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Effect of physical parameters in enhancing prodigiosin production and anti-MRSA activity of marine bacterium, *Serratia marcescens* IBRL USM84

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ABSTRACT

Aims: Marine bacteria have been reported to produce potential natural pigment with pharmaceutical properties and their growth can be manipulated in the laboratory to increase pigment production and their antimicrobial activity. Hence, this study aimed to enhance the prodigiosin production in *Serratia marcescens* IBRL USM84 by improving physical conditions.
Methodology and results: The quantification of the pigment produced by *S. marcescens* IBRL USM84, bacterial cell growth, and its antibacterial activity in the broth medium were determined using a spectrophotometry method. Meanwhile, the antibacterial effect of red pigment on MRSA cells was observed under a scanning electron microscope (SEM). This marine isolate produced the highest yield of prodigiosin (6.95 µg/mL) when cultivated in marine broth with the addition of 0.2% of agar, 25 °C incubation temperature, initial medium pH of 7, 150 rpm of agitation speed for 48 h of cultivation time under light illumination. There was an increment of 151.81% in prodigiosin production after enhancement compared to before the enhancement of cultural conditions. SEM observations revealed that severe damage to the cell's morphologies was exposed to red pigment as indicated by the formation of small dents, which led to completely collapse and eventually, cell death.
Conclusion, significance and impact of study: A positive correlation between pigment production and antibacterial activity was observed in the present study. The results supported the fact that marine bacteria are a reservoir of various pigments with antimicrobial properties. Also, the pigment production by *S. marcescens* and its antibacterial activity were significantly influenced by physical parameters.

Keywords: Marine isolate, physical parameter, prodigiosin, *Serratia marcescens*

INTRODUCTION

Marine microorganisms are believed to produce natural pigment with significant bioactive substances. Bacteria are one of the major groups that have been reported to produce natural pigment and various types of pigments found in marine heterotrophic bacteria, including carotenoid, flexirubin, xanthomonadine, and prodigiosin (Velmurugan *et al*., 2020). The rapid emergence of new infectious diseases and antibiotic-resistant bacteria such as vancomycin-resistant enterococci (VRE) and methicillin-resistant *Staphylococcus aureus* (MRSA) is getting worse since the drugs created are mostly analogues of existing compounds and has become extremely difficult to eradicate (Terreni *et al*., 2021). All of these cause problems in medical treatment, which encourage seeking new metabolites that are vigorous even against multi-resistant pathogens.

Prodigiosin is the first pigment identified by Gerber (1969) and recognized as one of the prodiginine derivatives. This pigment is mainly produced by *Bacillus prodigiosus* and is today known as *S. marcescens*. However, several bacteria have been reported to produce prodigiosin, including *Pseudomonas magnesiorubera*, *Vibrio psychrerythraea*, *Streptomyces variegatus,* and *Hahella chejuensis* (Lee *et al*., 2011). Prodigiosin has a diversity of biological properties, such as antibacterial, antiviral, antifungal, algicidal, antimalarial, antiparasitic, immunosuppressive, and anticancer activities (Islan *et al*., 2022). Moreover, a natural colorant such as prodigiosin derived from bacteria can be used in the pharmaceutical industry and colouring of food with natural colorant is gaining interest worldwide. Natural colorants are considered safe replacements for food use with the

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undesirable and unsafe markets of synthetic dyes (Darshan and Manonmani, 2015).

In this study, *S. marcescens* IBRL USM84 is a marine bacterium isolated from the marine sponge *Xetospongia testudinaria* and produces a significant natural red prodigiosin pigment with antibacterial activities. However, the production of prodigiosin is influenced by numerous factors such as bacterial species, physical parameters (incubation time, temperature and pH) and chemical parameters (inorganic salt, carbon and nitrogen sources) (Sumathi *et al*., 2014). Therefore, this study was carried out to enhance the prodigiosin production by *S. marcescens* IBRL USM84 by improving cultural condition parameters. The cultivation was carried out in a shake flask system. The physical parameters include incubation time, light illumination, initial pH of the medium, temperature, agitation speed, and percentage of agar towards the growth, pigment production and antibacterial activity of *S. marcescens* IBRL USM84 were studied.

MATERIALS AND METHODS

Bacterial strain

A pigmented bacterium, *S. marcescens* IBRL USM84, was previously isolated from the surface of a marine sponge, *Xestospongia testudinaria* at Pulau Bidong, Terengganu and deposited at the Industrial Biotechnology Research Laboratory (IBRL), School of Biological Sciences, Universiti Sains Malaysia, Penang, Malaysia was used in the present study. This isolate was maintained on Marine Agar (MA) (Difco, United Kingdom) slant and incubated at 25 °C for 24 h aerobically. For the test bacterium, methicillin-resistant *S. aureus* (MRSA ATCC 33591) was also provided by the IBRL and was grown on nutrient agar (NA) (Merk, Germany) slant at 37 °C for 24 h aerobically. Both slant agars were kept at 4 °C until further used and subculturing was done monthly to ensure their survival (Darah *et al*., 2014a).

Preparation of preculture inoculum

Serratia marcescens IBRL USM84 was grown in a 250 mL Erlenmeyer flask containing 50.0 mL of marine broth (Difco, United Kingdom) and incubated for 17 h at 26 °C in an orbital shaker at 120 rpm.

Cultivation medium

A volume of 2% (v/v) of preculture inoculum containing 17 h old bacterial inoculums at a concentration of 1×10^9 cells/mL was inoculated into a 250 mL Erlenmeyer flask containing 0.3% of agar in 100.0 mL of marine semi-solid medium (Difco, United Kingdom), initial pH of 7.5 and incubated at 25 °C in an orbital shaker at 120 rpm for 72 h (Giri *et al*., 2004). The samples were harvested at every 8 h intervals and analyzed for prodigiosin yield and anti-MRSA activity. The best cultivation time to produce the highest amount of prodigiosin was determined.

Enhancement of prodigiosin production and anti-MRSA activity

Enhancement of cultural conditions in the shake flask system for maximal prodigiosin production involves several physical parameters. They were light illumination condition (exposed and unexposed to light), initial pH medium (5, 6, 7, 8 and 9), temperature (20, 25, 30, 35 and 40 °C), agitation speed (0, 50, 100, 150 and 200 rpm) and agar concentration (0, 0.1, 0.2, 0.3 and 0.4%, w/v) were evaluated. All the experiments were performed in triplicates and the data values were presented as mean ± standard deviation (SD) and were analyzed using ANOVA with $p<0.05$ was considered significant (Darah *et al*., 2014a).

Growth determination, extraction and quantification of prodigiosin

The cell growth was determined at 620 nm using a spectrophotometer (Spectronic Unicam, Genesys 10UV) according to the method described by Gulani *et al.* (2012). The real cell growth rate was compared to the standard curve prepared prior to determination. For extraction, the bacterial culture was centrifuged at 4000 rpm for 45 min at 4 °C. The pellet was collected and extracted twice with acidified 2-propanol (4%, v/v of 1 M HCl) (Slater *et al*., 2003). The supernatant was then collected in the sterile vial. The prodigiosin extract was determined at 535 nm using a spectrophotometer (Spectronic Unicam, Genesys 10UV). The prodigiosin concentration was compared to the standard prodigiosin calibration curve prepared prior to determination. The pigment was extracted using a rotary evaporator (Heidolph, Laborota 4000). The pigment extract was dried under a fume hood until the dried pigment paste was obtained.

Assay for anti-MRSA activity

Anti-MRSA activity was determined according to the method described by Taufiq and Darah (2019). One loop full of MRSA colony at 24 h old was inoculated in a 20.0 mL universal bottle containing 5.0 mL of nutrient broth and incubated at 37 °C for 24 h. The 0.5 McFarland was prepared as a standard for visually adjusting the turbidity of MRSA inoculums. Only 0.1 mL of the MRSA inoculum was transferred into 7.9 mL nutrient broth, followed by the addition of 2.0 mL pigment extract that was extracted from *S. marcescens* IBRL USM84. The mixture was then incubated at 37 °C for 18 h. The control contained the materials that were equal to the test culture but with no addition of the pigment extracted from *S. marcescens* IBRL USM84; instead, it was replaced by the addition of another 2.0 mL of nutrient broth. The degree of inhibition of the MRSA was determined based on the reduction in the culture turbidity measured at 560 nm compared to the control. The antibacterial activity of the pigment extract was defined as one unit (U) of the antibacterial activity that resulted in the reduction of 1.0% of the growth of

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MRSA. The results were expressed as mean value ± standard deviation of the readings obtained with triplicates for each experiment.

Scanning electron microscopy (SEM) observation

The effect of crude extract from *S. marcescens* USM84 on MRSA was observed under scanning electron microscopy (SEM). For sample preparation, 20 µL crude extract was dropped on a Whatman antibiotic assay disc (6.0 mm diameter) that was placed on nutrient agar (NA). Prior to that, the NA was seeded with MRSA inoculum at a concentration of 5 × 10^5 CFU/mL. The plate was then incubated at 25 °C for 24 h. For SEM viewing, three agar blocks were cut from different sites (5.0 mm × 5.0 mm), as shown in Figure 1, followed by freezing in liquid nitrogen and then freeze-drying in a vacuum. The changes in bacterial cells were observed under SEM microscopy (Leica Cambridge, S-360, United Kingdom).

Statistical analysis

The SPSS Version 12.0 (ANOVA) was employed to compare the effect of each parameter on pigment production, bacterial growth and antibacterial activity. Statistically, significance was assumed at the 0.05 significance level (p<0.05). All the experiments were carried out in triplicate and the data were expressed as mean ± standard deviation.

RESULTS

Prodigiosin production, antibacterial activity and growth profiles before the enhancement of physical parameters

Figure 2 shows *S. marcescens* IBRL USM84 colonies with blood-red in colour on MA within 24 to 48 h of incubation at 25 °C aerobically. The effects of varying the incubation periods on the prodigiosin production, antibacterial activity and cell growth of *S. marcescens* IBRL USM84 are shown in Figure 3. The prodigiosin production was initiated and drastically increased during the logarithmic growth phase and continued until achieving its maximal value of 2.76 µg/mL during the 48 h of cultivation. The results also indicated that the anti-MRSA activity was highly correlated with the pigment production of *S. marcescens* IBRL USM84. The cell growth and the prodigiosin production were increased in a parallel pattern, which suggests that the cell growth was associated with the prodigiosin production. Hence, 48 h of cultivation time was selected to improve the prodigiosin production in the next physical parameters.

Effect of the light exposure

There was no significant effect on prodigiosin production, antibacterial activity and growth when incubated in the *S. marcescens* IBRL USM84 culture under both light and darkness conditions (Figure 4). The results showed the

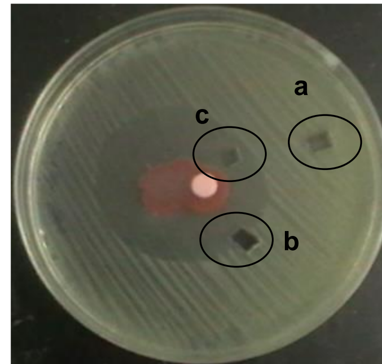

Figure 1: Agar blocks were cut at different sites. (a) Control [not exposed to crude extract], (b) Site with substantially exposed to crude extract, (c) Site with completely exposed to crude extract.

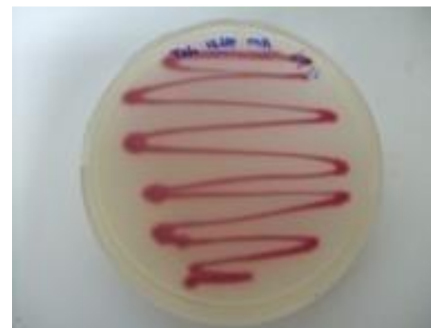

Figure 2: Reddish colony of isolate *S. marcescens* IBRL USM84 on marine agar plates after 48 h of incubation.

production of prodigiosin was 2.77 µg/mL in the light condition and 2.76 µg/mL in the dark condition after 48 h of incubation time. The cell growth for both light and darkness conditions was 0.61 and 0.60 g/L, respectively. The antibacterial activity of pigment extract in light and darkness conditions also did not show much difference (significance level, p<0.05) was 34.72 U/mL and 34.04 U/mL, respectively. Therefore, incubation under the light illumination condition was selected as the optimum condition for the bioactivity of *S. marcescens* IBRL USM84.

Effect of initial pH of the medium

The pH value plays a vital role in enhancing the bioactivity of microbial culture. As shown in Figure 5, the results revealed a significant effect of prodigiosin production of *S. marcescens* IBRL USM84 when cultivated at different initial pH of the medium. The highest production of prodigiosin and antibacterial activity was 3.31 µg/mL and 37.78 U/mL, respectively, when the pH value was 7.0. A pH of more or less than 7.0 reduced the pigment production and antibacterial activity of this strain significantly. Thus, pH 7.0 was selected as the best pH to cultivate *S. marcescens* IBRL USM84.

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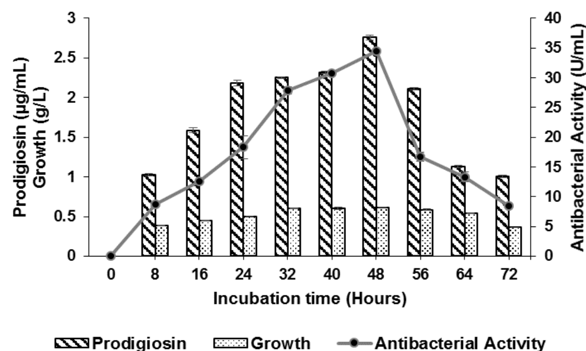

Figure 3: Effect of culture duration on prodigiosin production, antibacterial activity and growth of *S. marcescens* IBRL USM84.

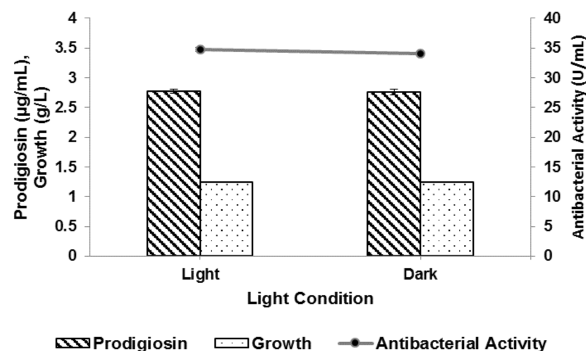

Figure 4: Effect of light on prodigiosin production, antibacterial activity and growth of *S. marcescens* IBRL USM84.

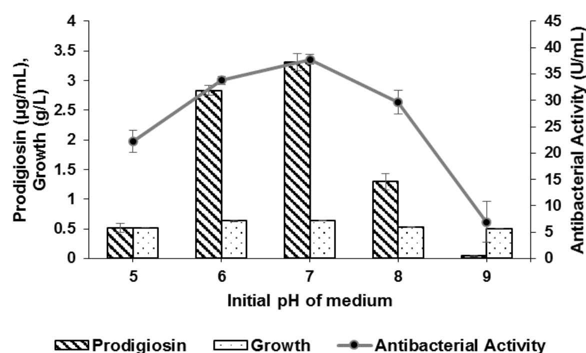

Figure 5: Effect of initial pH of the medium on prodigiosin production, antibacterial activity and growth of *S. marcescens* IBRL USM84.

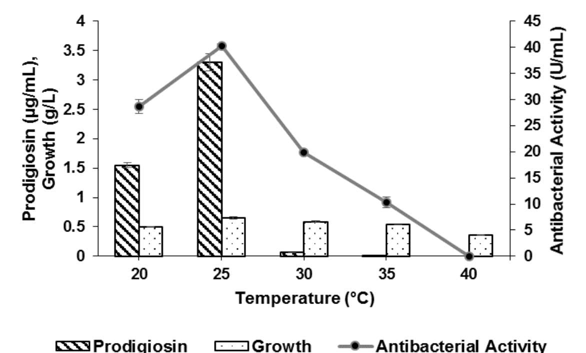

Figure 6: Effect of temperature on prodigiosin production, antibacterial activity and growth of *S. marcescens* IBRL USM84.

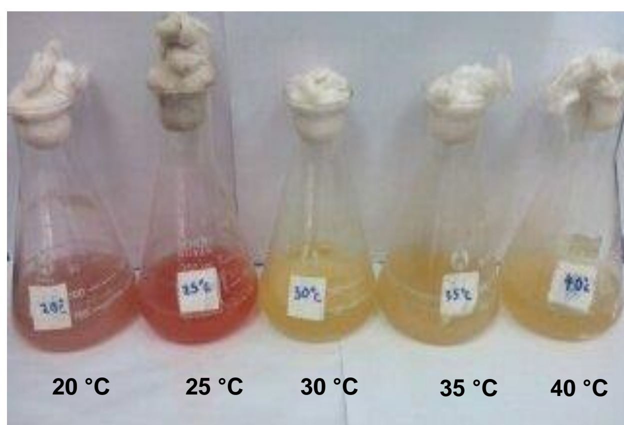

Figure 7: Culture of *S. marcescens* IBRL USM84 grown in marine broth for 48 h at different incubation temperatures (120 rpm).

Effect of temperature

Figure 6 shows the effect of cultivation temperature on prodigiosin production, antibacterial activity and cell growth of *S. marcescens* IBRL USM84. The highest pigment production and antibacterial activity were achieved at a temperature of 25 °C which was 3.31 µg/mL and 40.33 U/mL, respectively. The pigment production dropped drastically when the culture was incubated at 30 °C and above. The bacterial growth was detected in a broader range of temperatures (20 to 40 °C) compared to the range of temperatures for pigment production (20 to 30 °C). Thus, the incubation temperature of 25 °C was selected as the optimum temperature. Figure 7 shows the coloration of the MB-cultivated strain changed when incubated for 48 h at various temperatures. The colour of MB changed to red at temperatures ranging from 20 °C to 25 °C. The colour of MB turned yellowish-orange at a temperature of 30 °C and above. This condition indicated that the production of pigment by *S. marcescens* IBRL USM84 was temperature-dependent.

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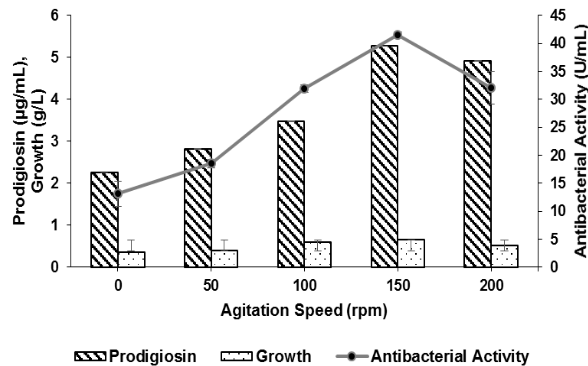

Figure 8: Effect of agitation speed on prodigiosin production, antibacterial activity and growth of *S. marcescens* IBRL USM84.

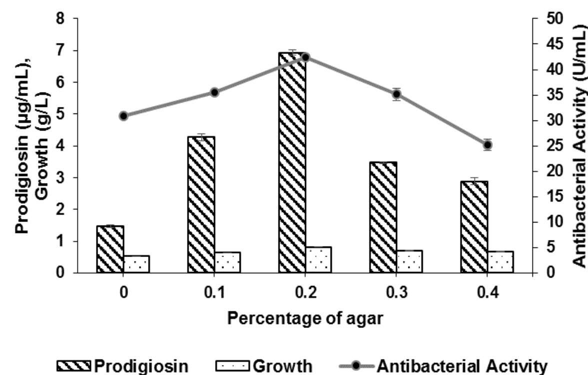

Figure 10: Effect of agar on prodigiosin production, antibacterial activity and growth of *S. marcescens* IBRL USM84.

Effect of agitation speed

The highest pigment production (5.27 µg/mL) and antibacterial activity (41.56 U/mL) were obtained at 150 rpm of agitation speed (Figure 8). Thus, the agitation speed of 150 rpm was chosen to enhance the prodigiosin production via a submerged fermentation process under optimum pH and temperature. The results showed that the agitation speed below and above 150 rpm affected the production of prodigiosin. At a static condition where the oxygen supply was limited, *S. marcescens* IBRL USM84 still can produce a low amount of prodigiosin (2.25 µg/mL). The cultivation at the static condition indicated the cultivation medium was obviously separated into double layers with red pigment production at the upper layer, as shown in Figure 9.

Effect of addition of agar into the medium

Figure 10 shows the effect of the addition of agar into the medium on prodigiosin production, antibacterial activity and bacterial growth. The amount of prodigiosin production and antibacterial activity increased as the amount of agar increased and then dropped after its maximal production was obtained. The addition of 0.2% of agar was sufficient to encourage the growth of *S. marcescens* IBRL USM84 by producing the highest yield of prodigiosin (6.94 µg/mL) in a semi-solid condition. Hence, the addition of 0.2% of agar into the medium was chosen as the best condition for the cultivation medium.

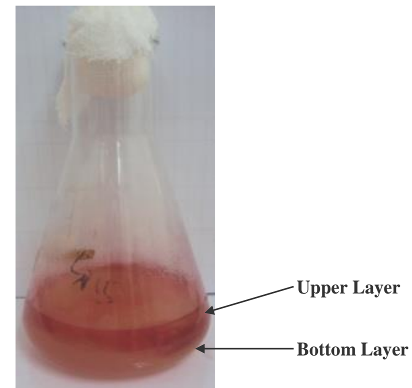

Figure 9: Culture of *S. marcescens* IBRL USM84 for 48 h cultivation period at 25 °C with a double layer formed under static condition (0 rpm).

Comparison of the growth, antibacterial activity and prodigiosin production before and after enhancement for physical parameter

The improved physical parameters had been incorporated and samples were taken at 8 h intervals for bioactivity determination. Figure 11 represents the results of the time-course study before and after enhancements. The optimal incubation temperature for the prodigiosin production was retained at 25 °C in the light illumination exposure for 48 h even after enhancement. However, other parameters changed after undergoing the enhancement process, as shown in Table 1. The highest prodigiosin production, antibacterial activity and growth yield were achieved at 48 h of incubation time. The percentage of increment obtained after physical parameters enhancement was 151.81% (2.76 µg/mL before and 6.95 µg/mL after enhancements) for prodigiosin production, 23.34% (34.45 U/mL before and 42.49 U/mL after enhancements) for antibacterial activity and 33.87% (0.62 g/L before and 0.83 g/L after enhancements) for growth yield.

Effectiveness of crude extract from *S. marcescens* IBRL USM84 as an anti-MRSA agent

Figure 12 shows the structural degeneration of bacterial cells exposed to the crude pigment extract. Figure 12a demonstrates the untreated MRSA ATCC 33591 cells with intact, typically spherical-shaped, smooth surface and undamaged cells. The cells were also observed growing actively which was indicated by compacted

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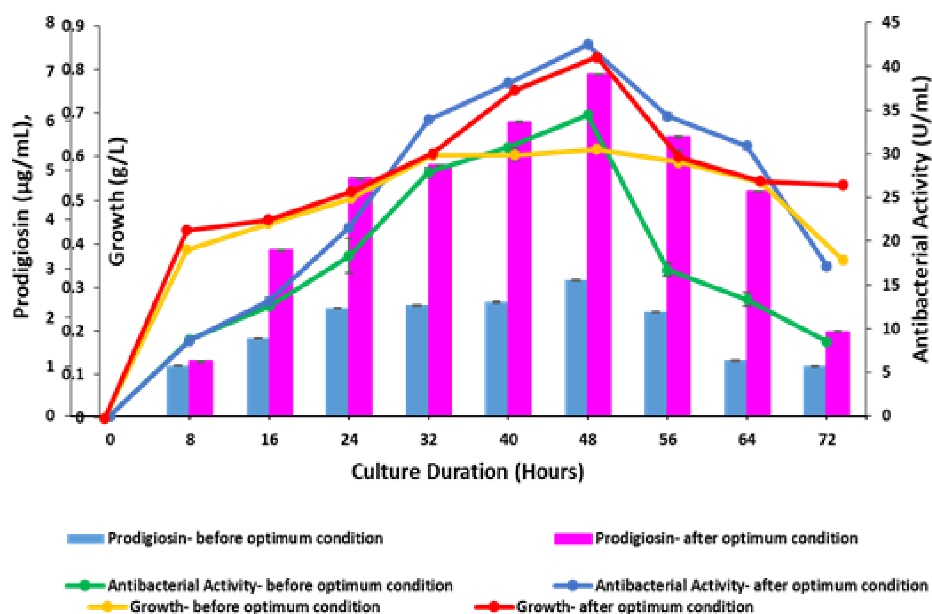

Figure 11: Profile of growth, prodigiosin production and antibacterial activity of *S. marcescens* IBRL USM84 before and after physical parameter enhancements.

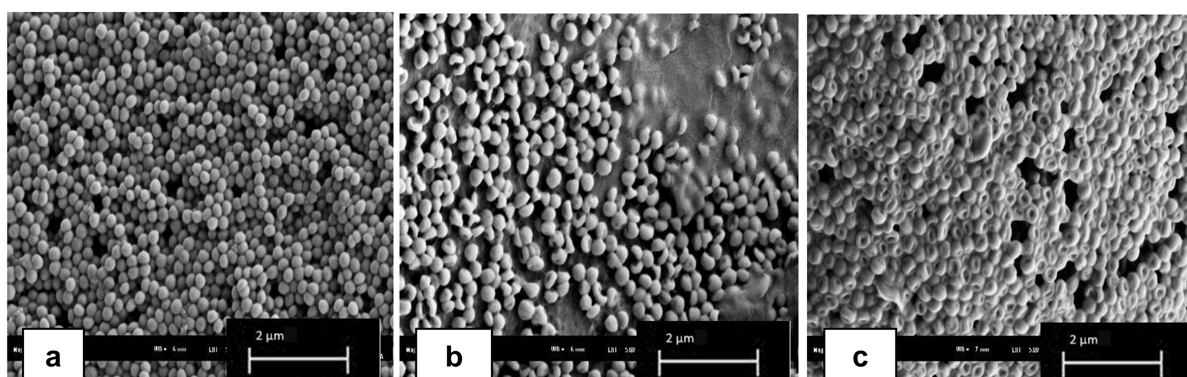

Figure 12: Morphology of bacterial cells exposed to crude extract (prodigiosin) at different inhibition sites. (a) Control [not exposed to crude extract], (b) Partially exposed to crude extract, (c) Completely exposed to crude extract.

Table 1: The summary of the culture condition before and after enhancements.

Parameters	Before	After
Incubation time (h)	48	48
Light exposure	Light	Light
Initial pH of medium	7.5	7.0
Incubation temperature (°C)	25	25
Agitation speed (rpm)	120	150
Percentage of agar (%)	0.3	0.2
Bacterial growth (g/L)	0.62	0.83
Increment (%)	-	33.87
Pigment production (µg/mL)	2.76	6.95
Increment (%)	-	151.81
Antibacterial activity (U/mL)	34.45	42.49
Increment (%)	-	23.34

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bacterial cells. After being partially exposed to the crude pigment extract, some bacterial cells were shrunken and became irregular in shape (Figure 12b). However, some bacterial cells were still in good condition. Figure 12c illustrates the structure of bacterial cells taken in the area that was completely exposed to the crude extract. Severe damage to the cell's morphologies was observed as indicated by the formation of minor dents, which led to complete collapse and eventually, cell death occurred.

DISCUSSION

Cultural conditions have a significant effect on microbial growth and the production of their secondary metabolites. Taufiq and Darah (2019) reported the improvement of cultural conditions, including pH, temperature, light intensity and the age of seed culture, could significantly enhance the anti-MRSA activity of the endophytic fungus, *Lasiodiplodia pseudotheobromae* IBRL OS-64. In this study, the physical parameters such as culture duration, pH, temperature, agitation speed and percentage of agar significantly influenced the production of prodigiosin by *S. marcescens* IBRL USM84. Méndez *et al.* (2011) stated that pH and temperature were among the physical parameters significantly affecting pigment production. MRSA was selected as a test bacterium for antibacterial analysis in this study since it is the most critical and life-threatening pathogen. MRSA is also known as a major nosocomial pathogen and has been proven to be the most common causative agent, exhibiting resistance to 50% of tested synthetic antibiotics (Darabpour *et al.*, 2011). This pathogen is responsible for the largest outbreak of hospital-acquired infection (HAI) worldwide (Fehér *et al.*, 2010). Therefore, the capability of prodigiosin extract from *S. marcescens* IBRL USM84 to inhibit the MRSA cells could be a great discovery in eliminating MRSA infection.

In the present study, the highest prodigiosin production of *S. marcescens* IBRL USM84 was at the end of the stationary growth phase. A similar finding by Wang *et al.* (2012) reported that the prodigiosin production from *S. marcescens* TKU011 started from the logarithmic growth phase and continued until the stationary growth phase. Siva *et al.* (2011) also stated that the prodigiosin production by *S. rubidae* achieved its maximal production at the stationary phase. However, prodigiosin production by some bacterial strains, such as *Serratia* sp. and *Vibrio* sp. showed maximal yield at the logarithmic growth phase (Alihosseini *et al.*, 2008; Bharmal *et al.*, 2012). The prodigiosin production varied among the bacteria strains since different bacteria could synthesize prodigiosin at different growth phases. Each prodigiosin producer has its own specific characteristics in pigment production.

Light intensity is believed to influence microbial growth and pigment production. However, the present finding revealed that the light and dark conditions did not significantly affect the production of secondary metabolites by *S. marcescens* IBRL USM84. Hence, incubation under the light condition was selected as the optimum condition. Similarly, Wang (2012) reported that *S. marcescens* TKU011 produced higher prodigiosin production under light illumination compared to under dark conditions. Contradictory, Ryazantseva *et al.* (1995) and Someya *et al.* (2004) revealed that light illumination significantly inhibited the production and stability of prodigiosin; however, prodigiosin has the ability to store the visible light energy, which is an essential factor in the pigment synthesis.

pH plays a vital role in buffering the medium to enhance pigment production (Darah *et al.*, 2014b). Solieve *et al.* (2011) reported that the enzymatic condensation of 2-methyl-3-n-amyl-pyrrole (MAP) and 4-methoxy-2,2-bipyrrole-5-carbaldehyde (MBC) precursors were initiated at pH 7.0 during prodigiosin biosynthesis. Tortora *et al.* (2004) also stated that the activity of the enzymes was hugely influenced by pH. The drastic changes in pH can affect the function of the amino acid (proline), which plays a crucial role in inducing the prodigiosin biosynthesis process (Bharmal *et al.*, 2012). In the present study, *S. marcescens* IBRL USM84 was observed to have optimum pH of 7.0 and this is due to the nature of the isolate that was previously isolated from the marine environment (pH of seawater ranging from 7.2 to 7.6); thus, this strain required neutral pH for the pigment production (Maithili *et al.*, 2014).

Incubation temperature greatly influences microbial growth and their secondary metabolite production. Extremely low and high temperatures could disrupt microbial growth and, thus, inhibit their secondary metabolite, including pigment. In the present study, incubation temperatures significantly enhance pigment production of *S. marcescens* IBRL USM84 and the maximal prodigiosin and anti-MRSA activity were obtained at a temperature of 25 °C. Similarly, Gulani *et al.* (2012) reported that the prodigiosin production achieved maximal yield at 25 °C. However, the production of prodigiosin completely ceased when the temperature was 35 °C and above since higher temperature may have an inhibitory effect on the expression of enzymes related to prodigiosin biosynthesis. Xu *et al.* (2014) studied 16 proteins after incubating *S. marcescens* JNB5-1 at 28 °C and 37 °C. They found that the O-methyl transferase and oxidoreductase, which acted as biosynthesis enzymes of prodigiosin and proteins related to the precursor substances involved in prodigiosin biosynthesis, including proline, methionine, serine, 2-Octenal and Malonyl-CoA were obviously down-expressed at 37 °C where the levels of mRNA transcriptional of O-methyl transferase, oxidoreductase and transketolase have decreased compared to 28 °C. They concluded that *S. marcescens* JNB5-1 could produce prodigiosin at lower temperatures and was inhibited at a higher temperature. This explanation agreed with Williams (1973), who revealed that maximal pigment production by *S. marcescens* was between 24 and 28 °C.

Aeration through agitation mixing is essential in the production of secondary metabolites. Prodigiosin production was decreased when the agitation speed was lower than the optimal speed level. This condition could be due to the low level of dissolved oxygen in the

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cultivation medium. A similar observation was reported by Pansuriya and Singhal (2011), who revealed that incomplete mixing and oxygen transfer might be the main factor of inferior production at lower agitation speeds. Meanwhile, a higher agitation speed would cause a shearing effect on the cells and result in disrupting the synthesis of secondary metabolites. The morphology of microorganisms is also affected by agitation speeds which eventually influence secondary metabolite production and growth of the microorganisms (Darah *et al.*, 2011). The present finding showed that bacterial isolate *S. marcescens* IBRL USM84 is able to produce red pigment at the top layer compared to the bottom layer under static conditions. The double layer formed because of the hydrophobic nature of prodigiosin, which is due to the resonance of its functional group electrons, non-polar dipole moment and also insoluble in water (Namazkar and Ahmad, 2013). Another factor for the bacterial culture accumulated at the top layer was a higher level of dissolved oxygen at the surface of the medium. This phenomenon has proven that *S. marcescens* IBRL USM84 is a facultative anaerobic bacterium where that can survive with less oxygen tension (Darah *et al.*, 2014b).

Serratia marcescens IBRL USM84 was previously isolated from the surface of the marine sponge *Xetospongia testudinaria*. This strain required a semi-solid agar condition to grow and enhance the prodigiosin production. However, *S. marcescens* IBRL USM84 still can grow and synthesize prodigiosin pigment in the broth medium (no agar added), but it's more tending to the semi-solid medium where it is mimicking the exact condition of its original habitat as described by Darah *et al.* (2014b). Besides, this semi-solid medium suggested that the motile bacterial cells need a substance to stick to or attach to in order to grow well and produce prodigiosin efficiently. According to Lemos *et al.* (1985) and Anand *et al.* (2006), almost all the bacterial strains isolated from marine organisms such as corals, sponges and seaweeds had recorded higher antibiotic production rates compared to the free-living bacteria that live in the marine environment. Hentschel *et al.* (2001) also found that sponges can host several marine microorganisms, including heterotrophic bacteria, archaebacteria, cyanobacteria and unicellular algae. Besides that, bacteria and algae mainly play a vital role in nutrition absorption and metabolic transport in sponges, as reported by Wilkinson and Garrone (1980). This could be the reason for *S. marcescens*'s IBRL USM84 on its ability to produce significant secondary metabolites since this strain is isolated from sponge-associated microorganisms.

In the present study, the effectiveness of the red pigment as an anti-MRSA agent was determined. The findings revealed the pigment caused severe damage to the bacterial cells. It is believed that the pigment was modified and interfered with the cell wall synthesis of the bacteria. This was indicated by the formation of small dents and pits. The result agreed with Jalil and Darah (2021), who revealed that MRSA cells exposed to secondary microbial metabolite experienced the formation of pits, the disintegration of the cell wall and leakage of cytoplasm that led to bacterial death. However, the cross-border area (Figure 1b and Figure 12b) that is partially exposed to the pigment extract showed a slight resistance of bacterial cells. This may be due to insufficient diffusion of pigment extract and the formation of biofilm. Jalil *et al.* (2021) reported the formation of biofilm facilitates the emergence of resistant MRSA cells and thus leads to bacterial regrowth. This statement was supported by Dakheel *et al.* (2016), who stated that the strains of MRSA can develop biofilm by releasing extracellular DNA (eDNA) to increase their biofilm stability and resistance to antibiotics.

The natural red pigment produced by marine isolate *S. marcescens* IBRL USM84 was previously identified as prodigiosin based on Ultra performance of Liquid Chromatography (UPLC) analysis by comparing this natural red pigment with the standard prodigiosin from *S. marcescens* (Sigma, Aldrich) (Teh Faridah, 2018). The present results revealed that the bacterial strain isolated from the surface of a marine sponge, *Xetospongia testudinaria*, at Pulau Bidong, Terengganu has a good potential as a prodigiosin producer with antimicrobial properties. Srinivasan *et al.,* (2021) reported that epiphytic marine bacteria are able to secrete secondary metabolites that act as antimicrobial agents to protect their hosts from pathogens. This natural red pigment with antibacterial activity can be used as a natural colorant and preservative in the food, beverage and cosmetic industries. However, the toxicity, efficacy and mode of action of the pigment must be further studied.

CONCLUSION

The production of prodigiosin by *S. marcescens* IBRL USM84 was influenced by several physical parameters. The maximal prodigiosin yield was achieved at 6.95 µg/mL, antibacterial activity at 42.49 U/mL and 0.83 g/L cell growth when using marine broth added with 0.2% (w/v) of agar, cultivation temperature of 25 °C, pH of 7 and agitation speed of 150 rpm for 48 h cultivation period under light illumination. However, further studies are necessary, especially on the bio-guided fractionation, since the present findings revealed the potential of red pigment extracted from *S. marcescens* IBRL USM84 as an antimicrobial agent, especially against MRSA. In addition, the exploitation of Malaysia's marine environment, especially on pigmented bacteria, could greatly benefit various sectors, including the cosmeceutical, pharmaceutical and biotechnological industries.

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CONFLICT OF INTEREST

The researchers report no conflict of interest. The researchers alone are responsible for the content and writing of the study.

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