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Pigments Production of Bacteria Isolated From Dried Seafood and Capability to Inhibit Microbial Pathogens

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Abstract: *Microorganisms are the rich sources of natural pigments and can be produced by bacteria, which are isolated from natural sources include food product. Some pigments have a capability to inhibit pathogenic bacteria. Purposes of this study were to isolate and select bacteria, which produce pigments colors, from 13 groups sample of dried seafood from Nongmon market, Chonburi province, Thailand and study their capability of pigments to inhibit pathogenic bacteria. The results showed that 29 isolated bacteria, which produced carotenoids pigments as yellow and orange colors. Of these 29 isolated bacteria, 8 isolated bacteria presented high degree of pigmentation were studied the capability of pigments to inhibit pathogenic bacteria by agarwell diffusion method. Carotenoids pigments from 7 isolated appeared antibacterial activity against Staphylococcus aureus ATCC 25923 (MSSA), S. xylosus, Bacillus macerans, Citrobacter divesus and Aeromonas schubertii. Taxonomic characterization was carried out and 7 isolates were identified as Bacillus sp., Corynebacterium sp., Kocuria roseus, Staphylococcus sp. and Brevibacterium sp.*

Keywords: *Dried seafood, Bacterial pigment, Carotenoids, Antibacterial*

I. Introduction

Natural pigments refer to pigment product of plant, animal, insect, microorganism and microalgae. Especially, natural pigments extracted from microorganisms have been used for the pharmaceutical, textile and food industry for decades. Many of microorganisms have been exploited for the production of color. The carotenoids are considered to be the key and most abundant pigment group, produced by a wide variety of organisms. They appear either yellow, orange or red and their biosynthesis of the carbon skeleton are based on condensation of isoprenyl units. Microorganisms, that produce yellow, orange or red colorant in carotenoids pigment are, for example, *Blakeslea trispora* (ß-carotene), *Rhodosporidium* (γ-carotene), *Mycobacterium lacticola* (astaxanthin), *Brevibacterium* (canthaxanthin), *Monascus purpureus*, *Streptomyces coelicolor* (polyketides) and Gram-positive micrococci [1]. Furthermore, *Agrobacterium aurantiacum*, *Paracoccus carotinifaciens, P. carotinifaciens*, *P. haeundaensis*, *Paracoccus* sp. strain MBIC 01143, *Halobacterium salinarium*, *Mycobacterium lacticola*, *Xanthophyllomyces dendrorhous* and *Haematococcus pluvialis* produce carotenoids pigment in the form of astaxanthin [2]. In general, microorganisms have the ability to produce natural pigments which can be used to inhibit microbial pathogen and microbial spoilage in food [3, 4]. The antimicrobial properties of microbial pigments have been observed in the recent years. Carotenoid pigments extracted from *Dunaliella* spp., *Phafia rhodozyma*, *Rhodotorula* sp. and othermicroorganism had the capacity to inhibit various pathogens associated with food borne pathogens such as *Salmonella typhimurium*, *S. enteritidis*, *Staphlococcus aureus*, *Escherichia coli* and *Bacillus subtilis* [5, 6, 7]. The purpose of this study is to isolate and select bacteria from dried seafood and seafood products which produced high yields of pigments and inhibited pathogenic bacteria.

II. Material And Method

2.1 Collection of sample

Thirteen groups sample of dried seafood and seafood products were collected from Nongmon market in Chonburi province. Samples were collected in sterile plastic bags, weighing 100 grams/sample. Then, all samples were transported to Microbiology Laboratory, Environmental Science Program, Faculty of Science, Burapha University, Thailand.

2.2 Isolation of Bacteria strains and identification

The bacterial strains were isolated from dried seafood and seafood products samples on nutrient agar (NA) (MERCK, Germany) by spread plate technique. NA plates were incubated at 35 °C for 24-48 hrs. Selected colonies, that produces pigment and purified on the same medium, were determined according to cell morphology and biochemical characteristic.

2.3 Bacteria pigment production

Loopfull of bacterial from NA plate transferred to 25 ml of nutrient broth (NB) (MERCK, Germany), incubated on a rotary shaker at 200 rpm for 24 hrs. Five ml of inoculum size was transferred to 250 ml Erlenmeyer flask containing 45 ml of NB at 30 °C on a rotary shaker at 150 rpm for 48 hrs. [8]. After that, a 1 ml of the culture was diluted to 10 ml by 25% NaCl solution to measure growth in terms of optical density (OD) at 660 nm using a spectrophotometer (GVC-Cintra40 UV visible spectrophotometer) [7].

2.4 Bacteria pigment extraction and analysis

The culture medium was harvested and centrifuged to remove cells at 9000 rpm for 10 min and the supernatant were discarded. Cell pellets were resuspended with deionized water, followed by centrifugation 9000 rpm for 10 min to recover the cells by discharging the supernatant again. The 10 ml of methanol (with a purity of 99.7%) was added to the cell for extracting bacteria pigment and the mixture was vortexed, extracted twice with the same solvent. The mixture of the cells and methanol was treated by ultrasonication (59 KHz, 35-40 °C, 90 min) and keep overnight in light protects. The pigment extract was separated by centrifugation at 9000 rpm for 10 min and filtrated by 0.45 µm filter. The pigment extracts were analyzed by scanning the absorbance in wavelength region of 300-650 nm using spectrophotometer (GVC-Cintra40 UV visible spectrophotometer). The solvent was evaporated to dryness in evaporator at 40 °C and crude pigment was collected in screw cap bottle and keep light protects at -20 °C [7, 8]. The Degree of cell pigmentation was calculated as the ratio of OD of carotenoid to OD of the growth (ODλmax/OD660) [8].

2.5 Antibacterial Activity

Antibacterial activity performed by agar well diffusion method. The pigment extracted was dissolved in DMSO and tested activity and using DMSO as a control against the 9 organisms viz. *Staphylococcus aureus* ATCC43300 (MRSA) and *S. aureus* ATCC25923 (MSSA) for control, *S. xylosus*, *Bacillus cereus*, *B. macerans*, *Escherichia coli*, *Klebsiella pneumoniae*, *Citrobacter divesus* and *Aeromonas schubertii*. on Mueller Hinton agar media and a well was prepared in the plates with sterilized cork-borer (0.6 cm). 30 µl of crude pigments extracted were poured into the well. The plates were incubated at 37 °C 24 hr, and the inhibition zones on bacteria culture plates are measured [9].

III. Result And Discussion

3.1 Isolation of Bacteria strains and identification

Many strains of bacterial, which produced pigments, were isolated from dried seafood and seafood products samples. The colony colors of bacteria were present in orange, yellow, dark yellow, orangish yellow and birth yellow (Fig. 1). The data show difference of 29 bacterial colony on NA. 29 isolated were collected to studied pigment production. The most predominant bacteria found in dry seafoods were identified as Gram-positive cocci bacteria 25 isolated and Gram-positive rod 4 isolated.

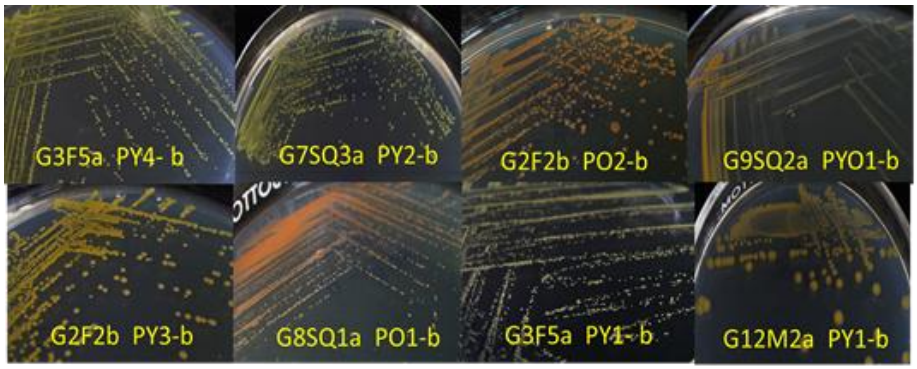


Figure 1 Colony of bacteria producing different colors on NA, isolated from dried seafood.

The results were similar to Nimrat et al. (2013) [10]. They studied to pathogenic bacteria-free in seafood products sale in Chonburi and total heterotroph bacteria were found highest (4.40×109 CFU/g) in Kobi squid. *Enterobacteriaceae* were found in Beka squid (1.70×104 CFU/g). The most predominant bacteria found in dry seafoods were *Staphylococcus*, followed by *Bacillus* and *Micrococcus*. Enterobacteriaceae were *Proteus*, *Enterobacter* and *Klebsiella*.

3.2 Bacteria pigments production

The 29 isolated of bacteria produced pigments in cells, after extraction with methanol (99.7%) the crude pigments extracted showed 5 differenced group of colors such as bright yellow, yellow, orangish yellow, orange, bright orange and greenish yellow (Fig. 2). The degree of cell pigmentation was calculated as the ratio of OD of carotenoid to OD of the growth (ODλmax/OD660) presented in Table 1. The 8 isolated of bacteria strains have highest of degree of cell pigmentation untial 0.587-0.160. Thus, bacteria isolated G12M2a PY1-b, G9SQ2a PYO1-b, G3F5a PY4- b, G3F5a PY1- b, G8SQ1a PO1-b, G2F2b PY3-b, G2F2b PO2-b, G7SQ3a PY2-b were selected to study the capability of pigments to inhibit pathogenic bacteria.

a b c d e

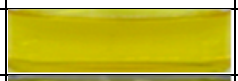
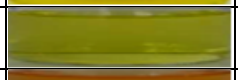
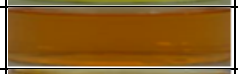
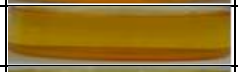
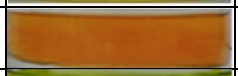
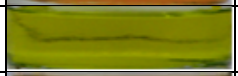
Figure 2 Five different colors groups of crude pigments extracted from bacteria isolated from dried seafood and seafood products after methanol extraction.

Table 1 Growth of and carotenoid production from bacteria 29 isolates by NB.

No.	Isolates	Growth (OD_{660})	Pigment ($OD_{\lambda max}$)	Degree of pigmentation ($OD_{\lambda max}/OD_{660}$)
1	G7SQ1a PO2-b	5.27	0.587	0.111
2	G3F5a PO1- b	2.68	0.293	0.109
3	G2F2a PP2-b	3.77	0.457	0.121
4	G12M2a PY1-b	3.25	0.519	0.160*
5	G9SQ2a PYO1-b	6.06	1.073	0.177*
6	G3F4a PY1-b	7.95	0.661	0.083
7	G3F5a PY2- b	7.51	0.882	0.117
8	G7SQ1a PO1-b	7.87	0.334	0.042
9	G7SQ1a PY2-b	9.11	1.220	0.134
10	G7SQ4a PY1-b	5.60	0.509	0.091
11	G7SQ1a PO3-b	7.51	0.311	0.041
12	G2F3a PY2-b	7.41	1.063	0.144
13	G3F5a PY4- b	2.26	1.328	0.587*
14	G2F6a PY1-b	6.16	0.472	0.077
15	G7SQ3a PY1-b	6.73	0.976	0.145
16	G2F4a PY2-b	8.62	1.030	0.120
17	G9SQ2a PY4-b	4.09	0.248	0.061
18	G2F4a PY1-b	6.77	0.660	0.098
19	G3F5a PY1- b	3.71	0.624	0.168*
20	G3F5a PY3-b	3.37	0.404	0.120
21	G9SQ2a PY3-b	7.97	0.540	0.068
22	G3F5a PP1- b	3.97	0.322	0.081
23	G8SQ1a PO1-b	5.16	0.887	0.172*
24	G2F2b PY3-b	3.33	0.586	0.176*
25	G2F2b PY4-b	7.52	0.331	0.044
26	G2F2b PO2-b	2.46	0.678	0.276*
27	G2F4b PY1-b	5.95	0.582	0.098
28	G7SQ3a PY2-b	2.10	0.815	0.388*
29	G10SQ1a PY3-b	4.18	0.317	0.076

Isolated G3F5a PY4- b, G7SQ3a PY2-b, G2F2b PO2-b, G9SQ2a PYO1-b, G8SQ1a PO1-b, G3F5a PY1- b and G12M2a PY1-b were determined according to cell morphology and biochemical characteristic. The characters of bacterial isolation are classified following Bergey's Manual of Systematic Bacteriology [11, 12] were similar as Genus *Bacillus* sp., *Corynebacterium* sp., *Kocuria roseus*, *Staphylococcus* sp., *Staphylococcus* sp., *Corynebacterium* sp., *Staphylococcus* sp., *Brevibacterium* sp. respectively). The results were consistent with Nimrat et al. (2013) about bacteria that contaminated in dry seafood such *Staphylococcus, Bacillus* and *Micrococcus.* Genus *Bacillus* sp., *Corynebacterium* sp., *Kocuria roseusv* (*Micrococcus roseusv*), *Staphylococcus* sp. and *Brevibacterium* sp can be produce carotenoid pigment [13, 14, 15]. Carotenoids are one of the most important groups of natural pigments. These are lipid-soluble, yellow–orange–red pigments found in microorganism (Table 2) [16].

Table 2 The ability of bacteria microorganisms to produce pigment.

Isolates	Degree of pigmentation	Crude pigments	Color	Microorganism*
G3F5a PY4-b	0.587		yellow	*Bacillus* sp.
G7SQ3a PY2-b	0.388		greenish yellow	*Corynebacterium* sp.
G2F2b PO2-b	0.276		orange	*Kocuria roseus*
G9SQ2a PYO1-b	0.177		light orange	*Staphylococcus* sp.
G2F2b PY3-b	0.176		yellow	*Staphylococcus* sp.
G8SQ1a PO1-b	0.172		orange	*Corynebacterium* sp.
G3F5a PY1- b	0.168		greenish yellow	*Staphylococcus* sp.
G12M2a PY1-b	0.160		light orange	*Brevibacterium* sp.

* The strains were determined according to cell morphology and biochemical characteristic.

3.3 Antibacterial activity of crude pigments extracted

The crude pigments extracted obtained from bacteria Genus *Bacillus* sp. (G3F5a PY4-b), *Corynebacterium* sp. (G7SQ3a PY2-b), *Kocuria roseus* (G2F2b PO2-b), *Staphylococcus* sp. (G9SQ2a PYO1-b)., *Staphylococcus* sp. (G2F2b PY3-b), *Corynebacterium* sp. (G8SQ1a PO1-b), *Staphylococcus* sp. (G3F5a PY1- b), *Brevibacterium* sp. (G12M2a PY1-b) were dissolved in DMSO and tested antibacterial activity by agar well diffusion technique and using DMSO as a control (Table 3).

Table 3 Antibacterial activity of crude pigment extracted from bacteria by using agar well diffusion method

Crude pigments extracted from isolate	Antibacterial activity (mm.)								
	S. aureus ATCC43300 (MRSA)	*S. aureus* ATCC25923 (MSSA)	*S. xylosus*	*B. cereus*	*B. macerans*	*E. coli*	*Klebsiella pneumoniae*	*Citrobacter diversus*	*Aeromonas schubertii*
G3F5a PY4- b	-	-	6.67	-	ac	-	-	6.37	-
G7SQ3a PY2-b	-	-	-	-	8.57	-	-	7.83	-
G2F2b PO2-b	-	-	-	-	ac	-	-	-	6.87
G9SQ2a PYO1-b	-	-	8.75	-	-	-	-	6.53	-
G2F2b PY3-b	-	-	-	-	-	-	-	-	-
G8SQ1a PO1-b	-	-	-	-	-	-	-	6.80	-
G3F5a PY1- b	-	-	ac	-	-	-	-	6.93	-
G12M2a PY1-b	-	7.00	-	-	-	-	-	ac	-
DMSO(control)	-	-	-	-	-	-	-	-	-

Crude pigment from 7 strains showed antibacterial activity on *S. aureus* ATCC25923(MSSA), *S. xylosus*, *B. macerans*, *Citrobacter diversus*, *Aeromonas schubertii* but no inhibitory effected on *S. aureus* ATCC43300 (MRSA), *B. cereus*, *E. coli* and *Klebsiella pneumoniae.* The results showed that crude pigment extracted from bacteria in form of carotene have the ability to inhibit microbial pathogen [17, 18].

IV. Conclusion

The results showed that 29 isolated bacteria can be produced carotenoids pigments as yellow and orange colors and crude pigment from 8 isolated, which have high degree of pigmentation, were studied the capability of pigments to inhibit pathogenic bacteria by agarwell diffusion method. Carotenoids pigments from 7 isolated also have antibacterial activity against *Staphylococcus aureus* ATCC 25923 (MSSA), *S. xylosus*, *Bacillus macerans*, *Citrobacter divesus* and *Aeromonas schubertii*. *Taxonomic characterization* was carried out

and 7 isolates were identified as *Bacillus* sp., *Corynebacterium* sp., *Kocuria roseus*, *Staphylococcus* sp. and *Brevibacterium* sp.

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