Isolation of red pigment-producing bacteria and product development from crude pigment

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Abstract

Six red pigment–producing bacterial isolates were isolated from soil samples collected from some areas of Chiang Mai University. To determine the optimal conditions for red pigment production, the isolates were cultured at different growth conditions and found that the highest pigment yield was obtained when grown at 30°C on nutrient agar (NA) at pH 6-7. The pigment was extracted using various ethanol concentrations with 70% ethanol provided the highest extraction efficiency and pigment solubility. The ethanolic extracted pigment solution was evaporated to obtain a dry crude pigment powder. The crude pigment powder was determined its stability using a heating and cooling method and found that the pigment had no moisture accumulation and no color changes for six cycles. The extracted pigment powder could not inhibit growth of Escherichia coli and Staphylococcus aureus. Furthermore, the crude pigment extract obtained could be developed into watercolor product with vivid pink and brownish-orange shades when adjusted to alkaline conditions using sodium hydroxide, demonstrating potential for further development to variety of color, enhance product value, and support creative applications in artistic fields in the future.

Introduction

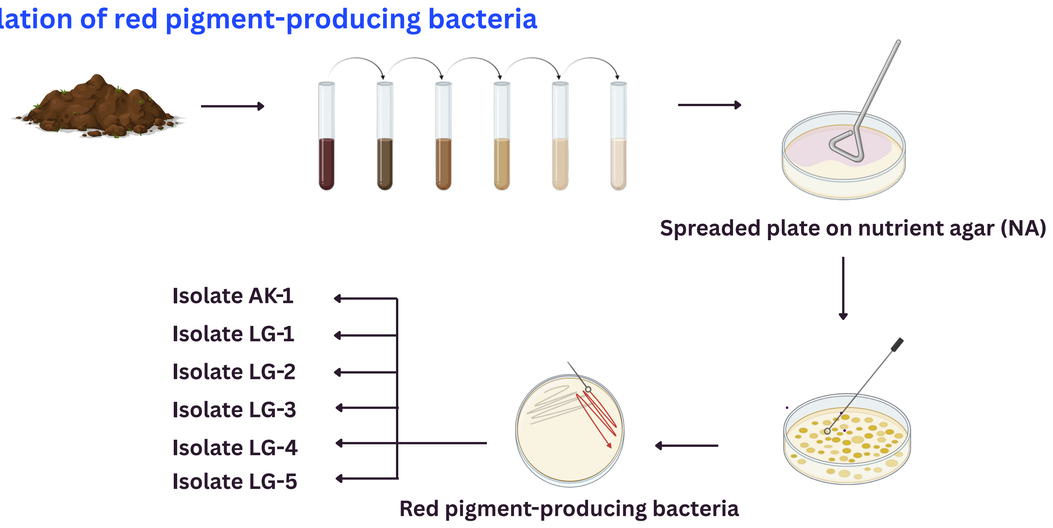
Currently, synthetic dyes produced by chemical synthesis are widely used in industry. These dyes contain heavy metals such as lead, mercury, arsenic, zinc, and chromium, which are health-risky to consumers. Therefore, producers are increasingly shifting towards natural dyes. This has led to the idea of developing a natural watercolor product from a red powder extracted from bacteria. This would enhance the creation of artwork and reduce the use of potentially harmful synthetic dyes.

Objectives

1. To isolate red pigment-producing bacteria from natural sources
2. To determine optimal conditions for pigment production
3. To extract and develop pigments into dry powder form for artistic application

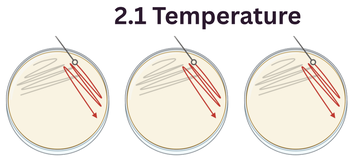
Methodology

1 Isolation of red pigment-producing bacteria

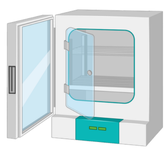


2 Optimization of growth conditions

2.1 Temperature



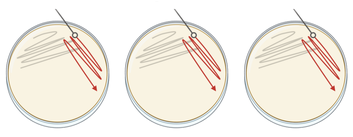
Serratia marcescens S1, Isolate AK-1 and LG-1 restreaked on NA



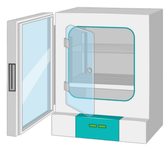
Incubated at 4, 25, 30, 37, 40 and 45°C for 168 hours

Restreaked on NA pH 7 incubated at 30°C and observed change of colony for 7 days

2.2 pH

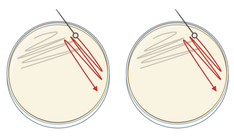


Serratia marcescens S1, Isolate AK-1 and LG-1 restreaked on NA pH 5-10



Incubated at 30°C for 168 hours

3 Pigment extraction



Serratia marcescens S1, Isolate AK-1



Using ethanol at various concentrations

Evaporated at 40°C



Dry crude pigment

- Solubilization
- Shelf life evaluation
- Antibacterial activity
- Watercolor product

Results

1 Isolation of red pigment-producing bacteria

Six red pigment–producing bacterial isolates were isolated from soil samples collected from some high humidity areas in Chiang Mai University.

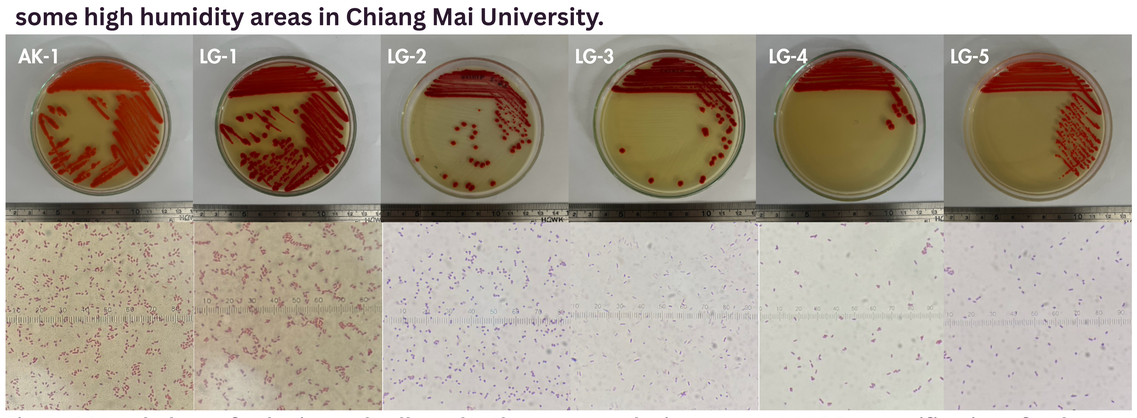


Figure 1 Morphology of colonies and cells under the compound microscope at 1000x magnification of red pigment-producing bacteria

Table 1 Soils collecting sites. The data presented number of soils, locations and number of isolate obtained.

Date obtained	No. of soil	Code	Location	Collection area			No. of isolate
				Subdistrict	District	Province	
25 Jun 68	1	SC	18°48′03.78"N, 98°57′20.54"E	Suthep	Mueang Chiang Mai	Chiang Mai	0
	2	EN	18°47′46.68"N, 98°57′04.57"E	Suthep	Mueang Chiang Mai	Chiang Mai	0
	3	MC	18°48′03.13"N, 98°56′52.21"E	Suthep	Mueang Chiang Mai	Chiang Mai	0
	4	AG	18°47′36.02"N, 98°57′33.32"E	Suthep	Mueang Chiang Mai	Chiang Mai	0
1 Jul 68	5	BIO	18°48′10.47"N, 98°57′09.77"E	Suthep	Mueang Chiang Mai	Chiang Mai	0
	6	DM	18°47′54.03"N, 98°57′15.11"E	Suthep	Mueang Chiang Mai	Chiang Mai	0
4 Jul 68	7	MH1	18°45′42.19"N, 98°55′54.11"E	Mae Hia	Mueang Chiang Mai	Chiang Mai	0
	8	MH2	18°45′57.34"N, 98°55′39.24"E	Mae Hia	Mueang Chiang Mai	Chiang Mai	0
	9	MH3	18°45′57.08"N, 98°55′39.57"E	Mae Hia	Mueang Chiang Mai	Chiang Mai	0
	10	MH4	18°45′45.82"N, 98°55′49.71"E	Mae Hia	Mueang Chiang Mai	Chiang Mai	0
	11	MH5	18°45′31.85"N, 98°55′46.01"E	Mae Hia	Mueang Chiang Mai	Chiang Mai	0

Table 2 Soils collecting sites that red pigment-producing bacteria were isolated.

Date obtained	No. of soil	Code	Location	Collection area			No. of isolate	Code of isolate
				Subdistrict	District	Province		
5 Jul 68	1	AK	18°48'28.64"N, 98°57'00.91"E	Suthep	Mueang Chiang Mai	Chiang Mai	1	AK-1
	1	LG	18°48'29.36"N, 98°57'01.41"E	Suthep	Mueang Chiang Mai	Chiang Mai	5	LG-1, LG-2, LG-3, LG-4, LG-5

2 Optimization of growth conditions

Pigment formation in bacteria is highly influenced by external factors such as pH, temperature, and available carbon and nitrogen sources. The optimal temperature and pH for pigment production was determined to be 30°C and pH 7.

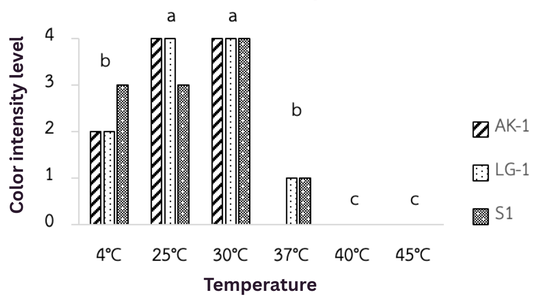


Figure 2 The bacteria produced red pigment after 168 hours at various temperatures

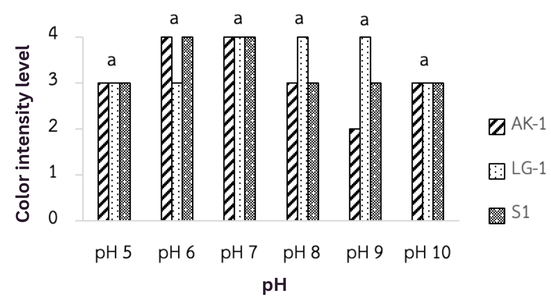
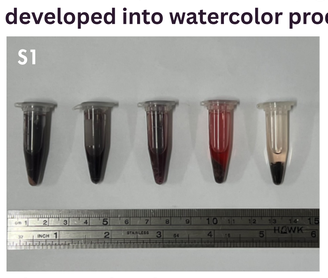


Figure 3 The bacteria produced red pigment after 168 hours at pH 5-10

3 Pigment extraction

70% ethanol provided the highest extraction efficiency and pigment solubility. The crude pigment powder was determined its stability using a heating and cooling method and found that the pigment had no moisture accumulation and no color changes. Furthermore, the crude pigment extract obtained could be developed into watercolor product with vivid pink.



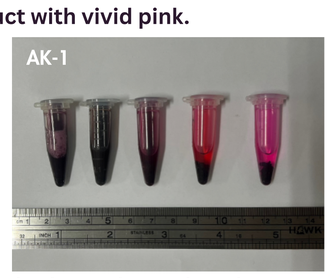


Figure 4 The pigment was extracted using various ethanol concentrations





Figure 5 Watercolor product

Conclusion

Six red pigment–producing bacterial isolates were isolated from soil samples collected from some areas of Chiang Mai University. The highest pigment yield was obtained when grown at 30°C on nutrient agar (NA) at pH 6-7. 70% ethanol provided the highest extraction efficiency and pigment solubility. Furthermore, the crude pigment extract obtained shows potential for development into a watercolor product.

Acknowledgements

This project has got an achievement because of support and advice from Asst. Prof. Dr. Narumol Thongwai, Dr. Patthanasak Rungsirivanich and all my family members. Furthermore, I would like to express my sincere gratitude to Chiang Mai University for the support during the experiment conducted at the university.

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