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# EFFECT OF PH AND NUTRITIONAL FACTORS ON BACTERIAL GROWTH AND CAROTENOIDS PIGMENTS PRODUCTION BY LOCAL BACTERIAL ISOLATES VIA VARYING ONE PARAMETER AT-A-TIME APPROACH

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## ABSTRACT

The local bacterial isolates, LO1 and LY1 were cultured in different pH media (pH 6, 7 and 8) and supplemented with 1% (w/w) of different nutritional factors, includes carbon sources (glucose, sucrose, starch), nitrogen sources (urea, casein, gelatine) and of inorganic salts (calcium chloride, sodium nitrate, sodium dihydrogen phosphate). It was found that the maximum orange pigment production was observed for LO1 local bacterial isolate cultivated in each modified LB media with pH 8, sucrose, gelatin and sodium dihydrogen phosphate respectively. Meanwhile, LY1 local bacterial isolate favoured to synthesis yellow pigment after being cultured separately in modified LB media with pH 8, glucose, casein and sodium dihydrogen phosphate.

**KEYWORDS:** Pigments; carotenoids; pH; nutritional factors; bacterial growth; local bacterial isolates.

## INTRODUCTION

Pigments with wide range of colour have been commercially used as food additives, colour intensifiers and antioxidants in foodstuffs for ages (Kumar et al. 2015). They can be obtained from natural sources such as plants, animals and microorganisms (known as organic pigments or bio-colours) or chemically synthesized (known as inorganic or artificial pigments). However, the natural pigments showed a great demand in food industry application due to the toxicity of artificial pigments (Usman et al. 2017).

Lately, awareness in human safety and environmental conservation has made an enthusiasm for natural sources of colours. The natural pigments from microbial sources are believed to be safe, non-toxic and biodegradable in nature (Usman et al. 2017). Besides, the microbial pigments production includes as one of the emerging fields of research since the microbes exhibited easy and fast growth in culture media (Heer and Sharma 2017).

Earlier study found that the bacteria isolated from Lala liquid could produce carotenoids pigments with yellow and orange bright colour, each recognized as Lala Yellow (LY1) and Lala Orange (LO1). The LY1 local bacterial isolate showed circular in form and viscid in texture with flat shape and smooth shinny surface. Meanwhile, the LO1 local bacterial isolate exhibited punctiform in form and dry in texture with convex shape and smooth surface (Hanina et al. 2018).

Most of the microbial pigments are carotenoids in nature. Carotenoids, naturally derived pigments with either yellow, orange or red in colour are present in wide variety of microorganisms (Kirti et al. 2014). The carotenoids with diverse group of lipophilic compounds, include β-Carotene, lycopene, lutein, and zeaxanthin are not only useful for the colouration, but they also exhibit distinctive phytochemical properties contributing to human health. They show important biological activities associated with antioxidant properties, include strengthening the immune system, reducing the risk of degenerative diseases as well as preventing the risk of cardiovascular disease and macular degeneration (Smitha and Nath 2017).

Naturally, bacteria must face and respond to numerous environmental conditions for their survival by producing signal transduction system. They could sense the environmental changes and control the coordinated expression of genes as cellular defence mechanism (Chung et al. 2006). It showed that the bacteria can create their own mechanism to ensure their growth and survival under hostile and challenging environment by changing their characteristics in term of gene expression pattern related to transcription and translation processes

for bio-products synthesis (Foster 2004). Hence, the different growth conditions include pH, temperature and nutrient availability could affect the bacterial growth and pigment production.

In cultivation or fermentation process, the bacteria will synthesize primary and secondary metabolites. The microbial secondary metabolites include pigments are produced during stationary phase which are usually derived from primary metabolites. The formation of microbial secondary metabolites is regulated by nutrients, growth rate, feedback control as well as enzyme inactivation and induction (Ali et al. 2018). Thus, optimization of fermentation process is very important to improve the microbial pigments production by bacteria. The optimization of microbial pigments production by bacterial strains can be performed based on conventional study using one variation of parameter, resulted in only an apparent set of optimal conditions (Venil et al. 2015).

## MATERIALS AND METHODS

### Bacteria preparation

Local bacterial isolates, orange pigment producing bacteria (LO1) and yellow pigment producing bacteria (LY1) were obtained from Microbiology Laboratory, Faculty of Science and Technology (FST), Universiti Sains Islam Malaysia (USIM). These bacteria were previously isolated from fresh seafood, seashells (*Lala*) by Siti Nabilah (2017). The bacteria were cultivated in Luria-Bertani broth (LBB, Merck, Germany) and maintained on Luria-Bertani agar (LBA, Merck, Germany) as stock culture and further incubated at 30 ºC for 24 hours according to method done by Shaikh (2016).

### Optimization of carotenoids pigments production via one parameter at-a-time approach

Optimization of carotenoids pigment production was carried out following conventional procedure of varying one parameter at-a-time approach according to method by Singh et al. (2017). In this approach, only one factor varied at a time while keeping others variable constant. The effect of pH of media (pH 6, 7 and 8) and 1% (w/w) of medium components such as carbon sources (glucose, sucrose and starch), nitrogen sources (urea, casein and gelatin) and inorganic salts (calcium chloride, sodium nitrate, sodium dihydrogen phosphate) on carotenoids production were evaluated separately.

### Cultivation of carotenoids pigment producing bacterial

Cultivation of carotenoids pigment producing bacterial was carried out based on method by Samyukhta and Mahajan (2016) with modification. A loopful colony of each local bacterial isolates, LO1 and LY1 was cultured in Erlenmeyer flasks containing Luria-Bertani Broth (LB, Merck, Germany) with different pH medium or nutrients supplementation separately and incubated at 30 ºC for 24 hours as seed culture. About 1 ml of each seed cultures was transferred into new Erlenmeyer flask containing 99 ml of Luria-Bertani broth (LB, Merck, Germany) of different pH (pH 6, 7 and 8) and nutrients supplementation respectively before being further incubated in shaking incubator (Sastec, USA) with 150 rpm at 30 ºC for 5 days. A culture without pH adjustment and nutrients supplementation served as a control. These set of experiments were performed in triplicates. The density of 5 days old bacterial cultures was then measured using Biophotometer (Eppendorf, Hamburg, Germany) at optical density (OD) of 600 nm for further analysis on degree of pigmentation.

### Carotenoids pigment extraction

Carotenoids pigments produced by local bacterial isolate were extracted based on method by Nor Aqeela (2017) with modification. Each 5 days old bacterial culture was harvested using centrifuge (Hanil Combi 514R, Korea) at 6000 x g at 4 ºC for 10 minutes to separate the bacterial cells (pellet) from culture media (supernatant). The pellet and supernatant were then collected for further extraction using different types of solvent. The supernatant was mixed with chloroform (Merck, Germany) in 1:1 ratio. The suspension was then vortexed and centrifuged at 6000 x g at 4 ºC for 10 minutes forming two layers of immiscible liquid. The clear pigmented supernatant was then collected. Meanwhile, the pellet was re-suspended in 10 ml of methanol (Merck, Germany). The mixture was vortexed before being further centrifuged at 6000 x g at 4ºC for 10 minutes. The clear supernatant containing pigments was then collected. The yield of dried pigments extracted from pellet and supernatant were measured before being further characterized using UV-Visible spectrophotometer.

### Carotenoids pigment characterization using UV-visible spectrophotometer

The extracted carotenoids pigments were analysed using UV-visible spectrophotometer (Varian Cary®50, Germany) based on method by Yip et al. (2014) with slightly modification. The extracted carotenoids pigments were scanned at the wavelength of 400 to 700 nm. Each yellow and orange pigment extracts was dissolved in 3 ml of methanol before being subjected to UV- Visible Spectrophotometer analysis. A 95% of methanol was used as a blank and the sample were scanned at wavelength of 400 to 700 nm to determine the maximum absorption spectra. The degree of pigmentation was then determined using an equation below:

Degree of pigmentation = Optical density of pigment / Optical density of bacterial growth

## RESULTS

### Optimization of carotenoids pigments production via one parameter at-a-time approach

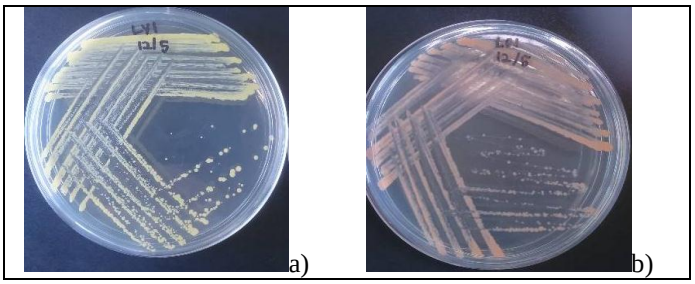
a) b)

**Figure 1: Pigment produced by local bacterial isolate.**

Note: a) Yellow pigment produced by LY1 local bacterial isolate; b) Orange pigment produced by LO1 local bacterial isolate.

### Effect of pH and nutritional factors on pigment producing bacterial growth

**Table 1: Effect of pH and nutrient supplementation of LO1 growth as being measured at optical density of 600 nm.**

| Types of fermentation media | | Bacterial cell density at absorbance $OD_{600}$ |
|---|---|---|
| [a]Control | | 5.23 ± 0.07 |
| [b]pH | pH 6 | 10.15 ± 0.28 |
| | pH 7 | 10.68 ± 0.06 |
| | pH 8 | 5.32 ± 0.35 |
| [c]Carbon sources (1% w/w) | Glucose | 10.43 ± 0.06 |
| | Sucrose | 5.78 ± 0.04 |
| | Starch | 10.56 ± 0.06 |
| [d]Nitrogen Sources (1% w/w) | Urea | 10.56 ± 0.06 |
| | Casein | 4.17 ± 0.07 |
| | Gelatine | 3.81 ± 0.20 |
| [e]Inorganic salts sources (1% w/w) | Calcium chloride | 5.78 ± 0.07 |
| | Sodium nitrate | 4.81 ± 0.06 |
| | Sodium dihydrogen phosphate | 2.01 ± 0.15 |

Note: [a]Control – LB media without pH adjustment or nutrient supplementation; [b]LB media with different pH adjustment; [c]LB media with different carbon sources; [d]LB media with different nitrogen sources; [e]LB media with different inorganic salts sources.

**Table 2: Effect of pH and nutrient supplementation of LY1 growth as being measured at optical density of 600 nm.**

| Types of fermentation media | | Bacterial cell density at absorbance $OD_{600}$ |
|---|---|---|
| [a]Control | | 5.50 ± 0.06 |
| [b]pH | pH 6 | 11.79 ± 0.72 |
| | pH 7 | 11.98 ± 0.40 |
| | pH 8 | 5.68 ± 0.26 |
| [c]Carbon sources (1% w/w) | Glucose | 5.12 ± 0.30 |
| | Sucrose | 8.23 ± 0.35 |
| | Starch | 5.82 ± 0.04 |
| [d]Nitrogen Sources (1% w/w) | Urea | 8.78 ± 0.12 |
| | Casein | 9.83 ± 0.08 |
| | Gelatine | 12.05 ± 0.10 |
| [e]Inorganic salts sources (1% w/w) | Calcium chloride | 12.12 ±0.06 |
| | Sodium nitrate | 6.03 ±0.15 |
| | Sodium dihydrogen phosphate | 5.77 ± 0.05 |

Note: [a]Control – LB media without pH adjustment or nutrient supplementation; [b]LB media with different pH adjustment; [c]LB media with different carbon sources; [d]LB media with different nitrogen sources; [e]LB media with different inorganic salts sources.

**Effect of pH and nutritional factors on carotenoids pigments production**

**Table 3: Analysis on degree of pigmentation of extracted pigment from LO1.**

| Types of fermentation media | | Bacterial cell density at absorbance, $OD_{600}$ | Pigment maximum absorption at absorbance, $OD_{400nm-700nm}$ | Degree of pigmentation |
|---|---|---|---|---|
| [a]Control | | 5.50 ±0.07 | 4.09 ±0.11 | 0.74 ±1.57 |
| [b]pH | pH 6 | 11.79 ±0.28 | 5.17 ±0.12 | 0.44 ±0.43 |
| | pH 7 | 11.98 ±0.06 | 4.05 ±0.07 | 0.34 ±1.17 |
| | pH 8 | 5.68 ±0.35 | 4.47 ±0.20 | 0.78 ±0.57 |
| [c]Carbon sources (1% w/w) | Glucose | 5.12 ±0.06 | 4.87 ±0.23 | 0.95 ±3.83 |
| | Sucrose | 8.23 ±0.04 | 3.81 ±0.12 | 0.47 ±3.00 |
| | Starch | 5.82 ±0.06 | 4.33 ±0.15 | 0.74 ±2.50 |
| [d]Nitrogen sources (1% w/w) | Urea | 8.78 ±0.06 | 3.86 ±0.11 | 0.44 ±1.83 |
| | Casein | 9.83 ±0.07 | 5.46 ±0.16 | 0.55 ±2.29 |
| | Gelatin | 12.05 ±0.20 | 5.16 ±0.08 | 0.43 ±0.40 |
| [e]Inorganic salts sources (1% w/w) | Calcium chloride | 12.12 ±0.07 | 4.69 ±0.12 | 0.39 ±1.71 |
| | Sodium nitrate | 6.03 ±0.06 | 4.69 ±0.12 | 0.78 ±2.00 |
| | Sodium dihydrogen phosphate | 5.77 ±0.15 | 5.16 ±0.20 | 0.89 ±1.33 |

Note: [a]Control – LB media without pH adjustment or nutrient supplementation; [b]LB media with different pH adjustment; [c]LB media with different carbon sources; [d]LB media with different nitrogen sources; [e]LB media with different inorganic salts sources.

**Table 4: Analysis on degree of pigmentation of extracted pigment from LY1.**

| Types of fermentation media | | Bacterial cell density at absorbance, $OD_{600}$ | Pigment maximum absorption at absorbance, $OD_{400nm-700nm}$ | Degree of pigmentation |
|---|---|---|---|---|
| [a]Control | | 5.50 ±0.06 | 4.09 ±0.14 | 0.74 ±2.33 |
| [b]pH | pH 6 | 11.79 ± 0.72 | 5.17 ±0.10 | 0.44 ±0.14 |
| | pH 7 | 11.98 ± 0.40 | 4.05 ±0.09 | 0.34 ±0.23 |
| | pH 8 | 5.68 ± 0.26 | 4.47 ±0.13 | 0.78 ±0.50 |
| [c]Carbon sources (1% w/w) | Glucose | 5.12 ± 0.30 | 4.87 ±0.23 | 0.95 ±0.77 |
| | Sucrose | 8.23 ± 0.35 | 3.81 ±0.09 | 0.47 ±0.26 |
| | Starch | 5.82 ± 0.04 | 4.33 ±0.04 | 0.74 ±1.00 |
| [d]Nitrogen sources (1% w/w) | Urea | 8.78 ± 0.12 | 3.86 ±0.06 | 0.44 ±0.50 |
| | Casein | 9.83 ± 0.08 | 5.46 ±0.08 | 0.55 ±1.00 |
| | Gelatin | 12.05 ± 0.10 | 5.16 ±0.18 | 0.43 ±1.80 |
| [e]Inorganic salts sources (1% w/w) | Calcium chloride | 12.12 ±0.06 | 4.69 ±0.06 | 0.39 ±1.00 |
| | Sodium nitrate | 6.03 ±0.15 | 4.69 ±0.16 | 0.78 ±1.07 |
| | Sodium dihydrogen phosphate | 5.77 ± 0.05 | 5.16 ±0.25 | 0.89 ±5.00 |

Note: [a]Control – LB media without pH adjustment or nutrient supplementation; [b]LB media with different pH adjustment; [c]LB media with different carbon sources; [d]LB media with different nitrogen sources; [e]LB media with different inorganic salts sources.

## DISCUSSION

### Optimization of carotenoids pigments production via one parameter at-a-time approach

The local bacterial isolates, LO1 and LY1 were previously isolated and purified from fresh seashells *(Lala)* (Siti Nabilah 2017; Hanina et al. 2018). Each bacterium could produce carotenoids pigments with yellow and bright orange colour, recognized as Lala Yellow (LY1) and Lala Orange (LO1) respectively as shown in Figure 1. The LY1 producing yellow pigment exhibited circular in form and viscid in texture with flat shape and smooth shinny surface. Whilst, the LO1 with orange pigment showed punctiform in form and dry in texture with convex shape and smooth surface (Hanina et al. 2018).

Carotenoids pigments are generally synthesized by certain pigments producing bacteria in response to environmental condition such as growth temperature, light, pH, nutrient availability and salt concentration (Smitha and Nath 2017). Study on the regulatory mechanisms of bacteria related to pigment production could provide an insight information on their adaptation toward the respective environment condition (Castro et al., 2018). It has been reported that red carotenoids

pigment synthesized by heterotrophic bacteria due to adaptation towards environmental stress as being cryo- and solar radiation protectants (Dieser et al. 2010). Therefore, optimization of cultivation conditions such as pH and nutrition availability need to be regulated in order to enhance the carotenoids pigments production. In this present study, the optimization of carotenoids pigments synthesis by two local bacterial isolates, LY1 and LO1 was conducted according one variation of parameter at-a-time approach. This typical optimization approach which is involved changing of one parameter or varying of several parameters at the same time resulted in only one apparent set of optimal conditions (Vishnu and Palaniswamy 2017).

Earlier study found that both LY1 and LO1 local bacterial isolates showed high bacterial growth with intense yellow-orange pigments production in Luria-Bertani compared to Nutrient and Peptone-glycerol synthetic growth medium (Hanina et al. 2018). However, there are some other factors need to be considered in modifying the bacterial growth media in order to increase the pigment production, include medium composition such as carbon, nitrogen and inorganic salts sources as well as physicochemical parameters like pH. It has been reported that carbon, nitrogen and inorganic salts sources are among the important variables that could affect the bacterial growth and microbial product synthesis such as pigments (Patil et al. 2015). In this experiment, LO1 and LY1 local bacterial isolates were cultured in Luria-Bertani broth containing different pH media (pH 6, 7 and 8) or supplemented with variable of nutrients, including 1% (w/w) of carbon (glucose, sucrose and starch); 1% (w/w) of nitrogen (urea, casein and gelatine) and 1% (w/w) of inorganic salts (calcium chloride, sodium nitrate and sodium dihydrogen phosphate) separately for 5 days with incubation temperature of 30 ºC in order to enhance yellow-orange pigments production.

Pigments are secondary metabolites produced during late-log and stationary of bacterial growth phases (Ali et al. 2018). Therefore, the carotenoids pigments may be internally synthesized or externally secreted in the growth media by LO1 and LY1 local bacterial isolates after being cultivated for 5 days of fermentation periods. The carotenoids pigments produced were then harvested using centrifugation at 6000 rpm and 4 ºC for 10 minutes to separate supernatant from pellet before being further subjected to pigments extraction. The selection of suitable extraction technique may affect the pigments yields. The extraction methods were selected according to the solubility of interest chemicals. Thus, the suitable extraction method of pigments must be considered as the target compounds may be non-polar or polar (Sasidharan et al. 2010). It has been reported that an organic solvent such as methanol and chloroform could give a good extraction yields of carotenoids or yellow-orange pigments (Saini and Keum 2018). In this experiment, each carotenoids pigment obtained from pellet and supernatant was extracted using methanol and chloroform respectively. Methanol may directly dissolve polar compounds in pellet, while chloroform may dissolve non-polar compounds in supernatant. Each pigment extracted from pellet and supernatant were collected, mixed and dried before being further characterized spectrophotometrically.

**Effect of pH and nutritional factors on pigment producing bacterial growth**

Environmental and nutritional factors are very important for controlling the developmental and physiological processes occurred in microbes. Therefore, there is a significant effect of medium composition as well as operating conditions such as pH on microbial cell growth, development, reproduction and metabolite production (Rehman and Dixit 2019). In this experiment, the density of each 5 days old bacteria cultivated in LB broth treated with different pH or variable of nutrient supplementation were determined using Biophotometer at optical density (OD) of 600 nm. The OD determination is used to evaluate the turbidity or cloudiness of bacterial culture that related to bacterial growth rate. It showed that high OD reading would indicate high bacterial growth.

Results indicated that different pH media as well as different nutritional factors could affect the bacterial growth of local bacterial isolate, LO1 and LY1 as shown in Table 1 and Table 2 respectively. Generally, both LO1 and LY1 local bacterial isolates grown best in normal pH 7 and nearly acidic pH media (pH 6) with high OD reading that related to bacterial density. The OD reading decreased when the local bacterial isolates cultivated in media with pH 8 indicated that nearly alkaline condition could reduce the bacterial growth. Bacteria may encounter an inclusive of environmental pH within its surrounding. However, they will adapt to the pH alteration by maintaining their cytoplasmic pH within a small range of pH to stabilize the nucleic acids and protein for the consequent growth (Krulwich et al. 2011).

Nutrients are required by bacteria for their growth and reproduction processes. The nutrients are being transported from environmental condition or growth media through cell walls and used up either by catabolic or anabolic processes to produce energy and create building block for cell growth (Rouf et al. 2017). Among the essential elements for the bacterial growth and metabolism are carbon, nitrogen and inorganics salts sources (Patil et al. 2015). The carbon sources are usually degraded at cellular level as monosaccharides, disaccharides before being further transferred and metabolized by bacterial cells (Poorniammal et al. 2015). Based on the recorded OD readings, LO1 bacterial cells were preferred to use glucose and starch as carbon source for their growth as shown in Table 1. However, LY1 bacterial cells showed opposite result in which sucrose was the most utilized carbon source compared to glucose and starch as summarized in Table 2.

The nitrogen sources which are commonly available in the surrounding environment either in low or high concentration may affect the growth system in most of microorganisms. The nitrogen gas such as nitrogen dioxide and nitric oxide in surrounding air are reported to give a positive impact in enhancing growth of bacteria (Patil et al. 2015). This present study indicated that each LO1 and LY1 local bacterial isolates is preferred to utilize urea and gelatine respectively for their growth as shown in Table 1 and Table 2.

Besides, the inorganic salts are also needed by some bacterial strains to carry out their cellular activities. The addition of inorganic salts into media performs several functions. Primarily they can help to retain the osmotic balance of the cells as well as can regulate the membrane cell potential by provision of sodium, potassium and calcium ions (Rouf et al. 2017). Therefore, the presence of inorganic salts at different concentration in growth media may affect bacterial growth. Even though some of the bacteria may grow without inorganic salts, but they are also able to tolerate with the small amount of salt introduce in growth media (Castro et al. 2018). Both LO1 and LY1 local bacterial isolates could tolerate with each three types of inorganic salts sources. However, they exhibited good growth in media with the addition of calcium chloride as inorganic salts sources.

**Effect of pH and nutritional factors on carotenoids pigments production**

Production of carotenoids pigments is strongly influenced by physical factors include pH, temperature and incubation time as well as media components especially carbon, nitrogen and inorganic salts sources (Patil et al. 2015). Therefore, a study on the effect of pH and nutritional factors was essential to find out the optimum culture media conditions and components for carotenoids pigments synthesize.

In present study, the carotenoids pigments produced by LO1 and LY1 were characterized using UV-visible spectrophotometer to determine the maximum wavelength absorption. The maximum absorption of yellow-orange pigment extracts produced by LO1 and LY1 cultivated in LB broth with pH adjustment or nutrient supply were obtained at wavelength of 400 nm – 700 nm as shown in Table 3 and Table 4 respectively.

In general, the carotenoids pigments produced by local bacterial isolates LO1 and LY1 cultured either in different pH media and nutritional factors with or without pH adjustment and nutrients added exhibited different extent of maximum absorption. Degree of pigmentation was calculated by dividing the readings data of pigment maximum absorption at absorbance of 400 nm – 700 nm over the reading data of bacterial cell density at absorbance of 600 nm (Sasidharan et al. 2013). In this study, high degree of pigmentation showed by LO1 and LY1 cultivated in media with pH 8 as presented in Table 3 and Table 4. The degree of pigmentation was decreased when both local bacterial isolates grown in media with neutral and nearly acidic pH. This revealed that a slight alkaline medium could affect the degree of pigmentation instead of affecting the bacterial growth. It has been reported that the optimum pH ranges from 7 to 9 could facilitate the pigmentation (Bhat et al. 2013).

Based on the degree of pigmentation, sucrose was the most utilized carbon sources for the orange pigment production by LO1 local bacterial isolate. Different result showed by LYI local bacterial isolate in which is preferred to use glucose as carbon sources for yellow pigment synthesis. It has been reported that carbon sources such as glucose, fructose, lactose and sucrose would give a remarkable influence on enhancement of biomass and pigment production (Pradeep and Pradeep 2013).

High degree of pigmentation was also observed for the LO1 local bacterial isolate cultivated in media with gelatine as nitrogen sources. However, LY1 local bacterial isolate is favoured to utilize casein as nitrogen sources for yellow pigment production. It revealed that gelatine and casein would give a significant effect on carotenoids pigments production by LO1 and LY1 local bacterial isolates. Inorganic salts sources could also affect the carotenoids pigments production by LO1 and LY1 local bacterial isolates. Both the local bacterial isolates cultivated in modified LB media containing sodium dihydrogen phosphate showed high degree of pigmentation.

## CONCLUSION

The present study revealed that growth rate and carotenoids pigments production by local bacterial isolate, LO1 and LY1 were influenced by physical factors especially pH as well as media components include carbon, nitrogen and inorganic acid sources. A thorough understanding on the effect of these factors and regulation of biosynthetic pathways for carotenoids pigments production will facilitate the development of bioprocess to increase the pigment synthesis, hence opening new avenues for further research.

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