EFFECT OF DIFFERENT CONCENTRATIONS OF CADMIUM NANOPARTICLE, PH AND SALINITY ON PRODUCTION OF ASTAXANTHIN IN HAEMATOCOCCUS PLUVIALIS

HONG YEE ZHEN
I14006021

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HONG YEE ZHEN	*Dr. CHENG WAN HEE*
I14006021	(SUPERVISOR)
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ABSTRACT

Haematococcus pluvialis is one of the potent microalgae for the production of secondary carotenoid, astaxanthin. Astaxanthin has great commercial value due to its high antioxidant properties. However, the mass production of astaxanthin is hindered by the low yield of astaxanthin obtained from the *H. pluvialis*. Various report have been used heavy metal to induce accumulation of astaxanthin in *H. pluvialis*, however, using nanoparticle to induce the production of astaxanthin in the same algae so far have not been investigated. In this present study, the objective was investigate the effect of Cadmium (Cd) nanoparticles on the accumulation of astaxanthin in *H. pluvialis* at optimal pH and salinity. *Haematococcus pluvialis* was cultured at different pH and salinity in order to find the optimized growth parameter for the growth of *H. pluvialis* and maximal astaxanthin production. Highest ($p<0.05$) of growth and astaxanthin production in the algae were found at pH 7 and 0.25% of salinity. The experiment was taken a step further by testing the algae at different concentration of Cd nanoparticle under the optimal growth condition which have been determined previously. Highest ($p<0.05$) accumulation of astaxanthin was found when the algae exposed at 10mg/mL of Cd nanoparticle. There were no correlation about the growth and astaxanthin formation. Even the Cd nanoparticle inhibit the growth of the algae, but the Cd nanoparticle still could induce astaxanthin accumulation in *H. pluvialis.* This is due to the ability of nanoparticle and metal could exerted stress on the algae by generating the reactive oxygen species (ROS), as a protective response against the detrimental effect of ROS, astaxanthin was being synthesized by the algae. Thus, we proposed possible mechanism was shared between Cd nanoparticle and Cd heavy metal in the induction of astaxanthin accumulation.

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LIST OF ABBREVIATION

BM	Basal Medium
C	Celsius
Cd	Cadmium
CDF	Cation Diffusion Facilitator
DNA	Deoxyribonucleic acid
g	Gram
HCl	Hydrochloric acid
kflux	Kiloflux
KOH	Potassium Hydroxide
M	Molarity
mg/L	Milligrams per Litre
mL	Millilitre
NaCl	Sodium Chloride
NADPH	Nicotinamide Adenine Dinucleotide Phosphate
nm	Nanometers
NP	Nanoparticle
OD	Optical Density
ROS	Reactive Oxygen Species
rpm	Rounds per Minute
UV	Ultraviolet
w/v	Weight over Volume

CHAPTER 1

INTRODUCTION

Astaxanthin is a bright red coloured secondary ketocarotenoid pigment. It is worth noted that natural astaxanthin is a potent antioxidant which can be synthesized by some aquatic organisms such as green microalgae, yeast, bacteria and plants (Higuera-Ciapara et al., 2006; Ranga et al., 2014). It is the best known substance to give red pigmentation to salmonids, lobsters, crayfishes as well as shrimps (Koller, Muhr, & Braunegg, 2014). Furthermore, astaxanthin is considered for a wide range of applications due to its powerful antioxidant properties, which is 10 times stronger than other carotenoids such as lutein, zeaxanthin, canthaxanthin, and beta carotenoids, over 100 times greater than tocopherol and 65 times stronger than vitamin C (Borowitzka, 2013; Pérez-Lópezetal., 2014; Koller et al., 2014; Cyanotech, 2015). Hence, it has been widely applied in nutraceutical, pharmaceutical and aquacultural industry (Lorenz & Cysewski, 2000). It has been reported that natural astaxanthin are safe for human consumption without any side effects (Yuan et al., 2011). Synthetic astaxanthin on the other hand has been proven to be 20 times less potent as an antioxidant than natural astaxanthin and its safety consumption for human has not been established (Lorenz & Cysewski, 2000; Li et al., 2011; Coller et al., 2014). Therefore, the production of natural astaxanthin is highly demanded in the market.

Microalgae is a well-known source of natural astaxanthin. Accumulation of commercial astaxanthin in green microalgae such as *Chlorella vulgaris* (Kong et al., 2011), *Chlorella zofingiensis* (Liu et al., 2014), and *Haematococcus pluvialis* (Kobayashi, Kakizono, & Nagai, 1993; He et al., 2007; Reyes et al., 2014) under stress has been well studied. Among carotenoid-producing microalgae, the freshwater microalga *Haematococcus pluvialis* is known to be a good producer of natural astaxanthin in the industry due to its ability to accumulate astaxanthin up to 3.8 % on the dry weight basis which relatively higher than other microorganisms when culture in unfavourable conditions (Ambati, Sarada, Basharan, & Gokare, 2009; Ranga, Raghunath, Baskaran, Sarada, & Ravishankar, 2010). The accumulation of astaxanthin is affected by various environmental factors such as light, temperature, nutritional starvation, salinity, pH, plant hormones and oxidative stress (Shah et al., 2016).

Nevertheless, various abiotic factors influencing level of accumulation of astaxanthin in have *H. pluvialis* been studied (Nagaraj, Arulmurugan, Rajaram, Sundararaj, & Rengasamy, 2012). Oxidative stress is one of the key factor involved in enhancement of biosynthesis of natural astaxanthin biosynthesis in microalgae (Mittler, 2002). In order to resist the damage caused by oxidative stress, antioxidant is produced as a defensive mechanisms to withstand the harsh condition. This have been documented in the literature where higher accumulation of astaxanthin in red cyst cells of *H. pluvialis* to resist reaction oxygen species (ROS) (Kobayashi et al., 1993). In regards of this scientific knowledge, nanoparticles may have the potential to increase ROS activity to trigger the production and accumulation of astaxanthin in *H. pluvialis* (Moos & Slaveykova, 2013). Up to date, there are no studies about using nanoparticles to stimulate the accumulation of astaxanthin as protective response to the oxidative stress on *H. pluvialis*. Thus, the objective of this study is to investigate the effect of Cadmium (Cd) nanoparticle on the accumulation of astaxanthin in *H. pluvialis* at optimum pH and salinity respectively.

CHAPTER 2

LITERATURE REVIEW

2.1 CHEMISTRY STRUCTURE OF ASTAXANTHIN

Astaxanthin (3,3′-dihydroxy-4,4′-diketo-β-carotene)) is a bright red, fat soluble secondary carotenoid which is synthesised *de novo* by various aquatic animals such as salmon, lobster, and fish eggs (Higuera-Ciapara, Felix-Valenzuela, & Goycoolea, 2006). Figure 2.1 showed the chemical structure of astaxanthin which is a member of the cyclic carotenoids that is consisted of two terminal rings with hydroxyl group and the rings is joined by a polyene chain (Higuera-Ciapara, Felix-Valenzuela, & Goycoolea, 2006). The presence of hydroxyl groups in astaxanthin enable it to react with fatty acids to form hydrophobic molecules which are soluble in fats (Britton, Liaaen-Jensen, & Pfander, 2004).

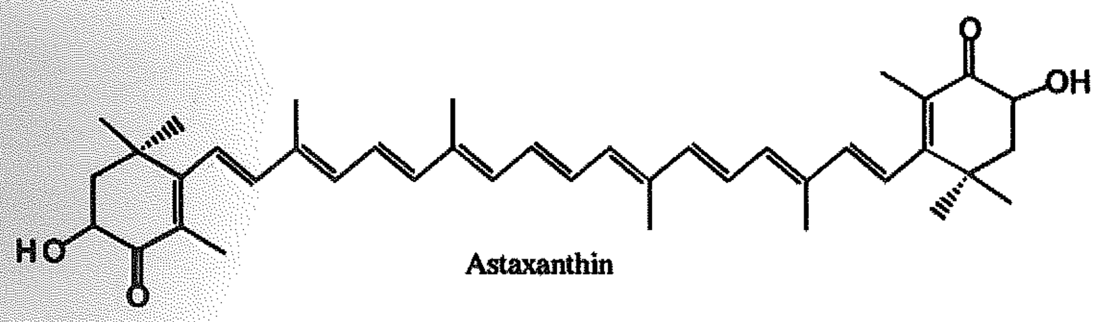

Figure 2.1 The structure of astaxanthin. (Higuera-Ciapara et al., 2006)

2.1.1 Difference Between Synthetic and Natural Astaxanthin

Three different stereoisomers, (3S, 3'S); (3'S, 3R), and (3R, 3'R) of astaxanthin is showed in Figure 2.2 The most valuable stereoisomer is in 3S, 3'S form which is predominantly biosynthesized by *H. pluvialis* (Yang, Kim, & Lee, 2013; Al-bulishi, Changhu, & Tang, 2015). Astaxanthin is preferably applied as feed additives because it has been reported to have higher pigmentation in rainbow trouts on a diet of natural astaxanthin as compared to a diet of synthetic astaxanthin with the same dietary concentration (Natural Algae Astaxanthin Association, 2015). Its safety for human consumption has been approved by several European countries, USA and Japan (Yuan, Peng, Yin, & Wang, 2011). Unlike synthetic astaxanthin which is synthesized from petrochemical, tend to have detrimental effects on health (Miao, Lu, Li, & Zeng, 2006; Holtin et al., 2009; Cysewski, 2014)

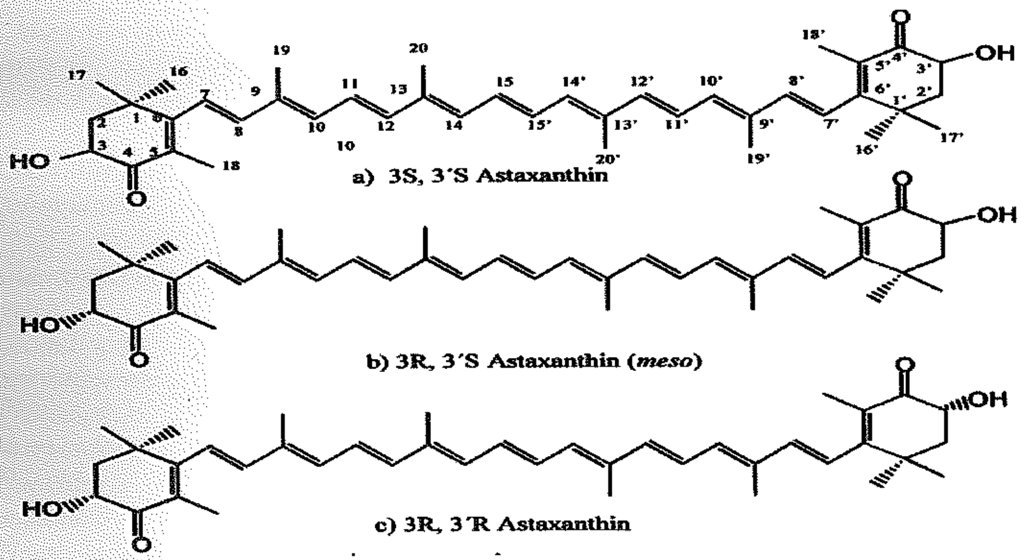

Figure 2.2 The three different stereoisomers of astaxanthin (Higuera-Ciapara et al., 2006).

Another disadvantage of synthetic astaxanthin is that it is 20 times lower in antioxidant activity than its natural counterpart. Hence, this makes natural astaxanthin become the key bioproduct in the market (Li et al., 2014)

2.2 *Haematococcus Pluvialis*

Haematococcus pluvialis is a unicellular green microalgae that found in freshwater (Boussiba & Vonhshak, 1991). *Haematococcus pluvialis* has gained popularity among researchers in this few years because of its capability to produce natural biological source of astaxanthin (Li et al., 2014). It have been choose for large scale natural astaxanthin production as it can accumulate up to 4% of astaxanthin of dry biomass (Zhekisheva, Khozin-Goldberg, Cohen, & Boussiba, 2005) as compare to other stains. Table 2.1 showed numerous strains of microalgae that have been reported to be potential feedstocks of astaxanthin production.

Table 2.1 Sources of astaxanthin from various microalgae.

Sources	**Astaxanthin (%) On The Dry Weight Basic**	**Reference**
Chlamydocapsa sp	0.04	(Leya, Rahn, Lütz, & Remias, 2009)
Scenedesmus sp.	0.3	(Qin, Liu, & Hu, 2008)
Chlorella zofingiensis	0.7	(Orosa, Torres, Fidalgo, & Abalde, 2000)
Chlorococcum sp.	0.7	(Ma & Chen, 2001)
Scotiellopsis oocystiformis	1.1	(Orosa et al., 2000)
Protosiphon botryoides	1.4	(Orosa et al., 2000)
Neochloris wimmeri	1.9	(Orosa et al., 2000)
Haematococcus pluvialis	4.0	(Aflalo, Meshulam, Zarka, & Boussiba, 2007)