ACCUMULATION OF NATURAL ASTAXANTHIN IN *HAEMATOCOCCUS PLUVIALIS* UNDER STRESS OF SILVER NANOPARTICLES

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ABSTRACT

Astaxanthin is a valuable product due to strong antioxidant properties. Astaxanthin have application in various type of industry such as food, medical, and poultry farming. Thus, astaxanthin has a high market demand and should be produced in a larger scale. *Haematococcus pluvialis* is a species of microalgae that produces the highest level of astaxanthin compared to other species. Thus, *H. pluvialis* was chosen in this study. Research findings have reported that the growth rate and astaxanthin production of *H. pluvialis* can be affected by exposure to silver (Ag) nanoparticles, hence this study served to investigate the maximum production rate of astaxanthin by exposing *H. pluvialis* to different concentrations (10 mg/L, 100 mg/L and 200 mg/L) of silver nanoparticles. The growth rate of *H. pluvialis* was observed at different concentrations of silver nanoparticles. The growth of *H. pluvialis* was influenced by silver nanoparticles through the interaction with protein and disruption of ATP synthesis which will then damage the DNA. Maximum astaxanthin production (0.458 ± 0.002 mg/L) was obtained by exposing *H. pluvialis* to 10 mg/L of Ag nanoparticles on day-14. Ag nanoparticles could inhibit the growth of microorganisms by the generation of free radicals from the surface of silver. To overcome the unfavorable conditions, *H. pluvialis* produced astaxanthin as protection by stopping the free radical process in the cell. In conclusion, 10 mg/L of Ag nanoparticles was able to induce the highest production of astaxanthin compared to the other concentrations of Ag nanoparticles. Thus, this study demonstrated that the growth rate of *H. pluvialis* was influenced by different concentrations of Ag nanoparticles and Ag nanoparticles were able to induce the astaxanthin production.

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LIST OF ABBREVIATIONS

Ag	Silver
ATP	Adenosine triphosphate
BBM	Bold Basal Medium
cells/mL	Total cells per Millilitre (Cell Density)
DNA	Deoxyribonucleic acid
g	Gram
g/mol	Gram per Mole
HCl	Hydrochloric acid
kg	Kilogram
L	Litre
mg/L	Milligrams per Litre
mL	Millilitre
mol	Mole
NaCl	Sodium Chloride
NaOH	Sodium Hydroxide
nm	Nanometre
OD	Optical Density
ppm	Parts per million
ROS	Reactive Oxygen Species
rpm	Revolution per Minute
UV	Ultraviolet

CHAPTER 1

INTRODUCTION

Astaxanthin is a red-orange carotenoid pigment which has been highly regarded as a potent antioxidant. Like any other antioxidants, astaxanthin acts as a defence system to shield the cell against oxidative damage. Astaxanthin is highly sought-after due to its powerful free radical antioxidant activity compared to vitamin E and other carotenoids (Miki, 1991; Shimidzu, Goto & Miki, 1996). Astaxanthin has also been reported to exhibit essential biological function such as stabilizing cell membranes and terminal free radicals in our body (Guerin, Huntley & Olaizola, 2003).

Study on astaxanthin accumulation in *Haematococcus pluvialis* is extremely important due to its potential application in various types of industries. In the food industry, it is generally utilized as a food dyeing agent or feed addition agent in poultry farming as well as in salmon or trout breeding in aquaculture (Bicas, Maróstica & Pastore, 2016). In the pharmaceutical sector, astaxanthin plays a vital role in human health by providing protection against UV-light, as an anti-inflammatory agent, and even as an anti-aging agent (Guerin et al., 2003). Several studies reported that astaxanthin could help the human immune system to combat various diseases such as diabetes, cancer and cardiovascular disease (Guerin et al., 2003; Lorenz & Cysewski, 2000; Yuan, Peng, Yin & Wang, 2010).

The freshwater algae, *H. pluvialis* is a main source of natural astaxanthin because it has highest production (40000ppm) of astaxanthin compared to other natural sources such as yeast, crab or prawn (Biswal, 2014). *Haematococcus pluvialis* has 3.8% higher amount of astaxanthin compared to other algae (Ambati, Phang, Ravi & Aswathanarayana, 2014). *H. pluvialis* was able to produce more than 30 g/kg dry weight basis of astaxanthin (Guerin et al., 2003). In fact, astaxanthin is naturally present in *H. pluvialis* but in low concentrations. *Haematococcus pluvialis* is able to accumulate astaxanthin, which served to protect its cells under harsh environmental conditions (Shah, Mahfuzur, Liang, Cheng & Daroch, 2016).

A lot of studies have been conducted to stimulate the production of astaxanthin by *H. pulvalis* under harsh condition (Boussiba & Vonshak, 1991). However, using nanoparticles to stimulate accumulation of astaxanthin has not been done before. Therefore, silver nanoparticle was used to induce accumulation of astaxanthin in *H. pluvialis* in this study. Previous studies also reported that metal could stress the cells, which stimulate the high production of astaxanthin in microalgae culture (Miazek, Iwanek, Remacle, Richel & Goffin, 2015). Silver nanoparticles could inhibit the growth of microorganism by the generation of free radical from the surface of silver. Formation of the free radical targets to membrane lipids and thus disrupting biological function (Kim et al., 2007).

Hence, the objectives of this research have, i) to observe growth pattern of the *H. pulvialis* with different concentrations of silver nanoparticles, ii) to obtain the maximum astaxanthin production by exposure *H. pluvialis* to different concentrations of silver nanoparticles.

CHAPTER 2

LITERATURE REVIEW

2.1. CHARACTERISTICS OF ASTAXANTHIN

2.1.1. Structure of Astaxanthin

Astaxanthin has the molecular formula of $C_{40}H_{52}O_4$ which is known as 3,3'-dihydroxy-β-carotene-4,4'-dione. It is classified under the xanthophyll family in the class of β-carotenoids. The structure of astaxanthin is composes of two terminal rings with a hydroxyl group connected by long conjugate double bond chain (Higuera-Ciapara, Felix-Valenzuela & Goycoolea, 2006). It consists of three types of stereoisomers which are (3S, 3'S), (3R 3'S) and (3R 3'R) as showed in Figure 2.1.1. The most valuable stereoisomer is 3S, 3'S stereoisomer which is mainly found in *H. pluvialis* (Guerin et al., 2003).

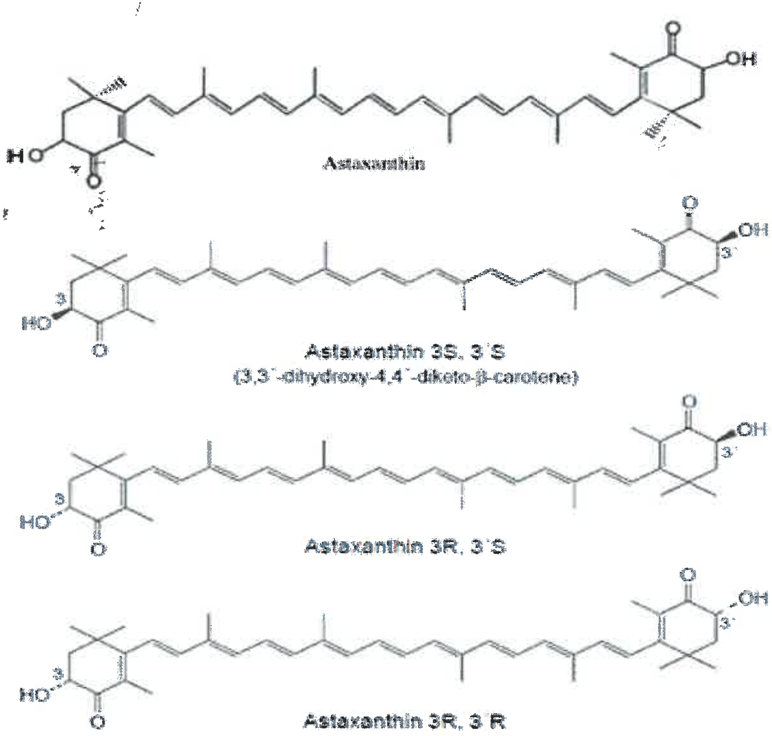

Figure 2.1.1 Structures and stereoisomers of astaxanthin (Higuera-Ciapara et al., 2006)

2.1.2. Antioxidant Activity of Astaxanthin

The strong antioxidant activity of astaxanthin is due to its structure. The presence of the hydroxyl group is responsible for the antioxidant process through conjugation with proteins or esterification with fatty acids to stabilize the molecule (Guerin et al., 2003). The antioxidant molecules present in astaxanthin possesses the ability to eliminate or inhibit the free radical process (Britton, 1995). To terminate the free radical chain reaction, long conjugated double bonds of astaxanthin release electrons and react with free radicals. The free radicals accept the electrons and cause the molecule to be resonance stabilized (Hussein, Sankawa, Goto, Matsumoto & Watanabe, 2006). In Figure 2.1.2, astaxanthin exists in both hydrophobic and hydrophilic region of the cell membrane providing a better protection against free radicals (Yamashita, 2013).

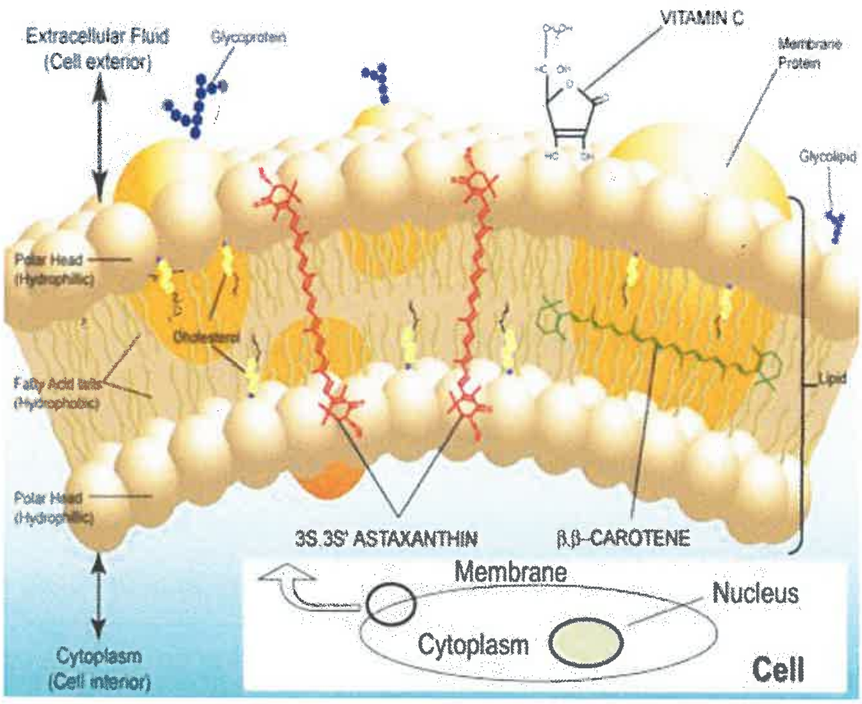

Figure 2.1.2 Position of astaxanthin in the cell membrane (Yamashita, 2013)

Strong free radical antioxidant property of astaxanthin has been demonstrated in several studies. In one of the study on antioxidant activity between α-tocopherol with different types of carotenoid, astaxanthin showed a 540× stronger activity compared to α-tocopherol and approximately 10× stronger than other carotenoids such as lutein, tunaxanthin, and halocynthiaxanthin (Shimidzu et al., 1996). Astaxanthin also been reported to be 100× better than the tocopherol form of vitamin E in carrying strong active forms of oxygen (Miki, 1991).

The antioxidant activities of astaxanthin have vital roles in human health due to its ability to stabilize cell membranes and terminal free radicals in our body that are produced as a result of unhealthy lifestyle (Guerin et al., 2003). Several studies have reported that astaxanthin has different biological function as stated in Table 2.1.

Table 2.1 Biological function of astaxanthin

Biological function	Reference
Protect body tissue from oxidative damage	(Yuan et al., 2010)
Protection against UV-light	(Lyons & O'Brien, 2002)
Anti-inflammatory	(Suzuki et al., 2006)
Anti-aging	(Guerin et al., 2003)
Induce immune response	(Chew & Park, 2004)
Antidiabetic	(Uchiyama et al., 2002)
Anticancer	(Higuera-Ciapara et al., 2006)
Prevention cardiovascular disease	(Jialal & Fuller, 1995)
Neuroprotection	(Wu et al., 2015)
Improve gastrointestinal system	(Liu & Lee, 2003)