**THESIS INFORMATION**

Thesis title: Study on creating mutant strains enhancing astaxanthin production from yeast *Rhodosporidium toruloides*

Speciality: Biochemistry

Code: 62420116

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**1. SUMMARY**:

Astaxanthin is an economically valuable carotenoid that has been approved by the US Food and Drug Administration (FDA) for use as a food coloring agent. In this study, the draft genome of yeast strain *Rhodosporidium toruloides* VN1 was published on DDBJ/EMBL/GeneBank with access code SJTE00000000. 4 mutant strains were created from wild type with 4 agents respectively (UV, gamma, benomyl and EMS) for higher astaxanthin production than the parent strain. Among them, the strain with the highest astaxanthin production was G17 (1990.26 µg/L), 5.4 times higher than the wild-type strain. Analysis of the differences in the gene sequences responsible for carotenoid biosynthetic pathway including *car*1 (*crt*I) and *car*2 (*crt*YB) genes between *R. toruloides* VN1 and *R. toruloides* G17 strains showed that deletions occurred at the start of both genes, analysis of the genomic data showed that between the parent strain and G17 there were gamma-ray-induced SNP and InDel sites. The RSM method with experimental design according to CCD model was used to optimize the three main components of medium including carbon source, nitrogen source and mineral source. The culture medium components were glucose 19.92 g/L, peptone 19.75 g/L and malt extract 13.56 g/L with the optimum result being 3012.68 µg/L. For medium contain molasses, the optimal value of four effect factors was obtained: molasses 49.596 g/L, urea 1.0 g/L, MgSO4 4.0909 g/L and seed ratio 9.9795 % with the optimal result of 1213.7 µg/L. column chromatography and HLPC were used to purification astaxanthin and astaxanthin stucture was analysis by NMR. The results showed that astaxanthin was obtained from the mutant yeast strain *R. toruloides* G17 with *all-trans* astaxanthin. In this research, astaxanthin crude extract and purified astaxanthin were evaluated for antioxidant activity by DPPH method, compared with vitamin C and vitamin E. IC50 value of purified astaxanthin was 0.97 ± 0.01 µg /mL, 9.4 times less than that of crude astaxanthin. It was also demonstrated the superior antioxidant activity of astaxanthin compared to the common antioxidant vitamin C (IC50= 41.97 ± 2.01 µg/mL, higher than 43.3). times) and vitamin E (IC50= 85.91 ± 0.91 µg/mL, higher than 88.6 times). In addition, astaxanthin also showed the ability to protect DNA against the effects of free radicals when DNA was preserved to 85.18 ± 5.98% at a concentration of 400 µg/mL. The protection of cell membrane lipids from the effects of oxidizing agents was 74.77 ± 0.88% at a concentration of 100 µg/mL asstaxanthin. Astaxanthin exhibits strong resistance to protein oxidation, being able to reduce carbonyl radical formation by 83.41 ± 2.22% at a concentration of 100 µg/mL. After 72 h of incubation with cells in culture medium, astaxanthin inhibited the growth of cancer cells in all cell lines of lung cancer A549, breast cancer MCF7 and cervical cancer HeLa and not cytotoxic to the normal cell line HK2 (98% of cells survived at a concentration of 100 µg/mL after 72 h of incubation). IC50 values ​​of cancer cell lines A549, MCF7 and HeLa after 72 h of culture were 56.38 ± 4.1 µg/mL, 55.60 ± 2.64 µg/mL and 69.07 ± 2.4 µg/mL, respectively, and were different from the control sample at p < 0.05.

**2. NOVELTY OF THESIS**:

- Genome sequencing of yeast strain *Rhodosporidium toruloides* VN1 isolated and identified in Vietnam for the ability to produce astaxanthin.

- 4 mutant strains were created from the wild yeast strain with 4 agents respectively (UV, gamma, benomyl and EMS) UV6, G17, B18 and E4 for higher astaxanthin production than parental strain.

- Enzymes in the astaxanthin biosynthetic pathway including phytoene synthase/lycopene cyclase and phytoene desaturase are affected by the oxidizing agent gamma-ray Coban60.

- Astaxanthin from *Rhodosporidium toruloides* G17 has an *all-trans* astaxanthin structure.

- Response surface methodology (RSM), CCD model is suitable for investigating the effect of media components on astaxanthin biosynthesis.

- Astaxanthin of *Rhodosporidium toruloides* G17 showed high inhibitory ability against cancer cells such as lung cancer A549, breast cancer MCF7 and cervical cancer HeLa.

**3**. **APPLICATIONS/ APPLICABILITY/ PERSPECTIVE**

- Study on the innoculation of *R. toruloides* G17 strain at pilot scale towards industrial fermentation to obtain astaxanthin for application in aquaculture feed or food colorant.

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| **SUPERVISOR**  **Asc.Prof. Ngo Dai Nghiep, PhD.** | **PhD STUDENT**  Tran Thi Tuyet Nhung |

**CERTIFICATION**

**UNIVERSITY OF SCIENCE**

**PRESIDENT**