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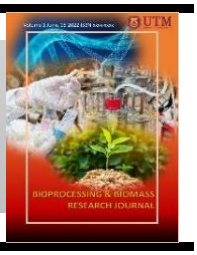

Research Article

Effects of Used and Fresh Palm Oil on Prodigiosin Production for Potential Sunscreen Agent

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ABSTRACT

Prodigiosin is a secondary metabolite produced by *Serratia* sp. that lives in habitat with high exposure to ultraviolet (UV) rays and has resistance to the UV rays. However, prodigiosin from *Serratia nematodiphila* requires high cost for its production and extraction. Fresh and used palm oils that are found abundantly and cheap in Malaysia can act as substrates or carbon sources that contribute to the prodigiosin production from *S. nematodiphila*. This study was conducted to investigate the effects of fresh and used palm oils to enhance prodigiosin production and potential of prodigiosin as sunscreen agent. The prodigiosin production was done through fermentation of *S. nematodiphila* with fresh and used palm oils at concentrations of 5, 6, 7, 8, and 9% (w/v). Prodigiosin was then extracted from fermentation broth with acidified methanol to determine in vitro sun protection factor (SPF). The highest yield of prodigiosin production with 8% (w/v) of used palm oil was 463.938 µmol/L while with 9% (w/v) of fresh palm oil was 518.84 µmol/L. The specific growth rate of *S. nematodiphila* with 8% (w/v) of used palm oil was 0.112 h^{-1} and doubling time was 6.18 h at incubation time of 46 h. Whereas for 8% (w/v) fresh palm oil, the specific growth rate and doubling time at incubation time of 40 h were 0.104 h^{-1} and 6.69 h, respectively. The SPF value with 1% (w/v) of prodigiosin was 3.68. This study provides a cheap and sustainable alternative of oil substrate for prodigiosin production and potential of prodigiosin as sunscreen agent.

INTRODUCTION

Nowadays, sunscreen has become a necessity in our daily life to protect our skins from ultraviolet (UV) rays. Mansuri et al. (2021) stated that UVA rays with longer wavelength of 320 – 400 nm can enter deeper into tissue cells to cause premature aging while UVB rays which have shorter wavelength of 280 – 320 nm can cause sunburn. Sunscreen could prevent skin cancer, immunosuppressive, pigmentation and photoaging due to exposure to UV rays (Choksi et al., 2020; Weig et al., 2019).

There are several bacterial pigments that have potential as sunscreen agents because they have better survival with UV radiation (Patki et al., 2021). For instance, Suryawanshi et al. (2015) stated that bacterial pigments such as violacein and prodigiosin can enhance the sun protection factor (SPF), which is a measurement of how well the UVB rays could be absorbed by a sunscreen product. Prodigiosin, a natural red by gram-positive and gram-negative bacteria within cell membrane, extracellular vesicles and intracellular granules (Paul et al., 2020; Srimathi et al., 2017). Other than being a colourant, prodigiosin has many properties such as

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anticancer, antimicrobial, anti-oxidant, immunosuppressant, and antimalarial (Elkenawy et al., 2017; Yip et al., 2019).

Prodigiosin is commonly found to be produced by *Serratia* sp. such as *Serratia marcescens*, *Serratia nematodiphila* and *Serratia rubidaea* (Bhombal et al., 2023). *Serratia* sp. can be found in air, water and soil, which are habitats with high exposure to UV rays. Thus, *Serratia* sp. are found to have resistant with UV radiation (Suryawanshi et al., 2015). Although prodigiosin brings many advantages, its production and extraction are costly (Liu et al., 2021). Therefore, many studies have been conducted previously to increase the prodigiosin production by optimising the fermentation conditions and microbial growth. For example, prodigiosin cannot be produced when there are metabolised sugars such as glucose because it can inhibit pigmentation through catabolic repression (Abdul Manas et al., 2020). This is because catabolism of glucose promotes extracellular acidification that decreases pH and interferes with synthesis of secondary metabolite like prodigiosin (Solé et al., 2000).

The carbon sources can be in powder, liquid, or oil form for the prodigiosin production. In recent studies, the carbon sources that are mainly used for prodigiosin production are vegetable oils such as peanut oil, soybean oil and palm oil. This is because oil can act as carbon sources to enhance prodigiosin production with its fatty acid content (Giri et al., 2004). Fatty acids in vegetable oils can act as carbon source to increase prodigiosin production as they yield precursors of prodigiosin, which are malonyl-CoA and 2-octenal in a high amount (Liu et al., 2021). Besides, it is found that higher amount of saturated fatty acid content can contribute to a rise in prodigiosin production (Shahitha & Poornima, 2012; Sundaramoorthy et al., 2009).

Malaysia is the major palm oil supplier, and palm oil is the cheapest among the other oils. Palm oil has a wide variety of usage such as a cooking oil, margarine, and soap. Embrandiri et al. (2011) stated that palm oil can be used as a base for shampoo, liquid detergents, lipstick, and wax. Since the demand of using fresh palm oil is increasing, the waste generated from palm oil also increases. Spalvins et al. (2020) stated that used palm oil could contaminate 1 million litre of water due to its very high carbon oxygen demand and biological oxygen demand. Therefore, water pollution can be greatly reduced by recycling or reusing the used palm oil to produce other products. Furthermore, there is only a study regarding the usage of fresh palm oil as carbon sources for fermentation of *S. nematodiphila* to produce prodigiosin and no studies on using used palm oil has been done yet. Hence, by using cheaper oil substrate during fermentation can reduce the cost of producing prodigiosin as sunscreen agent.

In order to produce high yield of prodigiosin production by *S. nematodiphila*, the effect of different concentrations of different carbon sources and the potential of prodigiosin as sunscreen agent were studied.

MATERIALS AND METHOD

Materials and Chemicals

The bacteria *Serratia nematodiphila* was previously isolated (Abdul Manas et al., 2020). Chemicals used were hydrochloric acid, methanol, ethanol, fresh palm oil and used palm oil.

Bacteria Culture

Firstly, Luria Bertani medium was prepared by mixing 10 g of tryptone, 5 g of yeast and 10 g of sodium chloride with 1 L of distilled water. Then, the culture medium was sterilised by autoclaving at 121 ˚C for 15 minutes before inoculating it with any bacteria.

Then, *S. nematodiphila* preculture was prepared by inserting sterile loop into the bacteria stock. The sterile loop was then gently streaked on the surface of Luria Bertani agar plate. This procedure was done near the Bunsen burner to avoid contamination. Next, the *S. nematodiphila* was cultured overnight (24 hours) at 37 °C for cell growth.

Lastly, a single colony bacterium from the agar plate was transferred to a 100 mL Erlenmeyer flask containing 25 mL of Luria Bertani broth in a shake flask for 48 hours at 200 rpm, pH 7 and 28 °C.

Effect of Different Concentration of Carbon Sources

The prodigiosin production was optimised by using different concentrations of carbon sources during fermentation of *S. nematodiphila*. The *S. nematodiphila* was fermented in two different carbon sources which were fresh palm oil and used palm oil. The concentration used for both fresh palm oil and used palm oil were 5, 6, 7, 8 and 9 (% w/v) to obtain high yield of prodigiosin. Then, both results for fresh palm oil and used palm oil were compared and analysed.

Quantification of Cell Growth

The cell growth was quantifed by measuring cell concentration in the medium. The absorbance at wavelength 600 nm was read using UV-Visible spectrometer The OD reading was plotted against time to observe the cell growth of *S. nematodiphila.*

Extraction and Quantification of Prodigiosin Pigment

After fermentation of *S. nematodiphila*, the prodigiosin was extracted from the fermentation broth by mixing with acidified methanol (1 N HCl and 96.0 mL of methanol). The mixture was vortexed for 5 minutes. The mixture was centrifuged again at 8, 000 g for 30 minutes. The supernatant of the medium was taken, and cell debris was removed. The supernatant was transferred to a fresh vial and the absorbance reading was measured by using UV-vis spectrophotometer at wavelength of 534 nm. Then, the absorbance reading was converted to concentration using **Equation 1** below to measure prodigiosin production (Verhoeven, 1996).

$$A = e\,c\,l \quad \textbf{(1)}$$

where,

A = Absorbance of the prodigiosin pigment
e = molar extinction coefficient (51.3×10^3 mol^{-1} L cm^{-1})
c = concentration of prodigiosin pigment (mol L^{-1})
l = length of the light path which is equal to the width of the cuvette (cm).

Fatty Acid Composition Analysis

Fatty acid composition analysis for used palm oil and fresh palm oil was done by using gas chromatography – flame ionizer detector (GC-FID). The used palm oil and fresh palm oil were analysed using AOCS Ce 2-66 (Reapproved 2009) and AOCS Ce 1a-13 (Approved 2013). The results analysed were from C6 to C22.

Growth Curve Analysis

Specific growth rate was referred to the steepness of the curve. The sample was taken at different hours. The biomass production yield and prodigiosin yield were analysed using graph curve to determine the relationship between prodigiosin yield and cell growth. Specific growth rate and doubling time were calculated using **Equation 2** and **Equation 3** below (Shuler & Kargi, 2002):

$$\mu = \frac{ln\ OD2 - ln\ OD1}{(t_2 - t_1)} \quad \textbf{(2)}$$

where,

μ = Specific growth rate (OD600/h)
OD600 = Optical density reading at point 1 and point 2
t = Time at OD reading was taken (hour)

$$t_d = \frac{ln\ 2}{\mu} \quad \textbf{(3)}$$

where,

t_d = Doubling time (hour)
μ = Specific growth rate (OD600/ h)

Determination of Sunscreen Protection Factors

The 40% ethanol solution was prepared by mixing 40 mL of ethanol with 60 mL of distilled water. The initial stock solution was prepared by taking 1% (v/v) prodigiosin in ethanol solution of 10 mL. Then, the stock solution of 0.01% was prepared by dilution. The absorbance value of each aliquot prepared was determined from wavelength of 290 to 320 nm at 5 intervals by using UV-Visible spectrophotometer. EE (λ) × I (λ) is a constant as shown in the **Table 1**. The in vitro SPF that were determined by **Equation 4** was described by Mansur mathematical model (Mansur et al., 1986).

$$Spectrophotometric\ SPF = CF \sum_{290}^{320} EE\ (\lambda)\ I\ (\lambda)\ \text{abs}\ (\lambda) \quad \textbf{(4)}$$

where,

CF = Correction factor (10);
EE (λ) = Erythmogenic effect of radiation with wavelength λ;
Abs (λ) = Spectrophotometric absorbance value of the solution; and
I (λ) = Solar intensity spectrum.

Table 1 The constant value of EE (λ) x I (λ) at wavelength of 290 to 320 nm with interval of 5 nm (Kumar et al., 2015)

Wavelength (λ)	EE (λ) x I (λ)
290	0.015
295	0.0817
300	0.2874
305	0.3278
310	0.1864
315	0.0839
320	0.018

RESULTS AND DISCUSSION

Effect of Different Concentration Carbon Sources

The prodigiosin production was done through the fermentation of *S. nematodiphila*. The production of prodigiosin was determined at different concentrations with different types of carbon sources. The carbon sources used were used palm oil and fresh palm oil with concentration of parameter at the range from 5 to 9% (w/v) at incubation period of 48 hours. **Figure 1** below shows the effect on different concentrations by different carbon sources.

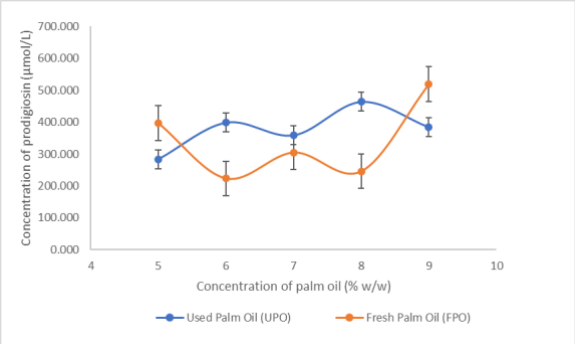

Figure 1 Production of prodigiosin at different concentrations of used palm oil and fresh palm oil.

Table 2 Comparison results of different concentrations of different oil substrates

Bacteria	Oil substrate (% w/v)	Prodigiosin production (g/L)	Reference
Serratia nematodiphila	Used palm oil 8%	0.1501	This study
Serratia nematodiphila	Fresh palm oil 8%	0.0795	This study
Serratia nematodiphila Y01	Palm oil 3%	0.0930	(Abdul Manas et al., 2020)
Serratia nematodiphila Y01	Olive oil 3%	0.0895	(Abdul Manas et al., 2020)
Serratia marcescens	Peanut oil 3%	2.72	(Shahitha & Poornima, 2012)
Serratia marcescens SMΔR	Sunflower oil 6%	0.79	(Liu et al., 2021)
Serratia marcescens BWL1001	Soybean oil 100 g/L	27650	(Liu et al., 2021)

Figure 1 shows that the optimum concentration for used palm oil was 8% (w/v) while for fresh palm oil was 9 % (w/v). The prodigiosin production increases when the concentration of oil substrates increases. This is because using palm oil as oil substrate causes double the yield of prodigiosin produced by fresh palm oil at concentration of 8% (w/v). However, the prodigiosin production drops at

concentration of 9% (w/v) for used palm oil and this might be due to unknown contents in it. There might be composition that inhibits the prodigiosin production such as glucose and fructose (Giri et al., 2004). In addition, Spalvins et al. (2020) stated that used palm oil may contain many degradation products such as peroxides, hydroperoxides, aldehydes and ketones. Although these products in used palm oil have not been tested on the prodigiosin production yet, these products could either enhance or negatively induce prodigiosin production. **Table 2** shows the comparison results of different concentrations of different oil substrates. Different concentrations of oil substrates cause different effects towards prodigiosin production. This might be due to the different amount of saturated fatty acid in the oil.

Table 3 below shows the fatty acid composition for different types of vegetable oils. Giri et al. (2004) stated that saturated fatty acid plays a role in enhancing cell growth as well as production of prodigiosin. Hence, prodigiosin production can increase due to high amount of saturated fatty acid content. Abdul Manas et al. (2020) stated that palm oil has higher production of prodigiosin compared to other types of oils because of its higher saturated fatty acid content. However, the amount of saturated fatty acid in used palm oil is less than in the fresh palm oil. Fresh palm oil has the highest saturated fatty acid content among the other oils based on **Table 3**. Therefore, fresh palm oil causes higher prodigiosin production at higher concentration due to higher amount of saturated fatty acid content. Besides, saturated fatty acid can act as a carbon and energy sources for *S. nematodiphila* as it has lipase activity for hydrolysis of oil to liberate fatty acid (Su et al., 2011).

Table 3 Fatty acid composition for different type of vegetable oils

Fatty acid	Chemical Formula	Type of fatty acid	Fatty acid composition (%)			
			Used palm oil	Fresh palm oil	Soybean oil	Peanut oil
	Reference		This research	This research	(Chowdhury et al., 2007)	(Matthäus & Musa Özcan, 2015)
Caproic acid	C6	Saturated fatty acid	<0.1	<0.1	-	-
Caprylic acid	C8	Saturated fatty acid	<0.1	<0.1	-	-
Capric acid	C10	Saturated fatty acid	<0.1	<0.1	-	-
Lauric acid	C12	Saturated fatty acid	0.1	0.1	-	-
Myristic acid	C14	Saturated fatty acid	0.8	0.8	-	-
Palmitic acid	C16	Saturated fatty acid	36.9	37.4	10	9.5
Palmitoleic acid	C16:1	Unsaturated fatty acid	0.3	0.2	-	-
Stearic acid	C18	Saturated fatty acid	4.0	4.2	4	3.2
Oleic acid	C18.1	Unsaturated fatty acid	45.4	43	18	52.5
Linoleic acid	C18.2	Unsaturated fatty acid	11.7	13.3	55	28.3
Arachidic acid	C20	Saturated fatty acid	0.4	0.4	-	-
Behenic acid	C22	Saturated fatty acid	<0.1	<0.1	-	-
Others			0.4	0.4	-	-
Saturated fatty acid			42.4	43.2	18.26	12.70
Unsaturated fatty acid			57.5	56.7	81.14	80.90

The higher production of used palm oil was due to the presence of amino acids because it enhances cell proliferation during growth. The used palm oil that was used for cooking with fish or vegetables might contain some of the amino acids from protein source even after filtering. For instance, proline is one of the non-essential amino acids which has been shown to induce prodigiosin production (Suryawanshi et al., 2014). In addition, essential amino acids such as methionine and non-essential amino acids such as cysteine were shown to increase prodigiosin production because methyl group on C6 of prodigiosin structure comes from methionine (Suryawanshi et al., 2014).

Relationship between Cell Growth and Prodigiosin Production

Bacteria require certain conditions such as temperature, pH, and carbon source to exhibit higher cell growth, but the growth conditions for each bacterium are not the same. The cell growth was illustrated in a growth curve to determine the growth phase. The growth phase consists of lag phase, exponential phase, stationary phase, and death phase. *S. nematodiphila* exhibits the same pattern of growth curve as the other bacteria. **Figure 2** and **Figure 3** show the relationship between cell growth of *S. nematodiphila* and prodigiosin production by using used palm oil and fresh palm oil as a carbon source respectively. Both figures were illustrated at concentration of 8% (w/v) of used palm oil and fresh palm oil.

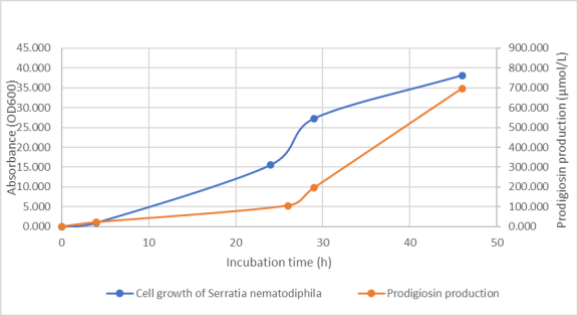

Figure 2 Relationship between cell growth of *Serratia nematodiphila* and prodigiosin production by using used palm oil as carbon source.

Figure 2 shows that the increase of cell growth caused an increase of prodigiosin. The prodigiosin produced by *S. nematodiphila* was at the later growth phase which was common for prodigiosin (Srimathi et al., 2017). The prodigiosin production increases exponentially at exponential phase of bacterial growth.

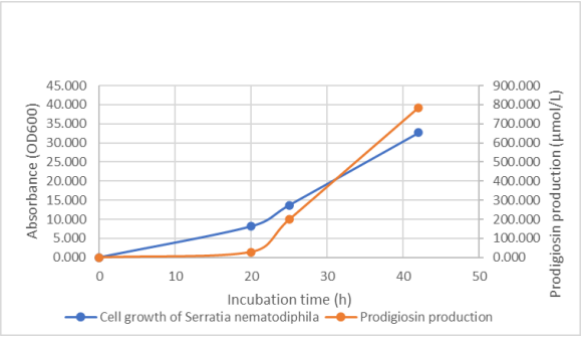

Figure 3 Relationship between cell growth of *Serratia nematodiphila and* prodigiosin production by using fresh palm oil as carbon source.

Figure 3 above shows the relationship between cell growth of *S. nematodiphila* and prodigiosin production by using 8% (w/v) of fresh palm oil as a carbon source. The prodigiosin produced by *S. nematodiphila* at the later growth phase is the same as in used palm oil, which is at the exponential phase of bacterial growth. The cell growth of S. *nematodiphila* increases exponentially after 20 hours as well as for prodigiosin production. Prodigiosin is commonly produced after incubation time of 20 hours. This is because prodigiosin is a secondary metabolite that is not required in primary metabolism for cell growth (Abdul Manas et al, 2020). Thus, the prodigiosin production increases only when the incubation time increases. Other than that, used palm oil has longer lag phase compared to fresh palm oil because used palm oil has higher amount of unsaturated fatty acid content as shown in **Table 3**. Hence, bacteria require more time to adapt to the composition of used palm oil (Haddix et al., 2008).

Table 4 Kinetic growth of *Serratia nematodiphila* with different type of carbon source at concentration of 8% (w/v)

Type of oil	Specific growth rate (h^{-1})	Doubling time (h)	Reference
Used Palm oil	0.112	6.18	This study
Fresh palm oil	0.104	6.69	This study
Fresh palm oil	0.255	2.72	(Abdul Manas et al., 2020)
Olive oil	0.241	2.88	(Abdul Manas et al., 2020)
Peanut oil	0.240	2.89	(Abdul Manas et al., 2020)

The data from **Figure 2** and **Figure 3** were further studied by determining the specific growth rate and doubling time and it was tabulated in **Table 4** above. The used palm oil as carbon source exhibits a slightly higher specific growth rate but slightly lower in doubling time compared to fresh palm oil. The growth rate was indirectly proportional to the prodigiosin production (Haddix et al., 2008).

The used palm oil induces higher growth but less prodigiosin yield when compared to fresh palm oil at a concentration of 8% (w/v). Haddix et al. (2008) stated that high growth rate causes decrease in prodigiosin production while low growth rate causes rapid production of prodigiosin. Thus, higher growth rate caused by used palm oil has negatively induced the prodigiosin production. The saturated fatty acid content in used palm oil is less than in the fresh palm oil based on **Table 3**. Su et al. (2011) stated that saturated fatty acids can act as carbon and energy sources during fermentation. Hence, higher amount of saturated fatty acid content in fresh palm oil can increase cell growth and prodigiosin (Giri et al., 2004). Furthermore, it was found that oil substrate provides twice the energy than glucose (Spalvins et al., 2020). Thus, oil substrate can provide sufficient energy for culturing microbes. Other than that, higher amount of unsaturated fatty acid in used palm oil could contribute to lower prodigiosin yield. This is due to the presence of double bond in unsaturated fatty acid which causes the carbon source to be less accessible (Giri et al., 2004). Although the exact used palm oil content other than fatty acid is unknown, used palm oil may contain carbohydrates and protein such as glucose, fructose, essential and non-essential amino acid derived from the cooked food. There are several sources that contribute to less prodigiosin production. For example, glucose and maltose are carbon sources that inhibit prodigiosin production through catabolite repression (Giri et al., 2004). However, proline, which is one of the non-essential amino acids, can give positive effect on prodigiosin production (Suryawanshi et al., 2014).

Potential as Sunscreen Agent

Some bacterial pigments have the potential of protecting skin from ultraviolet (UV) rays. Prodigiosin that is commonly found to be produced by *Serratia* sp. is one of the bacterial pigments which has the potential as sunscreen agent. This is because *Serratia* sp. lives in habitat with high exposure to UV rays, so it has photoprotective potential against UV rays. In addition, prodigiosin produced by *Streptopiomyces* sp. and *Vibrio* sp. are also associated with UV protection (Borić et al., 2011). The sun protection factor (SPF) is commonly used as a measurement of determining a good sunscreen. Mansuri et al. (2021) stated that SPF can be defined as the fraction of UV radiation needed to bring about the erythema on skin after the application of sunscreen to the amount of energy needed to bring the same effect on skin without application of sunscreen.

Table 5 Comparison on sun protection factor (SPF) on different concentration of bacterial pigment

Source of bacteria	Bacteria	Bacterial pigment	Concentration of pigment (% w/v)	$SPF_{in\ vitro}$	Reference
Waterfall	*Serratia nematodiphila*	Prodigiosin	1	3.68	This study
Soil	*Serratia marcescens*	Prodigiosin	0.92	2.10	(Cediel Becerra et al., 2022)
Soil	*Serratia marcescens*	Prodigiosin	19.7	30.90	(Cediel Becerra et al., 2022)
Surface water	*Serratia marcescens*	Prodigiosin	1	2.72	(Heshmatipour & Bana, 2017)
Rhizosphere soil	*Brevibacterium* sp.	Carotenoids (Orange)	10	5.311	(Patki et al., 2021)

The sun protection factor (SPF) was calculated using the in vitro spectrophotometer method. In this study, the SPF value of 1% (w/v) of prodigiosin is 3.68 as shown in Table 5. The prodigiosin with a higher concentration of 19.7% (w/v) by *Serratia marcescens* exhibits highest SPF value of 30.9 when compared to lower concentrations of prodigiosin based on **Table 5** (Cediel Becerra et al., 2022). Besides, the concentration of 10% (w/v) of carotenoids by *Brevibacterium* sp. has SPF value of 5.311 (Patki et al., 2021). Therefore, it is observed that SPF value increases as the pigment concentration increases. The higher SPF value gives higher protection on human skin from UVB rays. A higher concentration of bacterial pigment contributes to a higher SPF value. Other than that, Mansuri et al. (2021) stated that SPF level above 15 provides minimal protection; SPF 15 to 29 give average protection; SPF level of 30 to 49 for higher protection while SPF above 50 gives very high protection. If referring to the percentage of UVB radiation blockage, SPF 15 indicates 93% blockage; SPF 30 has 97% blockage while SPF 50 has 98% blockage (Mansuri et al., 2021). In short, comparison in **Table 5** suggests that the prodigiosin produced by *Serratia nematodiphila* has the potential of photoprotective activities.

CONCLUSION

In this study, prodigiosin was successfully produced from fermentation of *Serratia nematodiphila* by supplementation of used palm oil and fresh palm oil. The used palm oil enhanced the prodigiosin production and cell growth as well as fresh palm oil which showed a comparable result. The specific growth rate with respective used palm oil and fresh palm oil as substrates were comparable. The findings from this study showed that used palm oil confers a comparable effect than the fresh palm oil to enhance the prodigiosin production from *S. nematodiphila*. Hence, this study provides a cheap and sustainable alternative of oil substrate for production of prodigiosin as a potential sunscreen agent.

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