Short Communication

Extension of Life Span and Improvement of Vitality in *Drosophila melanogaster* by Supplementation with Lycopene Mycelium Powder from *Blakeslea trispora*

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The free radical theory of aging believes that free radicals are involved in the changes in cellular metabolism that lead to a time-dependent functional decline in the living organism. Antioxidant or free radical scavengers may retard the aging process. In the current study, we explored the anti-aging activity by lycopene in *Drosophila melanogaster*. The results from this study showed that lycopene mycelium powder have the ability to prolong the longevity of *Drosophila melanogaster*, enhanced its reproductivity and sexual capacity, had the ability to increase SOD level and decrease MDA level. From the above finding in the study, we concluded that lycopene can reduce the propagation of free radical reactions and limit the formation of new free radicals, therefore build a defense to protect the organism from the oxidative stress and slow the aging process.

Keywords: lycopene, free radical, *Drosophila melanogaster*, anti-aging

Introduction

Reactive oxygen species (ROS) are products of electron transport chains, enzymes, and redox cycling. The oxidative damage initiated by ROS is believed to be a major contributor to the functional decline associated with aging. Aging is the progressive intrinsic deterioration in physiological status that culminates in death (Arking, 2006). Although the underlying mechanisms of aging are still unclear, the free radical theory of aging seems to be a significant contributor in the process. Free radicals are highly reactive by-products of metabolism seeking stability by gaining an extra electron from any source. This very property leads to protein, lipid, and DNA damage (Zhao *et al.*, 2008). For aerobic organisms, they have two antioxidant systems to scavenge ROS and minimize their associated adverse effect (Droge, 2002.). First, they endogenously produce a group of antioxidant enzymes including superoxide dismutase (SOD), glutathione peroxidase (GPx), and catalase (CAT), which serves as a first line of defense against ROS. Second, exogenous antioxidants such as ascorbic acid, vitamins C, α -tocopherol and plant

flavonoid may build a secondary defense base to terminate the propagation of ROS reactions and slow the aging process. Both endogenous antioxidant enzymes and exogenous dietary antioxidants build a defense base to terminate the propagation of free radical reactions, limit the formation of new free radicals and slow down the aging process.

Carotenoids are a family of fat-soluble pigments that are prevalent in numerous fruits and vegetables especially in tomato and carrot. In recent decades, many researches have demonstrated that they have the potential of ameliorating oxidative stress (Agarwal and Rao, 2000; Cohen, 2002; Rao and Rao, 2007; Visioli *et al.*, 2003). Among the more than 600 different compounds founded in carotenoids, lycopene is proved to be the most prominent (Clinton, 1998; Giovannucci, 2002; Gupta, *et al.*, 2003; Jacob, *et al.*, 2008; Matos, *et al.*, 2002). Researches showed that lycopene plays an important role in health because of its highly efficient antioxidant scavenging activity against singlet-oxygen and free radicals (Cohen, 2002; Heber and Lu, 2002; Jonker *et al.* 2003; Stahl and Sies, 2003; Wertz, 2004).

The fruit fly of *Drosophila melanogaster* has been used as a model in aging research for decades. Flies are well suited for such studies for a number of reasons, as they share many

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conserved biological pathways and also because of their relative short life span, tiny body size, easy maintenance (Minois, 2006). Dietary modification is thought to be able to prolong the lifespan and slow age-related diseases, therefore adding different nutraceuticals such as some antioxidants into the daily diet has been recognized as a potential way to slow the aging process (Abbas and Wink, 2009; Grune, 2000; Peng *et al.*, 2009; Zhang, *et al.*, 2009). The current study was to investigate the anti-aging effect activity of lycopene when the lycopene from *Blakeslea trispora* were added into the diet of *Drosophila melanogaster*.

Material and Methods

Lycopene The test fermented lycopene (FL) (lycopene mycelium powder) was obtained from the fermentation of *Blakeslea trispora* in our lab. The strains of *Blakeslea trispora* ZJHD11 (+) and *Blakeslea trispora* ZJHD12 (–) were cultivated together (mated culture) in a fermentation medium that contained (in 1L), starch 41 g; soybean meal 20 g; soybean oil 35g; KH_2PO_4 2 g; $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ 1 g; thiamine 0.03 g; Triton x-100 1 mL; n-Dodecane 10 mL in distilled water. The initial pH of the medium was adjusted to 7.0 – 7.5. The components of medium were heated until completely dissolved before autoclaving. The fermentation was carried out in 500 mL flasks with 100 mL medium including 6% inoculum, and the inoculum was a 1:1 (volume ratio) mixture of *Blakeslea trispora* ZJHD11 (+) and *Blakeslea trispora* ZJHD12 (–). The cultures were incubated at 28°C in a rotary shaker at 200 rpm, then β -ionone (1.5 mL/L), isoniazid (0.006g/L) and 2.0% (v/v) tobacco extracts were added after fermenting for 44 h. Cultures were maintained for 144 h, then the cell mass was filtered through muslin, washed thoroughly with distilled water, and dried in a vacuum drier at 50°C under 0.08 MPa vacuum till constant weight. The production of biomass, lycopene and β -carotene were determined. The content of lycopene, β -carotene and γ -carotene in the mycelium powder was 1.87%, 0.032% and 0.021%, respectively. As the ratio of β -carotene and γ -carotene in the mycelium powder was low, we considered the effect of mycelium powder was from the lycopene. The synthesized lycopene (PL) was obtained from the Zhejiang NHU company Ltd., the content of lycopene was 99.99%.

Drosophila melanogaster The *Drosophila* strain used in current research was Oregon K, and was preserved by Institute of Materia Medica of Zhejiang Chinese medical school. The *Drosophila melanogaster* incubated within 8 h were anesthetized by diethyl ether and were cultured in the culture tube at 25 ± 1°C with the humidity of 45 – 75%.

Diet The basic nutrient diet was prepared as the following: corn starch 85 g, custer sugar 65 g, agar 10 g, dried yeast

7.5 g, propionic acid 7 mL, H_2O 1000 mL. Custer sugar, agar and corn starch were mixed with warm water and heated until they were fully dissolved, then the dried yeast and propionic acid were added into the mixture.

The treated medium 13.36 mg, 40.11 mg and 120.32 mg lycopene fermentation mycelium powder (FL) diluted with soybean oil were mixed with 100g basic nutrient medium respectively in the 50 – 60°C water bath, forming different levels of lycopene (2.5, 7.5, 22.5 ppm) in the nutrient medium. Pure synthesized lycopene (PL) diluted with soybean oil was also added in another basic nutrient medium forming the 7.5 ppm level of lycopene in the medium.

Effect of lycopene on Longevity of *Drosophila melanogaster* Newly emerged *Drosophila melanogaster* within 8 h were anesthetized by diethyl ether and were divided into five groups (n = 300 flies each with 150 males and 150 females). Group A was maintained on the basal diet, and group B was fed with the PL at 7.5ppm, whereas the C, D, E groups were fed one of the three diets containing 2.5, 7.5 or 22.5 ppm FL. The *Drosophila melanogaster* were raised in the incubator at 25 ± 1°C with the humidity of 45 – 75%. Dead flies were counted every day until all of the flies were dead. New nutrition medium containing the same diet was changed every 4 days. Mean longevity and the longest longevity were recorded and the lifetime extension was calculated. The mean longevity of the last 10 flies left in the trial was calculated as the longest longevity.

Effect of lycopene on the reproductivity of *Drosophila melanogaster* Newly emerged *Drosophila melanogaster* within 8 h were anesthetized by diethyl ether and were divided into five groups (n = 10 flies each with 5 males and 5 females). The flies were fed in the different diet like the above trial (Group A as control, Group B has 7.5ppm PL, Group C, D, E has 2.5, 7.5, 22.5ppm FL, respectively) and keep in the incubator at 25 ± 1°C with the humidity of 45-75%. After 7 days, the original 5 male and 5 female flies were removed, and the newly emerged flies were recorded daily. The flies emerged in the 7 days from the first fly were recorded as the amount of the first filial generation (F1) and the rate of increase was calculated.

Effect of lycopene on the sexual capacity of *Drosophila melanogaster* The same sexual *Drosophila melanogaster* newly emerged within 8 h were divided into five groups. The flies were fed in the different diet like the above trial (Group A as control, Group B has 7.5ppm PL, Group C, D, E has 2.5, 7.5, 22.5 ppm FL, respectively) and keep in the incubator at 25 ± 1°C with the humidity of 45 – 75% for 15 days. On the 15th day, the male and female flies were 1:1 matched and then the amount of mating flies, the incubation period of mating and the lasting time of mating in 30 mins were recorded.

Effect of lycopene on the SOD and MDA in *Drosophila melanogaster* The same sexual *Drosophila melanogaster* newly emerged within 8 h were divided into five groups and were fed in five different diets for 30 days. On the 15th and 30th day, 40 mg *Drosophila melanogaster* were anesthetized by diethyl ether, then 1 ml of the mixture of chloroform and methanol (2:1) were added and the flies were homogenated, then 1 mL normal saline were added and fully mixed. The samples were centrifuged at 3000 rpm for 10 min, and the SOD was determined using the supernatant liquid. An assay kit was used to quantify the SOD activity in fruit flies (Jiancheng, Nanjing, China).

The same sexual *Drosophila melanogaster* newly emerged within 8 h were divided into five groups and were fed in five different diets for 30 days. On the 15th and 30th day, 40 mg *Drosophila melanogaster* were anesthetized by diethyl ether, 0.5 mL normal saline were added and the flies were homogenated, then the sample were centrifuged at 3000 rpm for 10 min, and the supernatant liquid was collected for the determination of MDA. An assay kit was used to quantify the MDA activity in fruit flies (Jiancheng, Nanjing, China).

Statistical analysis Data were analysed using the general linear model (GLM) procedure of SAS (1997, SAS Inst., Inc., Cary, NC). All multiple comparisons among means were performed using Duncan's new multiple range test.

Results and Discussion

Effect of lycopene on longevity of *Drosophila melanogaster* The Life span of *Drosophila melanogaster* increased significantly when lycopene was added to the food. As showed in Table 1 and Table 2, the Average life and Maximum life of the treated group (including the PL and FL) were higher than the control. The mean life span and the maximum life span of the medium and high dose of FL were improved significantly, the mean life span extension rate were 11.6%, 17.4% for female flies and 13.0%, 17.8% for male flies, respectively; the mean maximum life span extension rate were 9.5%, 14.4% for female flies and 7.4%, 12.1% for male flies, respectively. Administering of 7.5 ppm PL significantly increased the mean life span and maximum life of female flies and the mean life span of males, but had no effect on maximum life of male flies. This indicated that lycopene had the ability to prolong the life span of *Drosophila melanogaster* and this ability was sexual dependent as to the mean life span and maximum life span.

As to the same 7.5 ppm level of lycopene added, the FL groups seemed have more profound effect on the mean life span and the maximum life span than the PL, this may because there were some other carotenoids like β -carotenoid and γ -carotenoid in the fermentation samples, and these carotenoids may also have the potential at ameliorating oxidative stress, and therefore have the potential way to slow the

Table 1. Effect of lycopene on the life-span of female *Drosophila melanogaster*.

Group	Average life (d)	Average life extension rate (%)	Maximum life (d)	Maximum life extension rate (%)
Control	48.2 $\pm$ 6.76 ^a	—	62.3 $\pm$ 1.64 ^a	—
PL(ppm) 7.5	52.1 $\pm$ 7.43 ^b	8.1	65.8 $\pm$ 2.10 ^{bc}	5.6
2.5	50.1 $\pm$ 7.34 ^a	3.9	64.7 $\pm$ 2.45 ^{ab}	3.9
FL (ppm) 7.5	53.8 $\pm$ 8.03 ^b	11.6	68.2 $\pm$ 3.25 ^c	9.5
22.5	56.6 $\pm$ 8.78 ^c	17.4	71.4 $\pm$ 4.12 ^d	14.4

a, b, c, d Means with different superscripts within the same row differ at $P < 0.05$.

Table 2. Effect of lycopene on the life-span of male *Drosophila melanogaster*.

Group	Average life (d)	Average life extension rate (%)	Maximum life (d)	Maximum life extension rate (%)
Control	46.0 $\pm$ 7.58 ^a	—	61.0 $\pm$ 2.21 ^a	—
PL(ppm) 7.5	48.2 $\pm$ 6.80 ^b	4.8	62.3 $\pm$ 1.64 ^a	2.1
2.5	47.4 $\pm$ 7.84 ^{ab}	3.0	62.1 $\pm$ 2.92 ^a	1.8
FL (ppm) 7.5	52.0 $\pm$ 7.46 ^c	13	65.5 $\pm$ 2.59 ^b	7.4
22.5	54.2 $\pm$ 7.46 ^c	17.8	68.4 $\pm$ 3.75 ^c	12.1

a, b, c, d Means with different superscripts within the same row differ at $P < 0.05$.

aging process and prolong the life span of *Drosophila melanogaster*.

Effect of lycopene on the reproductivity of *Drosophila melanogaster* Lycopene significantly and dose dependently ameliorated the reproductivity of *Drosophila melanogaster* when the flies were fed with different levels of lycopene. From Fig. 1, we noticed that medium or high dose of FL or 7.5 ppm PL significantly enhanced the amount of first filial generation, therefore improved the reproductivity of *Drosophila melanogaster*.

Effect of lycopene on the sexual capacity of *Drosophila melanogaster* The sexual capacity of *Drosophila melanogaster* was improved when the flies were fed with lycopene. Table 3 showed that the mating rate of all treated groups were higher than the control. We also found that the more FL was added, the more mating rates were gained, therefore we concluded that there was dose dependent effect of lycopene on the mating rate. As to the mating latency, administering of lycopene could shorten the incubation period of mating,

but there were no significant difference between the different dose levels. Administering of lycopene can prolong the mating duration time, but no dose related effect was found. Mating rate, mating latency and mating duration time are indexes of the sexual ability of the animals. Sexual function decline and less sexual activity is one of the main manifestations of aging. In current study, we observed that when the *Drosophila melanogaster* were fed with lycopene, their sexual capacity were improved, suggesting that lycopene have the ability of slowing down the aging process of *Drosophila melanogaster*.

Effect of lycopene on the SOD and MDA in *Drosophila melanogaster* One of the most prominent theories of aging is known as the free radical theory (Harman, 1957; Beckman and Ames, 1998). In this theory, they believed that an age-associated accumulation of macromolecules oxidatively damaged by ROS results in senescence. However, for aerobic organisms, they possess an antioxidant enzyme system as to terminate the propagation of free radical reactions, limit

Fig. 1. Effect of lycopene on reproduction ability of *Drosophila melanogaster*.

Table 3. Effect of lycopene on sexual potency of *Drosophila melanogaster*.

Group	Mating rate (%)	Mating latency (min)	Mating duration time (min)
Control	22.5 ± 2.08 ^a	12.9 ± 2.57 ^a	27.4 ± 3.86 ^a
PL (ppm) 7.5	26.0 ± 1.42 ^{bc}	9.8 ± 2.49 ^{ab}	30.8 ± 3.98 ^{ab}
2.5	25.5 ± 1.29 ^c	10.1 ± 2.22 ^{ab}	29.8 ± 4.34 ^{ab}
FL (ppm) 7.5	26.8 ± 1.25 ^c	9.6 ± 1.94 ^b	31.7 ± 3.75 ^{ab}
22.5	28.3 ± 1.89 ^d	8.95 ± 1.76 ^b	33.2 ± 3.82 ^b

^{a, b, c, d} Means with different superscripts within the same row differ at $P < 0.05$.

Table 4. Effect of lycopene on SOD activity and MDA content in *Drosophila melanogaster*.

Group		15d		30d	
		SOD (U/g)	MDA (umol/g)	SOD (U/g)	MDA (umol/g)
Control		582.5 ± 32.49 ^a	158.6 ± 4.45 ^a	647.3 ± 30.83 ^a	184.2 ± 6.31 ^a
PL (ppm)	7.5	795.9 ± 53.36 ^b	85.1 ± 5.35 ^b	1034.3 ± 73.28 ^b	94.4 ± 6.23 ^b
	2.5	782.8 ± 57.06 ^{bc}	96.1 ± 5.06 ^c	996.5 ± 54.46 ^{bc}	108.9 ± 6.50 ^c
FL (ppm)	7.5	955.1 ± 34.52 ^d	61.1 ± 5.63 ^d	1182.7 ± 74.94 ^d	68.9 ± 3.56 ^d
	22.5	1230.7 ± 46.71 ^e	29.6 ± 2.64 ^e	1424.9 ± 45.11 ^e	33.9 ± 2.48 ^e

^{a, b, c, d} Means with different superscripts within the same row differ at $P < 0.05$.

the formation of new free radicals (Droge, 2002). SOD is a group of metalloenzymes that plays a crucial antioxidant role and constitutes the primary defense against the toxic effects of superoxide radical in aerobic organisms (Kohen and Nyska, 2002). In this research, we found that SOD activities were significantly increased after lycopene was administered and this effect was associated with the decrease of MDA level (Table 4). MDA was reported as the major marker of endogenous lipid peroxidation. Thus, the increased level of SOD and the decreased level of MDA showed that lycopene had the ability to reduce the propagation of free radical reactions, limit the formation of new free radicals, and therefore build a defense to protect the organism from the oxidative stress and slow the aging process.

Conclusion

In the current study, we found that the lycopene mycelium powder fermented from *Blakeslea trispora* have the ability to prolong the longevity of *Drosophila melanogaster*, enhanced its reproductivity and sexual capacity, had the ability to increase SOD level and decrease MDA level, thus we believed that lycopene can reduce the propagation of free radical reactions and limit the formation of new free radicals, therefore build a defense to protect the organism from the oxidative stress and slow the aging process.

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