IOSR Journal of Environmental Science, Toxicology and Food Technology (IOSR-JESTFT)
e-ISSN: 2319-2402,p- ISSN: 2319-2399.Volume 9, Issue 1 Ver. I (Jan. 2015), PP 01-04
www.iosrjournals.org

Inductive Toxic-Mopping (IT-m) and Carotenogenic Bioconversion Properties of Thermotolerant *Rhodocista pekingensis* Sp. Nov

Akinnuoye Olawale Faith-Anthony[1,2,*], Nazlina Ibrahim[3], Akinnuoye Modupe Agnes[4,5], Ainon Hamzah[3]

[1]*Academic Research and Administration, MFM Group Sdn Bhd, Kuala Lumpur, Malaysia*
[2]*Formerly in School of Biosciences and Biotechnology, Faculty of Science, UKM 43600 Bangi, Malaysia*
[3]*School of Biosciences and Biotechnology, FST, National University of Malaysia, 43600 Bangi, Selangor, Malaysia*
[4]*Faculty of Social Sciences and Humanity, National University of Malaysia, 43600 Bangi, Selangor, Malaysia*
[5]*Faculty of Education, University of Waikato, New Zealand*
**faithanthony.ukm@gmail.com*

Abstract: *Environmental impurities and degrading substances are major causes of pollution. On-going efforts to cut the world's carbon emission have been an uphill battle with the unknown. Because of the importance of some photosynthetic bacteria, viable, efficient and environment-friendly biological remediation researches have therefore become inevitably important. In search of these criteria, there was the need for the logical merger of efficacy of the mop-up capability of the biological agents and productive beneficial end-results. We studied the previously identified thermophilic species of photosynthetic bacterium, Rhodocista pekingensis isolated from a local hot spring in Malaysia. Inductive Toxic-mopping (IT-m) technique was used to assess the production of bacteriocarotenoids by metabolic bioconversion process of eight toxic chemical compounds by Rhodocista pekingensis. Results showed a positive response to four of the toxic chemical compounds with the production of 65±5 mg/L, 63±3 mg/L, 59±3 mg/L and 53±6 mg/L yields of raw bacteriocarotenoid in DMSO, Methanol, Isopropyl alcohol and Dubai crude respectively when compared with 65±3 mg/l of raw bacteriocarotenoid produced in the Control experiment. Four of the toxic compounds were efficient to trigger carotenogenic processes needed in the production of beneficial bacteriocarotenoids, two were mild and two others were inadequate to trigger a substantial process. The improvement in carotenoid production with the assimilated toxic chemicals corroborated the efficiency of the Inductive Toxic-mopping Bioremediation pattern and beneficial convertibility of chemical wastes by Rhodocista pekingensis.*

Key words: *Bacteriocarotenoid, Toxic-mopping, Bioremediation, Carbon emission, Rhodocista*

I. Introduction

With the increase in greenhouse emission and high amount of toxic chemical wastes, the world is becoming a more dangerous place to live in. Potential man-made disasters may be looming over the continent as more of the dangerous chemicals and their derivatives are dumped in wrong places with impunity. The oxidation-reduction or "Redox" of pollutant chemicals with their component elements therefore, have become a focal point for Clinical Researchers, Biologists and Environmental Scientists in which phototrophic bacteria play important roles. Therefore, the bio-cycling of dangerous compounds and bioconversion of other organic matters by this group of bacteria in a manner that favors the eco-systems and the coexistence of all biological beings that share the planet Earth become more logical. Biologically, bacteria are very important in environmental bioremediation and rehabilitation especially in cheap treatment of waste water and other waste treatment systems [1][2]. The non-sulfur phototrophic bacterium *Rhodocista pekingensis* and many bacteria species in this group are facultative and grow either as anaerobic phototrophic under appropriate lighting condition or aerobic chemotrophic in the dark [3][4][5]. The anaerobic productions of bacteriocarotenoids through microbial oxidation-reduction processes have placed some bacterial species in unique positions in medical and health research. Results of direct enzymatic activities of *Rhodocista pekingensis* were confirmation of the ability to transform toxic chemicals in beneficial ways either anaerobically or otherwise under appropriate growth environment and conditions.

II. Materials And Methods

2.1 Bacterial culture and maintenance

This thermophilic strain of *Rhodocista pekingensis* (UKMP-WA) was cultured in malate yeast extract (MYE) broth as previously described [1][6][4]. The bacterial cells were observed by light and phase-contrast microscopes

(BX60 Olympus microscope), scanning electron microscopy (SEM) and ultrathin observations of bacterial cells were carried out as described and previously reported [4][1][7].

2.2 Toxic-mopping and assimilation trial

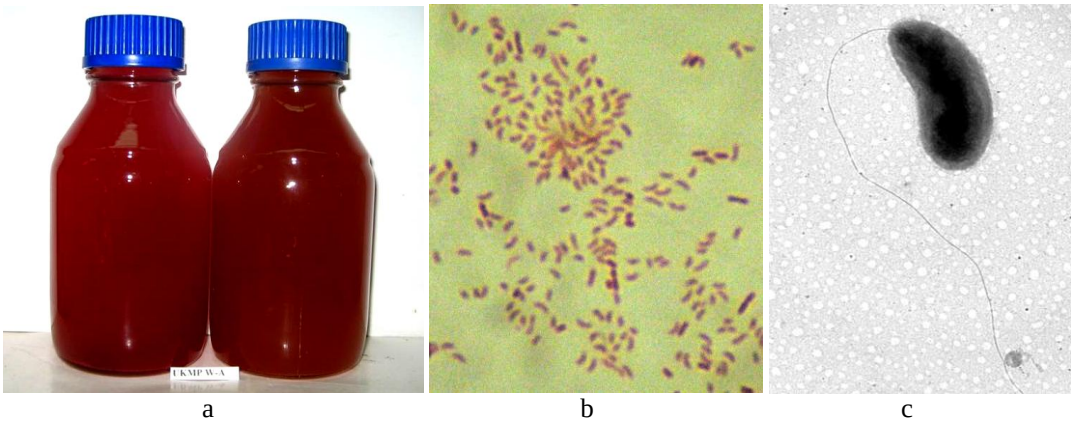
Toxic-mopping was carried out as outline by Akinnuoye et al [4] and minor modifications briefly described below: Fifteen milliliter volume of MYE broth was dispensed into 50mL-sized bottles with screw caps. The toxic chemicals were added to the medium after sterilization in ratio of 1% (v/v) of test bacterium. Five milliliters of culture was inoculated into the broth medium and incubated as previously cited (2.1). The control was grown in enhanced MYE broth containing ammonium chloride (NH_4Cl) 0.5 g; magnesium sulfate heptahydrate ($MgSO_4.7\ H_2O$) 0.4 g; calcium chloride di-hydrate ($CaCl._2.H_2O$) 0.05 g, sodium chloride (NaCl) 0.4 g, malic acid or sodium hydrogen malate 1.5 g, yeast extract 1.0 g in 1000mL of distilled H_2O.

2.3 Biomass, bacteriocarotenoid extraction and measurement

The determination of whole cell optical density ($OD_{0.5}$), inclusive cellular bacteriochlorophyll (Bchl) type together with the extraction of pigments and detection of absorption maxima (λ-max) of raw bacteriocarotenoid of bacterial cells were done as described [8] and slightly modified [4]. Peak-specific detection of bacteriochlorophylls in living cells and carotenoid extraction was done by spectrophotometric scanning of 1 mL of 14-day old broth culture at 600 nm for bacteriochlorophyll and at 480 nm wavelengths for carotenoid extract using Cary 50® spectrophotometer (Cary Australia). Treatment chemical compound were as follows: 1. Cumene, 2. Dimethyl sulfoxide (DMSO), 3. Ethylbenzene, 4. Iso-Amyl alcohol, 5. Methanol, 6. Amine, 7. Isopropyl alcohol (2-propanol), 8. Crude oil (Dubai) and 9. Control.

III. Results And Discussion

Pigmentation in *Rhodocista pekingensis* was deep pinkish to reddish color in MYE broth. Gram's test was negative and cells were motile when observed by hanging-drop method under a light microscope as previously reported [1]. Cells were motile. The shape was spiral or vibriod with length ranging between 0.6-0.8 µm. Negatively-stained cells were mostly singly flagellated with flagella attached to polar ends of the cells. Ultrathin cell observations revealed intracytoplasmic inclusions (Fig. 1)



a b c

Figure 1: Metabolic assimilation by Rhodocista pekingensis showing (a) reddish pigmented growth (b) Gram negative cells and (c) flagellated negatively-stained cell.

The growth of *Rhodocista pekingensis* as tabulated (Table 1) was equally normal with Dimethyl sulfoxide, Isopropyl alcohol, Methanol and Dubai crude (heavy crude oil). The growth with Amine and Ethyl benzene was mildly supported while Cumime and Isoamyl alcohol were less supportive to the growth of *Rhodocista pekingensis* and cellular bacteriocarotenoid production. The yields of raw bacteriocarotenoid varied slightly from the sequence of pigmentation as shown in Table 1.

Table 1: Bioconversion endpoints of Toxic-mopping inductive patterns showing Bchl, bacteriocarotenoid λ-max and yields of induced and normal (controlled) propagations

	Cum	Dimethyl sulfoxide	Ethyl-benzene	Isoamyl alcohol	Methanol	Amine	Isopropyl Alcohol	Dubai Crude	Control
$OD_{0.5}$ Cells	0.42	1.93	0.59	0.49	1.66	0.76	1.59	1.25	1.63
Bulk Caro	0.62	2.19	0.86	0.74	1.99	1.10	1.98	1.79	1.95
Bcaro.Yields mg/L	25±2	65±5	34±2	32±7	63±3	42±1	59±3	53±6	65±3

Results of Bchl with λ-max and yields of bacteriocarotenoid of normal and induced propagations did show the $OD_{0.5}$ of live cells at 1.93, 1.66, 1.59 and 1.79 with respective bacteriocarotenoid densities of 2.19, 1.99, 1.98 and 1.79. Subsequent yields were 65±5 mg/L, 63±3 mg/L, 59±3 mg/L and 53±6 mg/L of raw bacteriocarotenoids in DMSO, Methanol, Isopropyl alcohol and Dubai crude respectively (Table 1).

The absorption maxima (λ-Max) from spectral analyses of the bulk bacteriocarotenoids did show multiple point-specific peaks of specific carotenoids. The multiple peak detections were in the regions of 350 and 374; others were detected between 442 and 453, 461 and 478, while some were between 495 and 503 nm wavelengths in the spectral range of 300 to 700 nm while the wavelength range for Bchl was between 400 to 1200nm (Fig. 2).

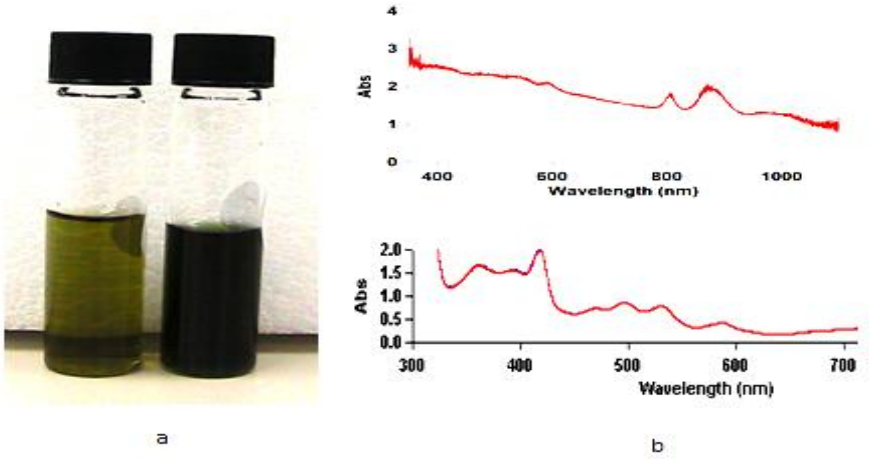


Figure 2: (a) Raw extracts of bacteriocarotenoid from *Rhodocista pekingensis*, (b) Spectrophotometric graphs of bacteriochlorophylls (top) and bacteriocarotenoids (bottom).

The results of chemical convertibility tests showed proportional increase in biomass production and resultant reciprocal high carotenoid yield (Table 1, Fig. 2). It was apparent from the $OD_{0.5}$ measurements, bacteriochlorophyll analysis and yields of bacteriocarotenoids, that there was a synchronized sequence of the pigment densities with bacteriocarotenoid yields of the bacterium. In other word, *Rhodocista pekingensis* efficaciously metabolized and/or degraded dimethyl sulfoxide, isopropyl alcohol, crude oil, and methanol with increase in biomass and subsequently increase in beneficial carotenoid production by the phototrophic bacterium.

In other word, these toxic chemicals were used as energy sources for growth by the bacterial cells resulting in the production of multiple bacteriocarotenoids, most of which could be of immense medical benefits. These assertions were corroborated by previous findings in which phototrophic bacteria were used in water treatment and in other bioremediation processes [9][10][11].

Moreover, the chemicals acted as enhancers for this organism in the production of bulk of raw carotenoids, the effects of which could be seen. Medical use of *Rhodocista pekingensis* was discovered in the ability of carotenoids of bacteria origins which have been known to be of immense advantage especially in antioxidant and anticarcinoma studies [11][12]. However, there was no significantly detectable change in both the external and the ultrathin structures of the bacterium as all the composite features were intact and appeared normal as in the controls of the trials. The photosynthetic materials and other intracellular materials did not display any physiological distortion. This showed the resilience of the bacterium in the face of damaging effects of toxic chemical compounds. In other word, the possibility of the organism to remain active till most or all recovery is done is certain.

IV. Conclusions

The prospects and the benefits of the bacteriocarotenoid produced by *Rhodocista pekingensis* through metabolic bioconversion are still being explored. However, it could be concluded that the convertibility of toxic chemical compounds and wastes cannot be overemphasized. The use of *Rhodocista pekingensis* in environmentally related cleansing and remediation coupled with the ability of the bacterium to translate the clean-up capability into medically beneficial bye-products such as carotenoids was overwhelmingly plausible.

Finally, the use of this bacterium in the treatment of environmental pollution attributable to lethal chemicals pollutants and their derivatives were evident and could be adopted in future studies on environmental bioremediation and rehabilitation.

Acknowledgements

The Authors express their appreciation to the Mountain of Fire Group, Malaysia and the National University of Malaysia for their support for this research.

References

[1]. O.F.A. Akinnuoye, O. Othman, I. Nazlina and H. Ainon. Ultramicrotomy and structural analyses of two thermophilic non-sulphur photosynthetic bacteria. Malaysian Journal of Microscopy 6, 2010, 23-29.

[2]. D. Zhang, Y. Huifang, W. Zhang H. Zhiyong and S.J. Liu. Rhodocista pekingensis sp. nov., a cyst-forming phototrophic bacterium from a municipal wastewater treatment plant. International Journal of Systematic and Evolutionary Microbiology 53, 2003,1111–1114.

[3]. O.F.A. Akinnuoye, I. Nazlina and H. Ainon. Application of Electron Microscopy and Energy Dispersive X-Ray Spectroscopy in The Characterization of Rhodomicrobium vannielii. Journal of Advanced Microscopy Research, 6, 2011, 1-7.

[4]. H. Ainon, C.J. Tan and S. Vikineswary, Biological characterization of Rhodomicrobium vannielli isolated from a Hot Spring at Gadek, Malacca,Malaysia. Malaysian Journal of Microbiology 2 (1), 2006, 15-21.

[5]. J.F. Imhoff and H.G. Truper. The genus Rhodospirillum and related genera. In The Prokaryotes, 2nd edn, vol. 1, pp. 2141–2155. Edited by A. Balows, H. G. Truper, M. Dworkin, W. Harder and K. H. Schleifer. Heidelberg:Springer. 1992.

[6]. O.F.A. Akinnuoye, O. Othman, I. Nazlina and H. Ainon. Comparison of ultrastructure of four thermophilic photosynthetic bacteria isolated from Malaysian hot springs. 10th Symposium of the Malaysian Society of Applied Biology, 2008.

[7]. K. Takai, T. Komatsu and K. Horikoshi. Hydrogenobacter subterraneus sp. nov., an extremely thermophilic, heterotrophic bacterium unable to grow on hydrogen gas, from deep subsurface geothermal water. International Journal of Systematic and Evolutionary Microbiology, 51, 2001, 1425–1435

[8]. H.J. Nelis and A.P. De Leenheer. Profiling and quantitation of bacterial carotenoids by LC and PAD. Applied and environ. Microbiol 55(12), 1989, 3065-3071.

[9]. K. Sasaki, T. Tanaka and S. Nagai, Use of photosynthetic bacteria for production of SCP and chemicals from organic wastes. In Martin, A. M. (ed.), Bioconversion of waste materials to industrial products, 2nd ed. Blackie Academic and Professionals, New York, p. 247–291, 1998.

[10]. H. Nagadomi, T. Kitamura, M. Watanabe and K. Sasaki, K., Simultaneous removal of chemical oxygen demand (COD), phosphate, nitrate and hydrogen sulphide in the synthetic sewage wastewater using porous ceramic immobilized photosynthetic bacteria. Biotechnol. Lett., 22, 2000, 1369–1374.

[11]. K. Sasaki, M. Watanabe, Y. Suda, A. Ishizuka and N. Noparatnaraporn, Applications of photosynthetic bacteria for medical fields. Journal of Bioscience and Bioengineering 100 (5), 2005, 481-488.

[12]. O.F.A. Akinnuoye, O. Othman, I. Nazlina and H. Ainon, Preliminary Evaluation of the Antioxidative and Anticarcinoma Activities of Bacteriocarotenoids from Bacterium Rhodopseudomonas palustris (UKM 2-5A). UKM/FST Post Gradaute Colloquium 2012(12), 2012, 182-185.