IOSR Journal of Dental and Medical Sciences (IOSR-JDMS)
e-ISSN: 2279-0853, p-ISSN: 2279-0861.Volume 13, Issue 12 Ver. VIII (Dec. 2014), PP 52-58
www.iosrjournals.org

Clinical Potentials of Bacteriocarotenoids: Rhodopin and β-Carotene from Phototrophic *Rhodopseudomonas palustris*

Akinnuoye Olawale Faith-Anthony[1,2,*], Nazlina Ibrahim[3], Ainon Hamzah[3]
[1]*Academic Research and Administration, MFM Group SdnBhd, Kuala Lumpur, Malaysia*
[2]*Formerly in School of Biosciences and Biotechnology, Faculty of Science,43600 Bangi, Selangor DE, Malaysia*
[3]*School of Biosciences and Biotechnology, Faculty of Science, 43600 Bangi, Selangor DE, Malaysia*
**faithanthony.medic@yahoo.com*

Abstract: *Bacteriocarotenoids, a group of carotenes isolated from bacteria, were extracted from Rhodopseudomonaspalustris, isolated from a hot spring in Pedas, Malaysia. The extracts were refined, then anticarcinoma efficacy and antioxidative potentials of two of the inclusive bacteriocarotenoids (β-carotene and Rhodopin) were tested against three cancer cell lines (MCF-7, MDA-MB and Hep-G2) and a Vero cell line. MTT([3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide, FRAP (ferric-reducing antioxidant power) and DPPH (2,2-diphenyl-2-picrylhydrazyl) assays were used along with in-vitro assays for cell proliferationmeasurement and antioxidative potentials. Results showed a reduction in proliferation of cells with low level of toxicity in the carcinoma cell lines. DPPH and FRAP tests did show antioxidant properties. Dose by time dependency analysis indicated a dosage of 25 µg/mL of refined bacteriocarotenoids virtually was as effective in 48 hours as with 12.5 µg/mL of bulk unseparated bacteriocarotenoids in 72 hours of treatment. These observations were noted in all tested carcinoma cell lines. Therefore at the dosage of 25 µg/mL of bulk-bacteriocarotenoids, results also suggested time-dependent inhibitory effects which were consistent with long but safe exposure time. Inhibitory effects were more pronounced at higher dosage, the effects were more pronounced in treatments with both isolate and bulk-bacteriocarotenoids at varied dosage.*

Keywords: *Anticarcinoma, Antioxidant, Bacteriocarotenoid, Phototrophic, β-carotene, Rhodopin*

I. Introduction

Cancer is one of the principal causes of death at any time with the risk for most type of cancer increasing with age. Cancer caused about 13% of all human deaths in 2007with 7.6 million deaths [1][2]. It was reported that breast cancer constituted 10.4% of all cancer incidences among women, making it the second most common type of non-skin cancer after lung cancer and the fifth most common cause of cancer deathworldwide [1]. Breast cancer was reported to have caused 519,000 deaths worldwide with 7% of total cancer deaths, amounting to almost 1% of all deaths. From research findings, breast cancer was about 100 times more common in women than in men, but survival rates were reported as equal in both sexes [3][4][5][6].As at 2012 reported new cases have risen to 37,400 from 32,000 in 2008 with over 21,700 deaths in 2012 as against 20,100 deaths in 2008. In 2012 the estimated 8.2 million deaths due to cancer did show an increase of 11% amounting to 14.1 million of new cases worldwide [7][8][9].

In the United States of America, cancer is the second leading cause of death, exceeded only by cardiovascular disease. About 1,372,910 new cancer cases were diagnosed in 2005. In addition, 570,280 Americans were projected to die of cancer, killing more than 1,500 people a day [10][1]. In the United States, cancer causes 1 of every 4 deaths and bladder cancer was the sixth most common diagnosed cancer.In 1997, around 39,500 men and 15,000 women were projected to be newly diagnosed with bladder cancer[11][1].According to WHO-IARC [9], 34.8 new cases of cervical cancer were reported per 100,000 women annually in sub-Saharan Africa while 22.5 per 100 000 women die from the disease. These figures compare with 6.6 and 2.5 per 100 000 women, respectively in North America.

In the report on Malaysian Cancer Statistical Data and Figure released by National Cancer Registry of Malaysian Ministry of Health[12], malignant neoplasms constituted 10.59% of death in Malaysia placing it third after septicaemia and Heart diseases with diseases of pulmonary circulation. A total of 21,773 cancer cases were diagnosed in 2006 alone comprising of 9,974 males and 11,799 females [12].This same report gave the age-standardised incidence rate (ASR) for year-round cancer cases for that year irrespective of gender,as 131.3 in 100,000. Most common cancer cases recorded in Peninsular Malaysia were breast, colorectal, lung, cervical and nasopharynx of at least 27 target sites identified in the report. In the 2007 staging report, 8,869 from 18,219 new cases of which 17%, 25%, 25% 32.7% were stages 1 to 4 respectively[13]. The need for cost effective readily available solutions to cancer remains as elusive as ever in spite of promising breakthroughs in recent years. For instancein the result of Paclitaxel versus Docetaxel for Early Breast Cancer reported in the New England Journal of Medicine[18], weekly administration of paclitaxel after standard adjuvant chemotherapy with doxorubicin and

cyclophosphamide improved disease-free and overall survival in women with breast cancer. In the five-year studies on the survival of metastatic pancreatic carcinoma by Ben and Chue[19], the median survival for patients with metastatic pancreatic adenocarcinoma was between 3 to 6 months. They reported that a long-term survival of a patient with this devastating malignancy was an illustration of a highly unusual case study of hope, courage and determination. Therefore, the efficacy and yet excellent tolerability of metronomic dosing of this and possibly other chemotherapies for metastatic pancreatic adenocarcinoma suggested a role for this type of treatment for other cancers and in other settings too. However, all these have not resulted in cost-effective cancer treatments. Hence the need for an alternative pathways with bacteriocarotenoid research initiative.

Bacterial pigments were discovered long before the discovery of bacteriochlorophyll (Bchl) in 1963.There have been researches on the intracytoplasmic membrane vesicles which are important contents of photosynthetic bacteria. The membrane vesicles areused by photosynthetic bacteria when necessary compounds are deficient[14][15][16][17], while embedded carotenoids have been discovered to possess some promising medicinal effects[14][21].This paper reports the results of a study on efficacy of bacteriocarotenoids on carcinoma and non-cancerous cell lines.

II. Materials And Methods

2.1 Growth andmaintenance of Rhodopseudomonas palustris

The culture of Rhodopseudomonas palustris was from the Department of Microbiology, School of Biosciences and Biotechnology, National University of Malaysia (UKM)[20][21]. The bacterium was sub-cultured on malate yeast extract (MYE) agar[20]. Establishment and maintenance of pure culture of Rhodopseudomonas palustris was done by colony-pick in MYE broth and incubated at 45^{o}C(±3) in INFORS HT® automated incubator (INFORS AG, Switzerland) with hydrogenated carbon dioxide (Oxoid® H_2-CO_2).

2.2 Standardizationof bacterial culture

Standardized culture was prepared from single colony of MYE agar-grown isolates [21]with slight adjustment as follows:Culture was grown in 1 liter-sized bottle instead of 18 mL of MYE broth and incubation was for 14 days instead of 7 days in preliminary experimentation with conventional non-phototrophic bacteria.Other growth and incubation factors remained same as previously reported [22][21]. Standardization of bacterial cells and optical density measurement ($OD_{0.5}$) at 600 nm wavelengths were done as previously reported using Cary 50 CONC UV-Vis® spectrophotometer (by Varian Australia Pty Ltd, Victoria, Australia). The standardized culture (10% v/v), was used in subsequent tests and procedures.

2.3 Growth and maintenance of cell lines

Frozen cell line was thawed by placing it in a water bath with a temperature of 37^{o}C for about 2 mins. The cell was placed in preconditioned DMEM medium supplemented with 10-20% FBS for about 10 mins at room temperature. Trypan blue cell count was done at a dilution of 1:4. The percentage of viable cell was with the formula:

No of cells x 10000 = cell/mL x volume of mL = number (%) of viable cells.

Cells were centrifuged at 1200 rpm for five to seven mins at room temperature and then suspended to a concentration of 10^5 cells/mL. The cells were then cultured by placing 2.5x10^6per culture flask with the lid slightly closed. The culture was incubated at 37^{o}C with 5% CO_2 checking the medium daily for physiological or character changes in the medium and for cell confluence. Cells were sub-cultured by removing the medium and washing with phosphate buffered saline (PBS) and dislodged by adding 0.025 to 0.25% trypsin and incubated for about 10 mins, checking and tapping intermittently for quick detachment of cells from the surface of the culture flask. After detachment of the cells, trypsin was inactivated by adding serum-supplemented MEM or DMEM medium. The cells were centrifuged as above and viability count was made as described above. Average of 2.5 x 10^6cells were plated in cell flask and incubated as described earlier. The cell lines used were carcinoma non-carcinoma cell lines (MCF-7, MDA-MB, Hep-G2) and a normal Vero cell line, grown in 24 mL-sized flask according to the protocol described by Betancur-Galvisetal[23]. Incubation was at 37^{o}C in 5% CO_2 and cell growth conditions were observed every 36 hours until confluent cell formation was attained. Physiology of the study-cell lines was studied microscopically.

2.4 Assessmentof Bacteriochlorophyll and Bacteriocarotenoid purification

Assessment and determination of the type of bacteriochlorophylls (Bchl) in live bacterial cells was done as described by Lorquine et al [24].Processmodifications have been reported [21].The methods used for carotenoid extraction and for the detection of the absorption maxima (λ-Max) of bulk bacteriocarotenoid wereadopted from previously described method [24]. Bacteriocarotenoid types were determined by thin-layer chromatography (TLC), spectral and high-performance liquid chromatography (HPLC)analyses of the bacterial

extracts. Summarily extracts from the bacterium were processed into dried forms and quantified by pipetting 15 mL of the extracts into bottles, transferred into a desiccator, connected to a GAST® electrical vacuum pump. The solvent was dried under pressurized vacuum for 72 hours in the dark until no liquid was observed. The extract was dried in vertical automated liquid nitrogen pressurized drier and dried for 7 days. The dried samples were stored at -20^{o}C until needed. Silica gel coated aluminium plates (TLC silica gel 60F_{254}®, Merck KG_GA®, Germany) were used. Spotting of TLC plates with the bacteriocarotenoid samples and development of spots were done in the dark.

2.5 Antioxidative and Anticarcinoma efficacy trials

The method of Arai et al [25] was adopted in the preparation of stock solution of extract samples. The method was modified as follows:Fresh 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT)solution was prepared by adding 5 mg/mL of MTT into PBS, then filtered and kept in the dark by wrapping the container with aluminium foil. Acidic isopropanol was an alternative to dimethyl sulfoxide (DMSO) used for solubilizingformazan in MTT assay. It was prepared by adding 0.1N hydrochloric acid (HCl) in absolute isopropanol.Determination of antioxidant activity was done using 2,2-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assaydescribed by Vaya and Aviram[26],ferric-reducing antioxidant power also known as ferric reducing ability of plasma (FRAP) assay as described by Benzie and Strain[27]. Pictorial data were taken with computer-enhanced JVC® camera attached to a Leica® microscope.

III. Results And Discussion

The inoculated bottles were initially incubated at a starting temperature of 37^{o}C. The initiation of growth at 37^{o}C was done to avoid stressful thermal shock in the first six hours of incubation. The bacterialculture was grown and maintained from freezing temperature of 0 to 4^{o}C because it wasstored in refrigerator prior usage while isolation was initiated by gradual increase of the incubating temperature to 45^{o}C while measuring the light intensity based on the distance of incubated culture to light source. Red pigmented growth was observed in 72 hours of incubation in all the broth bottles.

For the avoidance of thermal shock, temperature was gradually increased in an automated incubator, while full, dense pigmented growth was observed within ten days the culture produced the required high optical density, high biomass indicator at each of the selected temperature gradients, Fig 1.

ab

Figure 1: Pictorial representations of incubation and growthshowing (a) Automated incubator with mechanised adjustable functionality and (b) Anaerobically grown pure culture of Rhodopseudomonas palustris in MYE broth

Bchl a was present in the bacterial cells with detected peaks located at wavelengths from 374-376, 590, 800-806 and 831-890 nm The absorption maxima (λ-max) from spectral analyses of the bulk bacteriocarotenoids showed multiple but point-specific peaks of different bacteriocarotenoids. The multiple peak detections were in the regions of 350 and 374; others were detected between 442 and 453, 461 and 478, 495 and 503 nm wavelengths in the spectral range of 300 to 700 nm and 350-1000 nm for bacteriocarotenoids and Bchl respectively, Fig 2.

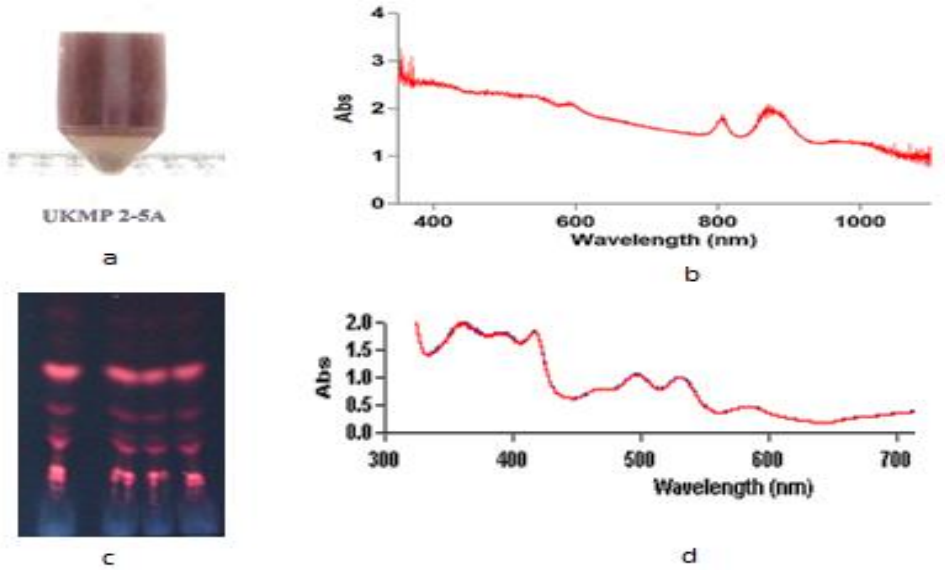

Figure 2: Images of (a) Bacteriocarotenoid extract (b) Spectrograph of bacteriochlorophyll (c) TLC separation (d) spectrograph of bulk-bacteriocarotenoids

Three cell lines out of the four used for anti-proliferation and MTT toxicity tests, were malignant carcinoma cell lines: MBA-MB (malignant carcinoma cell line from a pleural effusion of breast cancer), MCF-7 (malignant carcinoma cell line from malignant adenocarcinoma of the breast in a pleural effusion) and Hep-G2 (malignant cancer cell line from the liver tissue with a well differentiated hepatocellular carcinoma) while the fourth, Vero cell, was a normal epithelial cell line from the kidney of African green monkey.

The bulk-bacteriocarotenoid extracts were tested on the cell lines and results were reported alongside those obtained from tests with isolate-bacteriocarotenoids. Both the bulk-bacteriocarotenoids and consequent isolate-bacteriocarotenoids administered in serialized doses in accordance with the IC_{50} (or LC_{50}) model of the National Cancer Institute. Results presented a consistent uniformity in most cases with bulk-bacteriocarotenoids showing reduction in viability of the cells with increased dose treatment within short exposure time. This observation was recorded in treatment with elongated exposure time and lower doses. Time-dependent effects were observed from the IC_{50} of the extracts needed to reduce viability by 50%.

Table 1: Efficacies of raw and isolate bacteriocarotenoids with $IC_{50}$by exposure time, showing the anti-proliferation propertyof the bacteriocarotenoids

Bacteriocarotenoid Source	IC_{50} (µg/mL)				
	MBA-MB	Hep-G2	MCF-7	Vero	Conc./Time
Bulk-bacteriocarotenoid	1.17	0.70	2.33	2.50	12.5 µg/mL/48h
Rhodopin	3.45	3.77	3.62	3.89	25µg/mL/72h
β-carotene	3.22	1.48	1.72	3.83	25µg/mL/72h

In Table 1 the reactions of the treated cell lines to rhodopin and β-carotene treatments varied in a consistent manner. The IC_{50} values obtained from treatments have shown relatively close and similar response by the four cell lines. The results of rhodopin at the same treatment dosage of 25 and 12.5 µg/mL were similar. The IC_{50} results of 3.45, 3.77 and 3.62 µg/mL respectively for MDA-MB, Hep-G2 and MCF-7 at 72 hours of exposure were obtained at 25 µg/mL. With treatment of cell lines with 25 µg/mL β-carotene, results of IC_{50}obtained were 3.22, 1.48 and 1.77 µg/mL for MDA-MB, Hep-G2 and MCF-7 and Vero at 72 hours.

Extract of β-carotene showed inhibition on Hep-G2 and MCF-7 carcinoma cell lines with low toxicity. There was a weak biphasic response on this cell line in 48 hours of exposure to the bulk-carotenoids and 72 hours of exposure to isolate-bacteriocarotenoids in more than half of the studied cases. Rhodopin showed inhibition with low toxicity level in all the cells. However, with the administration of individual component-carotenoids, effects were more of dose-dependent viability in order to reduce viability of the carcinoma cell lines. This gave same effects as in treatments obtained by elongated exposure time with low doses of bulk-bacteriocarotenoid extracts. It was also observed that the bulk-bacteriocarotenoid extracts showed toxic activity against MDA-MB and MCF-7 cells according to the calculated $IC_{50}$with mild toxic response in Vero cell, Fig 3.

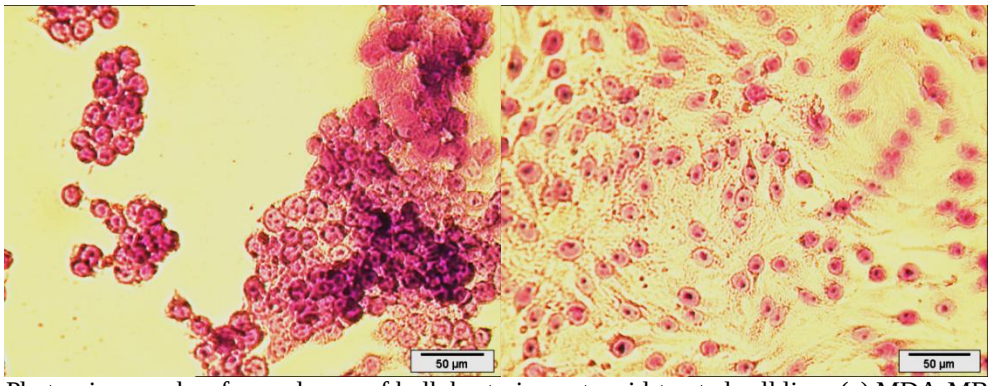

Figure 3: Photomicrographs of monolayers of bulk bacteriocarotenoid-treated cell lines (a) MDA-MB and (b) Vero. Bar 50µm

In summary, generally β-carotene was more anti-proliferative on MCF-7 and Hep-G2 than rhodopin. β-carotene was anti-proliferative on Hep-G2 and MCF-7 but slightly on MDA-MB. Results from both the tested raw bacteriocarotenoid extracts and individual isolate-identified bacteriocarotenoid did show β-carotene was more anti-proliferative on MCF-7 and Hep-G2 than rhodopin with inhibitory characteristics on most of the tested carcinoma cell lines in both dose-dependent and time-dependent patterns and a combination of both. The value of IC_{50} obtained from cells treated with 12.5 µg/mL raw bulk-bacteriocarotenoid ranged from 0.79 to 2.50 µg/mL. High inhibitory effects were observed in Hep-G2 and MDA-MB with 0.70 µg/mL and 1.17 µg/mL respectively within 48 of 72 hours of treatment. The IC_{50} of bulk-bacteriocarotenoid extracts on MCF-7 and Vero cells were 2.33 µg/mL and 2.50 µg/mL respectively with exposure to same dose of bulk-bacteriocarotenoid for the same number of hours. The IC_{50} values reduced with increase in concentration of the bacteriocarotenoids. Results obtained from treatments with bulk-bacteriocarotenoids (raw extracts) were consistently uniform in most cases, showing reduction in viability of the cells with increase of doses of administered extracts or with extended exposure time where dosage was lower. Some of the cells lost their membrane integrity, with disruption of metabolic activities. Results from the bacteriocarotenoid extracts showed the percentages of inhibition as 88.2%, 78.9% and 81.2% in cell treated with 25 µg/mL isolate-bacteriocarotenoids incubated for 72 hours and 89% inhibition by bulk raw-extracts were recorded in about 72 hours of exposure. Treatment with 12.5 µg/mL of bulk-bacteriocarotenoids and 25 µg/mL of isolate-bacteriocarotenoids showed inhibition after 48 hours of exposure to the bacteriocarotenoids with fairly consistent reduction in viability of all the cell lines. Necrosis was observed at maximum dosage of isolate-bacteriocarotenoids (25 µg/mL). This was a better result than earlier reports of Spudich and Luecke[31] and Bedard et al [32] in which a lower carotenoid potency was obtained.

Moreover, the improved results likely due to the adopted optimisation methods which were divergent from some of the conventional methods.Moreover, the proliferative effects of the bacteriocarotenoid extracts were more of dose-dependent than of elongated exposure time except for bulk-bacteriocarotenoid extracts in which effects were pronounced at as low as 12.5µg/mL. This probably was due to multiple or consortium of identifiable bacteriocarotenoids and other "occult" compounds that were unknown and not identifiable. Reports attesting to the findings have been published bySakhietal [28], found a significant positive association between post-radiotherapy plasma carotenoids especially beta-carotene) and progression-free survival in patients suffering from head, neck squamous cell carcinoma (HNSCC). In the work of Gallicchio et al[29]in which the associations of β-carotene supplementation was suggested to be the result of the function of carotenoid, this finding became one of the parameters in the measurements as a peculiarity marker in the study of specific carotenoids and alent of support to the results herewith obtained.

The DPPHand FRAP assays results from the efficacy trials of the bacteriocarotenoid extracts did show the prospect of high potency of the all extractsas antioxidants. It should also be noted that under normal condition, the bulk-bacteriocarotenoid was better. However, risk of toxicity at high doses may be difficult to overcome at this stage. Results with both DPPH and FRAP complimented each other, Table 2 and Fig 4.

Table 2: Comparison of antioxidant activity using DPPH and FRAP methods

Extracts	Antioxidant Activities	
	DPPH	FRAP
1.Bulk-bacteriocarotenoid	37.91 ±3.2	34.01±0.8
2. β-Carotene	26.60±2.4	22.28±2.7
3. Rhodopin	18.06±3.1	19.33±0.6

The above findings and assertions were corroborated with the first large randomized trial on antioxidants and cancer risk, reported by Blot et al [30], the effect of a combination of β-carotene, vitamin E and selenium on cancer was investigated in healthy people at high risk for gastric cancer. Results of the study confirmed that a consortium of beta-carotene, vitamin E and selenium significantly effected a reduction in the incidence of both gastric cancer and cancer in general. The respective MTT assay IC_{50} values of β-carotene, rhodopin and bulk-bacteriocarotenoids for toxicity on MDA-MB, MCF-7 and Hep-G2 cancer cell lines and Vero benign cell line indicated inhibitory capabilities of these extracts, Fig 4.

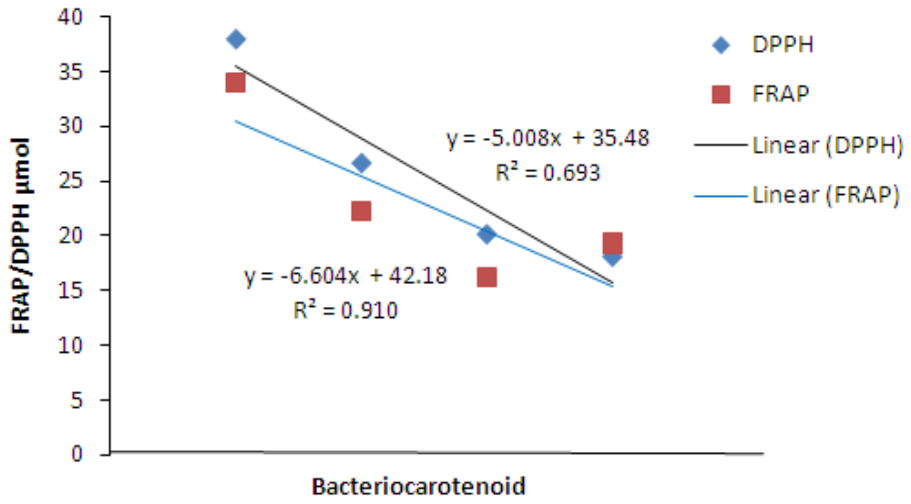

Figure 4: Trendline equations of FRAP and DPPH showing the efficacies of both methods in evaluation of the anti-oxidative potentials of bacteriocarotenoids.

IV. Conclusion

In conclusion, the lowest percentage of cell viability resulted from higher treatment doses, elongated exposure time and a combination of both factors for isolate-bacteriocarotenoids. With exposure up to 72 hours better inhibition was observed. On the other hand with consortia of bacteriocarotenoids making up the bulk-extracts it was possible to attain same results at lower dosage with emphasis constitutes a short-coming in the use of bulk-bacteriocarotenoid extracts instead of refined ones such as β-carotene and rhodopin from this bacterium. Therefore, the study-photosynthetic bacteria produced high yield of bacteriocarotenoids at the minimum required growth temperature and within the shortest possible incubation time.The effects of physiologic concentrations of one of the isolate bacteriocarotenoids, β-carotene, were assessed on MCF-7 breast cancer cells. The incubation of this cancer cells with the bacteriocarotenoid showed significant reductions in proliferation (18–89%) when compared to control cultures. The non-viability of MCF-7 was both dose-dependent and time-dependent. β-Carotene was inhibitory to MCF-7 cell line but not to Vero. The in-vitro results were indications that β-carotene may be cytotoxic to the cancer cell lines in-vivo. It should be noted that the effects of bulk bacteriocarotenoid extracts from this bacterium, were more pronounced even at lower concentrations than they were in individual isolate-bacteriocarotenoids. However, all the extracts were less toxic to Vero cell line than they were to carcinoma cell lines. This reason for this observation therefore is a case study for the next steps in this study.It is therefore suggested that due to ready availability of carotenogenic photosynthetic bacteria in hot and normal water, salt water and ponds around, large scale production of beneficial bacteriocarotenoids becomes easier, time and cost efficient with emphasis though on sponsorship, machinery adaptation and the will power to continue where this novel work has reached.

Acknowledgements

Appreciation to the National University of Malaysia for partly supporting this research work and MFM Group SdhBhd for full scholarship and supports.

References

[1]. American Cancer Society-ACS. 2007. Report sees 7.6 million global Cancer Deaths. American Cancer Society.Atlanta Georgia, USA.

[2]. World Health Organization. 2013: Latest world cancer statistics; Global cancer burden rises to 14.1 million new cases in 2012: Marked increase in breast cancers must be addressed. International Agency for Research on Cancer.Press Release 223.http://www.iarc.fr/en/media-centre/pr/2013/pdfs/pr223_E.pdf (Retrieved 25-11-2014 4.37pm).

[3]. K.W. KinzlerandB. Vogelstein, Introduction. The genetic basis of human cancer (2nd, illustrated, revised edition.).(New York: McGraw-Hill, Medical Pub. Division.2002.

[4]. D.A. Nelson,T.T. Tan, A.B. Rabson, D. Anderson, K. DegenhardtandE. White, Hypoxia and defective apoptosis drive genomic instability and tumorigenesis. Genes &Development18(17), 2004, 2095–2107.

[5]. A.J. Sasco, M.B. Secretan, andK. Straif, Tobacco smoking and cancer: a brief review of recent epidemiological evidence. Lung cancer.45(2), 2004, (S3–9).

[6]. L.M. Merlo, J.W. Pepper, B.J. Reid and C.C. Maley, Cancer as an evolutionary and ecological process.National Review on Cancer6(12), 2006, 924–35.

[7]. J. Ferlay, I. Soerjomataram, M. Ervik, R. Dikshit, S. Eser, C. Mathers, M. Rebelo, D.M. Parkin, D. Forman, F. Bray, GLOBOCAN 2012 v1.0, Cancer Incidence and Mortality Worldwide:IARC CancerBase No. 11 [Internet]. Lyon, France: International Agency for Research on Cancer. Available from http://globocan.iarc.fr. 2013.

[8]. F. Bray, J.S. Ren, E. Masuyer, J. Ferlay, Global estimates of cancer prevalence for 27 sites in the adult population in 2008. Int J Cancer, 132(5), 2013, 1133–1145.

[9]. WHO-IARC. Cancer Incidence and Mortality Worldwide: Cancer Base No. 11 [Internet]. GLOBOCAN 2012 v1.0.Ferlay J, Soerjomataram I, Ervik M, Dikshit R, Eser S, Mathers C, Rebelo M, Parkin DM, Forman D, Bray(eds.). F. Lyon, France: International Agency for Research on Cancer. http://globocan.iarc.fr, 2013.

[10]. American Cancer Society. 2005. Cancer Facts and Figures 2005.American Cancer Society, Atlanta Georgia, USA.

[11]. L.A. Ries, B.A. Miller, B.F. Hankey, C.L. Kosary, A. HarrasandB.K. Edwards (eds.), SEER Cancer statistics review 1973-1997. National Institute of health, National Cancer Institute. Report No DHHS, Puplication No NIH 97-2789, 1997.

[12]. Z.A. Omar, Z.M. Ali andN.S.I. Tamin, Malaysian cancer statistics-data and figure peninsular Malaysia 2006. National Cancer Registry, Ministry of Health, Malaysia, 2006.

[13]. Z.A. Omar and N.S.I. Tamin,Malaysian cancer statistics-data and figure peninsular Malaysia 2007. National Cancer Registry, Ministry of Health, Malaysia, 2007.

[14]. O.F.A. Akinnuoye, I. Nazlina and H. Ainon, Application of electron microscopy and energy dispersive x-ray spectroscopy in the characterization of Rhodomicrobiumvannielii. Journal of Advanced Microscopy Research 6 (1), 2011, 46-57.

[15]. Z. AksuandE.A. Tugba, Carotenoids production by the yeast Rhodotorulamucilaginosa: Use of agricultural wastes as a carbon source, Process Biochemistry 40 (9), 2005, 2985-2991.

[16]. [16]F.S. Lang, and D.Oesterhelt, Gene transfer system for Rhodopseudomonas viridis.Journal of Bacteriology, 171, 1989a, 2827-2834.

[17]. T.W. Goodwin and H.G. Osman, Studies on carotenogenesis: General cultural conditions controlling carotenoid (Spiriloxanthine) synthesis in the photosynthetic bacterium Rhodospirillumrubrum. Biochemical Journal 53, 1953, 541-546.

[18]. J.A. Sparano, M. Wang, S. Martino, V. Jones, E.A. Perez, T. Saphner, A.C. Wolff, G.W. SledgeJr, W.C. Wood and N.E.Davidson, Weekly paclitaxel in the adjuvant treatment of breast cancer.New England Journal of Medicine358(16), 2008, 1663-1671.

[19]. M. Ben. andM.D. Chue, Five-Year Survival of Metastatic Pancreatic Carcinoma: A Study of Courage and Hope. Gastrointest Cancer Research 3(5), 2009, 208–211.

[20]. 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.Malaysian Society of Applied Biology, Malaysia, 2008.

[21]. 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.

[22]. 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.

[23]. L.A. Betancur-Galvis, G.E. Morales, J.E. Forero, and J. Roldan, Cytotoxic and antiviral activity of colombian medicinal plant extract of the EuphorbiaGenus.Memorias do InstitutoOswaldo Cruz, Rio de Janeiro 97(4), 2002, 541-546.

[24]. J. Lorquin, F. Moloubaand B.L Dreyfu, Identification of the carotenoid pigment Canthaxanthin from Photosynthetic Bradyrhizobium Strains. Appl. Environ. Microbiol.63(3), 1997, 1151-4.

[25]. K. Arai, S. Takano, T.Teratani, Y. Ito, T. Yamada, and R. Nozawa, S100A8 and S100 Overexpression is associated with poor pathological parameters in invasive carcinoma of the breast. Current Cancer Drug Target 8, 2008, 243-252.

[26]. J. Vaya, andM. Aviram, Nutritional antioxidants: mechanism of action, analyses of activities and medical applications. Current Medicinal Chemistry–Immunology Endocrinology and Metabolic Agents1, 2001, 99–117.

[27]. F.F. Benzie, and J.J. Strain, The Ferric Reducing Ability of Plasma (FRAP) As a Measure of Antioxidant Power. Analytical Biochemistry. 239(1), 1999, 70-76.

[28]. A.K. Sakhi, .S.K.Bohn, S.Smeland,M.Thoresen, G.B. Smedshaug, J. Tausjo, A. Svilaas, A. Karlsen,K.M. RussnesT. Svilaas and R. Blomhoff,Postradiotherapy plasma lutein, alpha-carotene, and beta-carotene are positively associated with survival in patients with head and neck squamous cell carcinoma. Nutrition and Cancer.62(3), 2010, 322-328.

[29]. L. Gallicchio, K. Boyd, G. Matanoski, X.G. Tao, C. Liwei, T.K. Lam, M. Shiels, E. Hammond, K.A. Robinson, L.E. Caulfield, J.G. Herman, E. Guallar, E. and A.J. Alberg, Carotenoids and the risk of developing lung cancer: a systematic review. American Journal of Clinical Nutrition 88(2), 2008, 372-383.

[30]. W.J. Blot, J.Y. Li and P.R. Taylor, Nutrition intervention trials in Linxian, China: supplementation with specific vitamin/mineral combinations, cancer incidence, and disease-specific mortality in the general population. Journal of National Cancer Institute. 85, 1993, 1483–91.

[31]. J.L. Spudich,and H. Luecke, Sensory rhodopsin II: functional insights from structure. Current Opinion in Structural Biology 12, 2002, 540–546.

[32]. D.L. Bedard, G.V.S. Jerzak, U. Nubel, M.M. Bateson and D.M. Ward, Novelthermophilic green sulfur bacteria discovered in hot springs in two regions of Yellowstone National Park. American Society for Microbiology General Meeting, Salt Lake City, Utah Abstract 159. 2002.