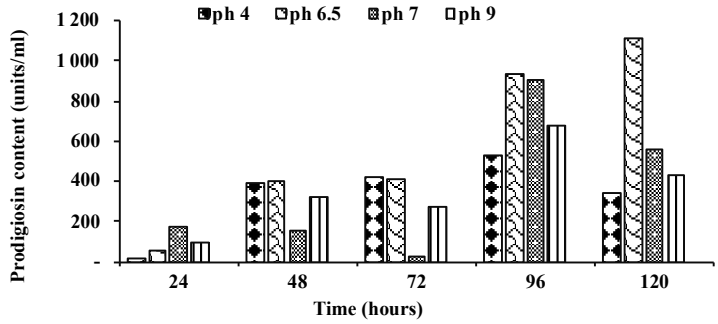
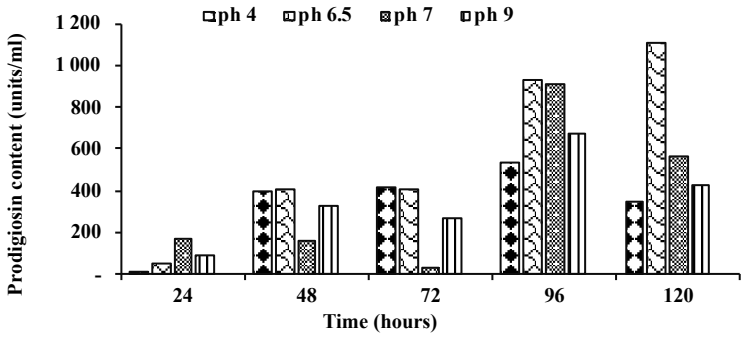


c)



d)

Figure 2. Optimization of prodigiosin production by varying combined stress factors. Bars denote mean ± standard deviation (n=3). a) *S. marcescens* in nutrient broth (Nb) at 25ºC; b) *S. marcescens* in nutrient broth (Nb) at 30ºC; c) *S. marcescens* in lysogeny broth (Lb) at 25ºC; d) *S. marcescens* in lysogency broth (Lb) at 30ºC.

Compounds that serve as carbon source, nitrogen source and inorganic salts are influencing factors in pigment production. Typical nutrient broth contains peptone, meat extract, sodium chloride (NaCl), and glucose in comparison to Lb media which contains tryptone, yeast extract and NaCl. Tryptone and yeast extract in Lb media are sources of nitrogen which highly influence the growth of cells as well as pigment production of prodigiosin. Trypton encourages higher metabolic activity increasing pigment production (Wei and Chen, 2005). A study conducted by Gulani *et al*. (2012), established that the role of glucose in production of prodigiosin was critical. Glucose being the carbon source in Nb broth was found to produce relatively lower yield. Glucose was suggested to inhibit pigment production as it causes acidification of the media. The relatively lower yield in prodigiosin production is due to the presence or addition of glucose which has also been reported by various other studies (Oller, 2005; Gulani *et al*., 2012).

Growth rates and robust prodigiosin production is well supported by Lb medium. Wei and Chen (2005) further went on to describe that increase in prodigiosin production can be facilitated by modification of the composition of Lb media. It was found, when tryptone and yeast extract were increased, that there was a profound increase in pigment yield, especially when sodium chloride (NaCl) levels were reduced, suggesting that NaCl may pose an inhibitory effect on pigment production. The presence of excessive NaCl concentrations are said to stop or slow down the activity of condensing enzymes which is responsible in the regulation of the terminal condensation step in prodigiosin production (Silverman and Munoz, 1973).

pH

pH influences production of prodigiosin by either affecting the nutritional properties of the media such as the solubility of the nutrients, transportation, and ionization, in addition to influencing the overall stability of the pigment. Maximum production of prodigiosin (1332.67 unit/cell) was observed at pH 6.5 in Lb broth at 120 h. As seen in Figure 2c prodigiosin is also capable of being synthesized at pH 7 which was reported in many studies. The pH range supporting the production of prodigiosin was reported to be between 5 to 8 (Das *et al*., 2018), however as seen in Figure 2a and 2d, *S. marcescens* in Nb broth at pH 4 also produces prodigiosin at varying lower levels. It was suggested that, when the selected media becomes too acidified, an inhibitory effect limits the use of the carbon source. This causes a reduction in levels of prodigiosin production (Sole *et al*., 1997). This can be observed in Figure 2b and 2d. A similar effect is observed when there is an increase in alkalinity of media, which results in decrease of cell viability and production of prodigiosin (Das *et al*., 2018). It may be concluded that pH is a determining factor contributing to either an increase or decrease in prodigiosin yield.

Duration of pigment production

Maximum prodigiosin production was previously reported to occur in the 96 h in a study conducted by Ramani *et al*. (2014). The present study revealed that maximum production of pigment was observed after 96 h, as seen in Figure 2c.

This result was supported by the commencing of an increase in pigment production which was observed to occur towards the stationary growth phase, and the quantity of pigment reached peaks after 4 days of incubation which can be observed in Figure 2. According to a study conducted by Williams (1973), following 24 h of incubation there was little increase in cells. Between 48 and 96 h maximal production of prodigiosin occurs due to the end of exponential phase. It was observed that prodigiosin production generally occurs at the end of exponential growth phase, during the more stationary growth phase where cells cease to divide (senescent cells). A study conducted by Elkenawy *et al*. (2017) revealed that incubation period ≥6 days produced better yield of prodigiosin and the pigment appeared brighter and intensified.

Temperature

Temperature is a critical factor, although microorganism may be supplied with the necessary nutrients, its growth is highly dependent on the incubation temperature. The temperature determines and influences the cells ability to absorb nutrients required for production of pigment and also influences the degradation and denaturing of cells. Figure 2a and 2b shows that *S. marcescens* incubated at a temperature of 25°C, at varying pH produced quantitatively higher prodigiosin in comparison to cultivation at 30°C as seen in Figure 2c and 2d. The growth of most bacteria are facilitated at a temperature of 37ºC, however in *S. marcescens* at 37ºC the growth of bacteria is favored over the production of pigment. Various studies showed that at temperatures higher than 30ºC *S. marcescens* appeared white and there was a complete block in prodigiosin production (Haddix and Werner, 2000; Sundaramoorthy *et al*., 2009). The white pigment, which is suggested to be non-profiling, could be a response to a lost in activity of enzymes that assist in producing the distinctive red color. Biosynthesis was established over a narrow temperature range of 24 to 30°C (Casullo de Araújo *et al*., 2010).

Extraction of pigment

Extraction was successfully carried out by the use of 95% methanol. The pigment extracted in the solvent did not display a change in color when dissolved. In a study conducted by Vora *et al*. (2014) was examined the extraction of the pigment with different solvents including chloroform, ethyl acetate, acetone and methanol. Acetone and methanol proved to have the capacity to extract the pigment from the cell, however the largest extraction of the pigment was shown to be achieved in methanol. A presumptive test was carried out on the extracted pigment, to qualitatively determine if the pigment extracted was prodigiosin. A sharp pink color change was observed upon the addition of 1M HCl and a distinguishing yellow when 1M of NaOH was added. Both indicated a positive result. This corresponded to results obtained in a study by Gulani *et al*. (2012).

Spectral analysis and TLC purification

The pigment was subjected to spectral analysis and the maximum wavelength at which prodigiosin peaked was at 533 nm, which corresponded to results reported by Nakashima *et al*. (2005). The spectrophotometric data obtained for the red

pigment complex was in agreement with most studies conducted. The wavelength range where prodigiosin was reported to peak was 499, 530, 533 and 535 nm. However it was also found to be very depended on the solvent utilized for the extraction of the pigment. The purification was carried out using the solvent system chloroform:methanol (95:5 v/v). The Rf value obtained was 0.875 which corresponded to results obtained by Vora *et al*. (2014) who also obtained various shades of fractions such as green, blue, yellow and dark orange with respectively different Rf values.

Bunting (1940) also suggested the presence of purple fractions, resulting from the purification of prodigiosin. These different fractions are most often a result of the elution solvents as well as also the type of strain isolate.

Stability of extracted pigment

pH stability of prodigiosin

Upon the addition of the respective buffers a color change was noted - from red to pink in acidic condition and from red to yellow as the pH increased. Alkaline conditions lead to faster reduction in prodigiosin stability as seen in Figure 3 at pH 11. These color changes corresponded to a study conducted by Namazkar and Ahmad (2013). At extreme acidic pH, the protonation of pyrrole group occurs on one of the carbon atoms of the second position in the ring and not on nitrogen atom, therefore becoming nonaromatic (Struchkova *et al*., 1973). Fading in prodigiosin is caused by this protonation. In extreme alkaline pH, the OH- groups deprotonates the amine group in the structure thus resulting in the formation of an anion. Both conditions proceed to destroy the very conjugated system of double bonds and hence being responsible for pigment degradation (Namazkar *et al*., 2013). As mentioned upon the addition of the respective buffers there was an immediate color change. It was observed that in conjunction to a color change the spectral peak of prodigiosin changed and depicted various peaks as seen in Figure 4. This was in agreement to a study conducted by Faraag *et al*. (2017). It is suggested that prodigiosin exists in two dissimilar forms. However, this is dependent on the hydrogen ion concentration of the solution. A sharp spectral peak within the range of 535 nm was observed when the pigment was subjected to acidic conditions, further acidic conditions leading to the pigment depicting a spectral peak at 510 nm. The pigment exhibited orange to yellow color in alkaline conditions peaking at 470 nm as discussed by idem.

Evaluation of prodigiosin stability to light

Chemical mechanisms that trigger fading of color are highly depended on the pigments nature. Oxidation, a combination of oxidation and hydration, and metal ion loss and oxidation are accelerated when pigments are exposed to light irradiation. As seen in Figure 5, when prodigiosin was irradiated with light there was significant reduction in stability over the respective duration as compared to when prodigiosin was kept in the absence of light. A gradual reduction in pigment stability when exposed to light was also reported by Namazkar and Ahmad (2013). In the presence of light, the pigment molecules are described as excited and in an

energetic state suitable to increase their ability to react. This reaction leads to the destruction of color due to the delocalization of the pyrrole group in prodigiosin, which contributes to the characteristic reddish color.

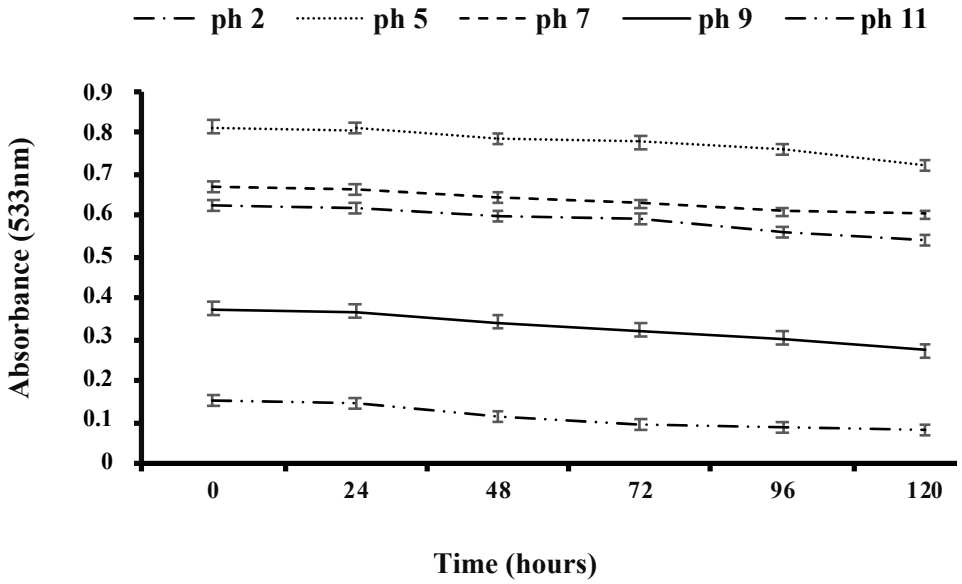


Figure 1. Gradual reduction in pH stability of crude prodigiosin pigment upon the addition of respective buffers. Data represents mean ± standard deviation (n=3).

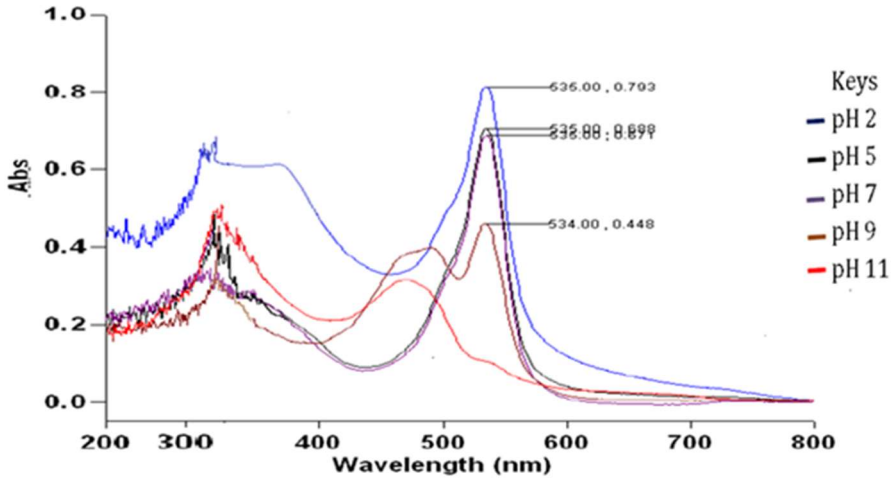


Figure 2. Spectral peaks presented by prodigiosin at various pH values

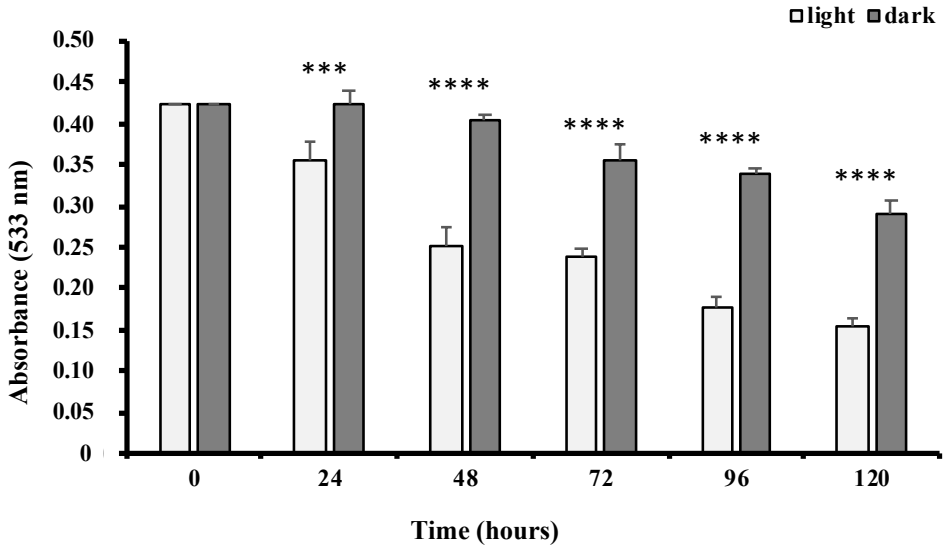


Figure 3. Effect of light exposure on prodigiosin stability. Bars denote mean ± standard deviation (n=3).

Evaluation of temperature on prodigiosin stability

Prodigiosin is fairly stable over a temperature of 20-60°C as seen in Figure 6. However, as the temperature increased a reduction in color stability was observed. This reduction in color could be due to the supposed destruction of pyrrole groups forming part of the prodigiosin structure. Temperature reduction was observed due to the high sensitivity of the monopyrrole and bipyrrole (Williams, 1973). These results were in correspondence to a study conducted by Rocha *et al*. (2012). The instability of natural extracted pigment to temperature often results to the alteration of their chemical structures which thereafter poses an effect on their absorption spectra as well as their color properties (Fernández-López *et al*., 2013).

Thermal degradation kinetics

As seen in Figure 7 direct relationship can be observed between the temperature and the pigment stability: as the temperature increases, the pigment stability is reduced. In a study conducted by Fernández-López *et al*. (2013), it was suggested that degradation pattern was highly dependent on temperature and time. Observations made suggest that high thermal conditions bring about visible reduction color changes in the pigment.

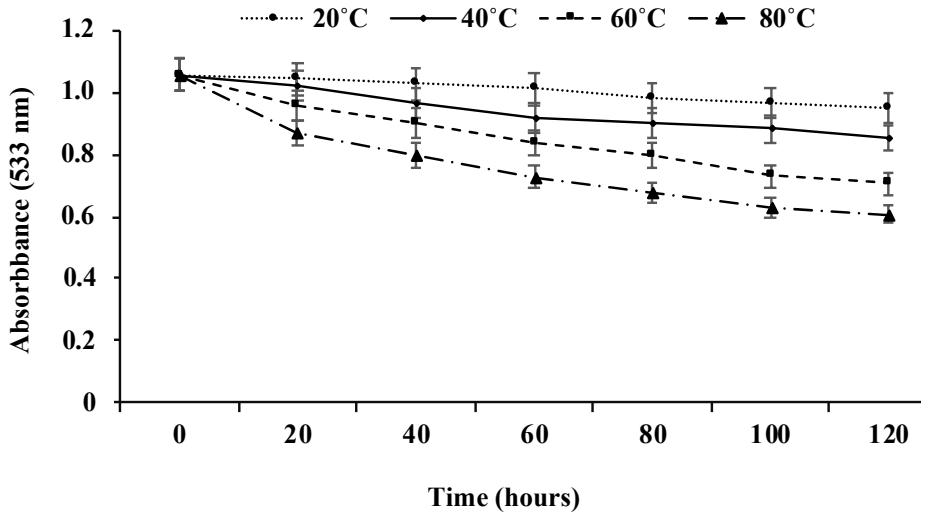


Figure 4. Prodigiosin stability at various temperature range over time. Data represents mean ± standard deviation (n=3).

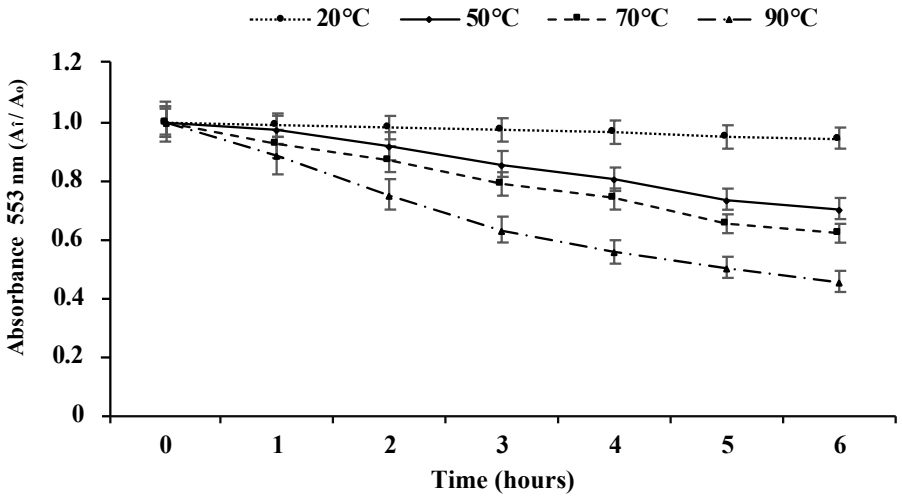


Figure 5. Thermal degradation of prodigiosin at 20, 50, 70 and 90°C. Data represents mean ± standard deviation (n=3).

Colorimetric evaluation of prodigiosin

According to Whetzel (2015), ΔL* value represents lightness vs. darkness, Δa* value represents the level of redness to greenness, and Δb* value is an indication of yellowness *versus* blueness. The LAB values are parameters which are indication of the overall difference in color between the sample and standard (ΔE). ΔE is an overall color difference that classifies the sample as either a pass or fail. When ΔL* is lower than 50, the sample is regarded as being dark, when higher (51-100), the sample is classified as being light. As seen in Table 1, prodigiosin presented a low

ΔL* value (1.11±0.5), indicating that prodigiosin is darker. The Δa* value (0.87±0.07) indicated that prodigiosin was overall red, while Δb* value indicated that prodigiosin (1.40 ± 0.9) had more yellowness. The overall color difference between prodigiosin and the standard was 0.2. Whetzel (2015) suggested that when ΔE ≤ 1, the sample is closely related to the standard. ΔE value generally range from 0 to 100. In this case the difference showed that prodigiosin was closely related to the standard.

Table 1. Clorimetric analysis of prodigiosin in comparison to a commercial synthetic red colorant

	ΔL*	Δa*	Δb*	ΔE
Prodigiosin	1.11±0.5	0.87±0.07	1.40±0.9	1.98
Ponceau 4R (Standard)	1.22±0.03	1.44±0.005	0.07±0.01	1.78

Data represents mean ± standard deviation (n=3)

Conclusions

Like all naturally sourced pigments, prodigiosin also depicted gradual reduction in stability parameters such as pH, light and temperature. The color degradation observed in prodigiosin can be attributed to the destruction of its natural chemical structure. However, this study does not condone the use of prodigiosin in foods as a potential natural colorant. Modification of prodigiosins chemical structure or methods such as encapsulation of the colorant could support its addition to foods.

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