Molecular and Biochemical Characterization of Pink-Pigmented Thermophile Bacteria (GDG IX) from GedongSongo Hot-Spring in Bandungan–Semarang

by Endang Kusdiyantini

Submission date: 09-Apr-2019 07:39PM (UTC+0700)
Submission ID: 1108914304
File name: Pink_bacteria_ASL.pdf (1,015.07K)
Word count: 1506
Character count: 8543

RESEARCH ARTICLE

Advanced Science Letters
Vol. 23, 6421–6423, 2017

Molecular and Biochemical Characterization of Pink-Pigmented Thermophile Bacteria (GDG IX) from GedongSongo Hot-Spring in Bandungan–Semarang

Endang Kusdiyantini[1,*], Ahmad Edi Darmawan[1], DyahWulandari[2], Alfana Bagus Kusuma[2], and Anto Budiharjo[1,2]

[1]*Department of Biology, Faculty of Science and Mathematics, Diponegoro University, Jl. Prof. Soedarto, SH, No.1 Tembalang, Semarang, 50275, Indonesia*
[2]*Bacteriology Laboratory-Central Laboratory of Research and Science Diponegoro University, Jl. Prof. Soedarto, SH, No.1 Tembalang, Semarang, 50275, Indonesia*

Microorganisms become an important source of natural pigments, especially isolates from extreme environments. Pink-pigmented thermophile bacteria has been isolated from GedongSongo hot spring in Bandungan–Semarang. Morphological, biochemical and molecular analysis were used to identify the isolate. The isolate had coccus shape, GRAM positive, moderate size, irregular shape, raised elevation, and entire margin. Biochemical tests showed that the isolate had indole positive, catalase positive, methyl red negative, urease positive and voges-proskove test negative. Molecular identification based on 16S rRNA gene showed that the pink-pigmented isolate GDG IX had 96% homology with *Rhodococcus* sp. Chr-9.

Keywords: Pink-Pigmented Thermophile Bacteria, 16S rRNA, *Rhodococcus* sp.

1. INTRODUCTION

Natural pigments are secondary metabolites produced by plants, animals and microorganisms. Amongst many natural pigment sources, microorganism is the most potential source to be explored and developed. Microorganisms produce pigments from their metabolic activities. Pigment-producing microorganisms are interesting to be scale-up in industrial scale due to several reasons for instance short growth, easy to be engineered to increase the production and have long been used to produce other secondary metabolites such as enzymes, antibiotics, vitamins, amino acid and etc.

Bacteria have a big potential to produce different kind of pigments e.g., *Vogesella* sp. producing blue pigment,[3] Cyanobacteria producing phycobilin pigment and *Serratiamarcescens* producing prodigiosin pigment.[11] In addition to bacteria, several microalgae (*Haematococcuspluvialis*) and yeast (*Phaffiarhodozyma*) produced astaxanthin pigment.[4]

The use of carotenoid natural pigments as a dye has increased its application due to its safety nature. Many of natural pigments have commercially potency as antioxidant. Exploration of pigment-producing microorganisms, including bacteria has been done continually to find out potential isolates for industrial application.[1,7,10] Extreme environment, such as hot spring is one of the places that are worth exploring for pigmented bacteria. Bandungan is one of the districts in Semarang which has unique environment and used as tourist destination. It is located at 1200 m above the sea and has sulfuric hot spring. Thermophilic bacteria (GDG IX) isolated from sediment of GedongSongo hot spring produced a pink pigment. Molecular, biochemical and microbiological identifications are needed to determine its characteristic. Based on 16S rRNA molecular identification, phylogenetic tree will be analyzed.

2. EXPERIMENTAL DETAILS

2.1. Bacteria Isolate

The pink-pigmented bacteria was cultured on Nutrient Agar (NA) at pH 6 and incubated at room temperature. This culture was conserved at temperature 4 °C.

2.2. Molecular Identification

2.2.1. DNA Extraction

DNA extraction was done by Chelex method (Walsh et al., 2013). Three loops overnight bacteria culture were placed in a tube containing 100 μL ddH_2O. 1 mL 0,5% saponin was added. Then, it was incubated overnight at 4 °C before being centrifuged for

*Author to whom correspondence should be addressed.

Adv. Sci. Lett. 23, 6421–6423, 2017

Table I. Macroscopic, microscopic and biochemical characteristics of pink-pigmented thermophile bacteria.

	Morphological		
Test	Colony	Cells	Biochemical
Color	Pink	–	
Size	Moderate	Coccus	
Shape	Irregular	–	
Elevation	Raised	–	
Margin	Entire	–	
GRAM	–	Positive	
Indole test			Positive
Catalase test			Positive
Methyl red test			Negative
Urease test			Positive
Voges-proskove test			Negative

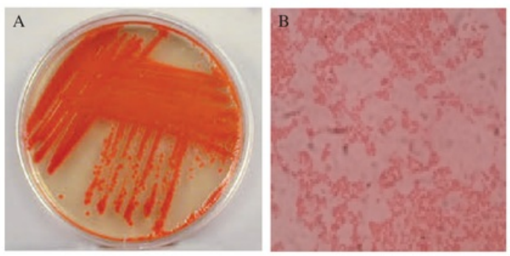

Fig. 1. Colony (A) and gram stain of the pink-pigmented bacteria cells (B).

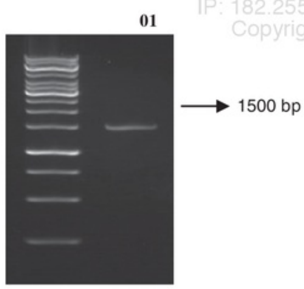

Fig. 2. Visualization of amplification of 16S rRNA gene of pink-pigmented thermophile bacteria in agarose gel concentration 1% (M = marker; 01 = GDG IX).

10 minutes at 12.000 rpm. The supernatant was removed, and 100 μL ddH_2O and 50 μL chelex 20% was added to the pellet. The mixture was then boiled for 10 min with vortexing every 5 min, and centrifuged. The DNA would be in the supernatant and kept at 4 °C.

2.2.2. *DNA Amplification*

DNA amplification was performed using the following program: pre denaturation at 95 °C for 3 minutes, denaturation at 94 °C for 1 minute, annealing at 55 °C for 1 minute, extention at 72 °C for 1 minute and a final extention at 72 °C for 7 minutes. Primers used were 27F (5′-AGAGTTTGATC**M**TGGCTCAG-3′) and 1492R (5′-GG**Y**TACCTTGTTACGACTT-3′).[8] The PCR mixture contained 3 μL DNA template, 1.5 μL of each primer, 25 μL KAPA 2GFAST Kits and 19 μL ddH_2O. Visualization of the PCR product was done using gel electrophoresis with 1% agarose concentration, run at 100 V for 30 minutes. The band of PCR product was seen on gel documentation.

2.2.3. *Sequences Analyze*

Sequencing of PCR product was done at PT. Genetika Science, Indonesia. The sequence was analyzed by base alignment using Basic Local Alignment Search Tool (BLAST) to establish the percentage of base pair similarity with reference isolates from the GenBank.

2.2.4. *Phylogenetic Tree*

Phylogenetic tree was made using MEGA 5 software. Sequence of isolate was analyzed and compared with sequence of bacteria found in gene bank. Phylogenetic tree was constructed by test of Neighbour-joining tree and test with Bootstrap method.

2.2.5. *Morphological and Biochemical Identifications*

The isolate was cultured on Nutrient Agar media, incubated at room temperature, for 24 hours. The isolate was identified for its colony characters, cell shape, and Gram staining. Biochemical tests included indole, methyl red, catalase and urease test.

3. RESULTS AND DISCUSSION

3.1. Characterization of Pink-Pigmented Thermophile Bacteria

Identification of pink-pigmented bacteria based on macroscopic and microscopic indicated that the bacteria had coccus shape,

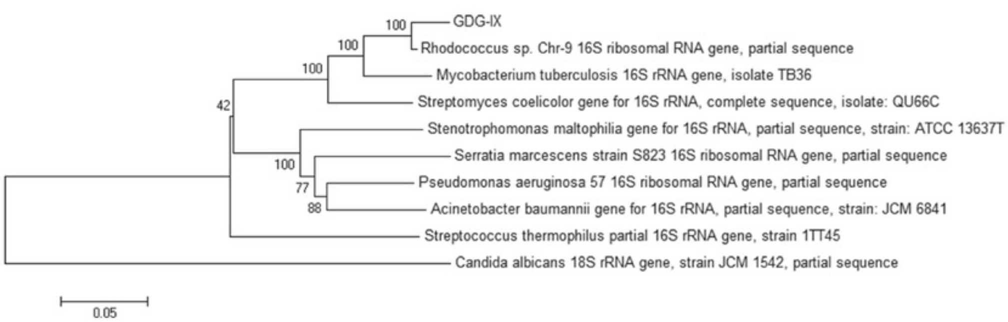

Fig. 3. Phylogenetic tree of isolate GDG IX.

positive Gram stain, irregular shape, raised elevation, and entire margin. Biochemical tests showed that the isolate has positive indole, positive catalase, negative methyl red, positive urease and negative voges-proskove test (Table I). Figure 1 shows the colony and Gram stain of the pink-pigmented thermophile bacteria.

3.2. Molecular Identification

DNA extraction of pink-pigmented thermophile bacteria using Chelex method resulted DNA concentration of 87,5 ng/uL and purity of 1,89. This result indicated that the chelex method was suitable for extraction of DNA from bacteria. The 16S rRNA gene was used for molecular identification as this gene present and conserved in all bacteria. In addition, the gene is long enough (1.500 bp) to be used as barcode gene.[6] Osborne et al.[8] stated that primer 27F and 1492R which were used for amplifying the gene of 16S rRNA able to amplify almost all of bacteria. Figure 2 is visualization of the amplification of the 16S rRNA gene of pink-pigmented thermophile bacteria at agarose gel concentration 1% with low mass ladder marker.

DNA Sequence of the pink-pigmented thermophile bacteria was analyzed using Basic Local Alignment Search Tool (BLAST). The result showed that the bacteria had 96% homology with *Rhodococcus* sp. Chr-9. Phylogenetic tree of the pink-pigmented thermophile bacteria GDG IX showing appropriate affiliation is shown in Figure 3. The phylogenetic tree was constructed by comparing the sequences of GDG IX isolate with sequence of other pigmented-producing bacteria such as *Serratiamarcescens*.

References and Notes

1. I. Arulselvi, S. Umamaheswari, S. G. Ranandkumar, C. Karthik, and C. Jayakrishna, *J. Food Process Technol.* 5, 292 **(2014)**.
2. M. Arya, K. Gopal, K. G. Joshi, G. K. Atul, A. Kumar, and A. Raturi, *Ann. Microbiol.* 65, 1457 **(2015)**.
3. V. Cardona-Cardona, D. Arroyo, J. Scellekens, and C. Rios-Velazquez, Characterization of blue pigmented bacteria isolated from Puerto Rico in current research, Technology and Education Topics in Applied Microbiology and Microbial Biotechnology, edited by A. Mendez-Vilas **(2010)**.
4. A. Gramza-Michałowska and B. Stachowiak, The Antioxidant Potential of Carotenoid **(2010)**.
5. Extract from phaffiarhodozyma, *Acta Sci. Pol., Technol. Aliment.* 9, 171 **(2010)**.
6. J. M. Janda and S. L. Abbott, *J. Clin. Microbiol.* 45, 2761 **(2007)**.
7. R. Muthezhilan, R. Raguh, A. C. Pushpam, R. L. Narayanan, and A. J. Hussain, *Biosciences Biotechnology Research Asia* 11, 271 **(2014)**.
8. C. A. Osborne, M. Galic, P. Sangwan, and P. H. Jansen, *FEMS Microbiol. Lett.* 248, 183 **(2005)**.
9. N. Sundaramoorthy, P. Yogesh, and R. Dhandapani, *Indian Journal of Science and Technology* 2 **(2009)**.
10. J. Tkačova, K. Furdikova, T. Klempova, K. Ďurčanska, and M. Čertik, *ActaChimicaSlovaca* 8, 34 **(2015)**.
11. J. U. Vora, N. K. Jain, and H. A. Modi, *Int. J. Pure App. Biosci.* 2, 160 **(2014)**.

Received: 5 September 2016. Accepted: 13 December 2016.

Molecular and Biochemical Characterization of Pink-Pigmented Thermophile Bacteria (GDG IX) from GedongSongo Hot-Spring in Bandungan–Semarang

ORIGINALITY REPORT

5%	4%	3%	2%
SIMILARITY INDEX	INTERNET SOURCES	PUBLICATIONS	STUDENT PAPERS

PRIMARY SOURCES

1	jjmicrobiol.com Internet Source	2%
2	academic.oup.com Internet Source	1%
3	Submitted to Higher Education Commission Pakistan Student Paper	1%
4	www.sussex.ac.uk Internet Source	1%
5	www.omicsonline.org Internet Source	1%
6	Gabriele Berg, Annette Krechel, Michaela Ditz, Richard A. Sikora, Andreas Ulrich, Johannes Hallmann. "Endophytic and ectophytic potato-associated bacterial communities differ in structure and antagonistic function against plant pathogenic fungi", FEMS Microbiology	1%

Ecology, 2005

Publication

Exclude quotes	Off	Exclude matches	Off
Exclude bibliography	Off		

Molecular and Biochemical Characterization of Pink-Pigmented Thermophile Bacteria (GDG IX) from GedongSongo Hot-Spring in Bandungan–Semarang

GRADEMARK REPORT

FINAL GRADE

/0

GENERAL COMMENTS

Instructor

PAGE 1

PAGE 2

PAGE 3