

Comparative β -Carotene content of *Spirulina* Strains at different days of Incubation

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ABSTRACT

Carotenoids are synthesized *de novo* by all the photosynthetic organisms and by some microorganisms. The β – carotene content was ranged from 171.1 to 231.7 ($\mu\text{g g}^{-1}$ dry weight) in different *Spirulina* strains. The four top ranked strains based on LSD grouping were Sp₄ (231.7), Sp₇ (212.8), Sp₃ (185.9) and Sp₂ (182.90 $\mu\text{g g}^{-1}$ dry weight). There was a gradual increase in β carotene accumulation with the peak observed at 15th day of incubation followed by a slow decline there after upto a period of 25th day of incubation. Strain x days of incubation interaction studies were significant and the top ranked combinations were Sp₄ at 15th day (295.6 $\mu\text{g mL}^{-1}$), 20th day (289.5 $\mu\text{g mL}^{-1}$) and at 25th day of incubation (282.4 $\mu\text{g mL}^{-1}$). High carotenoid production by Sp₄ can be exploited as natural food colouring additive.

Key words: β -carotene, Carotenoids, HPCL, *Spirulina*

Spirulina, a filamentous non-heterocystous *Cyanobacterium* belonging to order Nostocales and family Oscillatoriaceae has been commercially exploited in several countries in health foods and therapeutic preparations because of its valuable constituents, particularly proteins and vitamins. *Spirulina* contains high protein of 60-70 % of its dry weight and carotenoids and xanthophylls 0.55% of the organic weight. Maximum total carotenoid production of cell cultures of *Spirulina platensis* was measured under white light at an irradiance of 432 $\mu\text{Em-2s}^{-1}$, which is the onset of light saturation for this organism. But unfortunately information on β -carotene is limited and scattered in the available literature. The contents of this important metabolite may vary depending upon the growth conditions and environmental factors. In view of this an investigation was carried out in *Spirulina*, to quantify the β -carotene present in different strains during different incubation periods.

MATERIAL AND METHODS

Organism

The different strains of *Spirulina* employed in this investigation were procured from the germ plasm collection of CCUBGA, IARI, New Delhi. The identification of the *Spirulina* cultures was authenticated based upon the keys given by Desikachary (1959). The details regarding sources of these strains and their accession numbers are as under :

Strain No. Cultures and Source (CCC No.)

Sp ₁	<i>Spirulina platensis</i> (west Germany)
Sp ₂	<i>Spirulina platensis</i> (Israel)
Sp ₃	<i>Spirulina platensis</i> (Vietnam)
Sp ₄	<i>Spirulina platensis</i> (CFTRI, Mysore, wild)
Sp ₅	<i>Spirulina maxima</i> (CFTRI, Mysore)
Sp ₆	<i>Spirulina lonar</i> (China)
Sp ₇	<i>Spirulina platensis</i> (Mutant of Sp ₄)

β -carotene content:

β -carotene was estimated using HPLC system (Waters, 501) based upon reversed phase liquid chromatography. Reversed phase liquid chromatography (RPLC) is a form of high performance liquid chromatography (HPCL) where the stationary phase is a non polar compound and the mobile phase is a polar solvent.

Stationary phase : Octadecylsilane (ODS)
Mobile phase : Degassed carbinol and acetonitrile in 9:1 ratio

HPLC system : Isocratic system
High -pressure pump : Waters 501 HPLC Pump Millipore

Carbinol and the β -carotene were quantified by using the method of Braumann and Griemme (1981).

RESULTS AND DISCUSSION

β -carotene content ($\mu\text{g g}^{-1}$ dry weight)

The β carotene content was ranged from 171.1 to 231.7 ($\mu\text{g g}^{-1}$ dry weight) in the different

Table 1. Comparative β - carotene content ($\mu\text{g mL}^{-1}$) of *Spirulina* strains at different days of incubation.

Strains	Days					Mean
	5	10	15	20	25	
Sp ₁	86.5	152.3	211.5	216.5	213.5	176.1
Sp ₂	92.2	164.7	222.1	219.5	215.9	182.9
Sp ₃	89.4	170.3	226.8	222.9	219.6	185.9 ⁱⁱⁱ
Sp ₄	97.4	193.5	295.6 ⁱ	289.5 ⁱⁱ	282.4 ⁱⁱⁱ	231.7 ⁱ
Sp ₅	76.7	144.8	211.7	213.0	209.5	171.1
Sp ₆	77.5	148.5	213.7	211.8	206.4	171.6
Sp ₇	81.9	182.7	270.4	265.8	263.1	212.8 ⁱⁱ
Mean	86.0	165.3	236.1	234.1	230.1	

	SE(m) \pm	CD (P=0.05)
Strains (S)	0.74	1.48
Day (D)	0.63	1.25
Strains x Days	1.67	3.32

* i, ii,iii, indicates top three ranking

Spirulina strains. The four top ranked strains based on LSD grouping were Sp₄ (231.7), Sp₇(212.8),Sp₃ (185.9) and Sp₂ (182.90 $\mu\text{g g}^{-1}$ dry weight) (Table 1).

There was a gradual increase in β carotene accumulation with the peak observed at 15th day of incubation followed by a slow decline there after upto a period of 25th day of incubation. The β carotene content showed a typical sigmoid behaviour in *Spirulina* strains with incubation.

Strain x days of incubation interaction studies were significant and the top ranked combinations were Sp₄ (*Spirulina platensis*) at 15th day (295.6 $\mu\text{g mL}^{-1}$), 20th day (289.5 $\mu\text{g mL}^{-1}$) and at 25th day of incubation (282.4 $\mu\text{g mL}^{-1}$). Strains Sp₅ (*Spirulina maxima*) at 5th day of incubation showed lowest β carotene content (Table 1). Bramley and Mackenzie (1988) reported that carotenoid biosynthesis is a regulated process and various environmental and developmental factors control carotenoid synthesis and accumulation in prokaryote as well as in eukaryotes.

High β -carotene production by Sp₄ can be exploited and can be used as natural food colouring additive to enhance the colour of flesh of fish, egg yolk and may have an importance for the health and fertility of low fed cattle (Jackson *et al.*, 1981).

Commercial mass cultivation of micro algae possessing the ability to accumulate simultaneously large quantities of β -carotene, vitamin E and Vitamin C has not been reported as yet. The present investigation revealed possibility of β carotene

production on a large scale by using the strain Sp₄ (*Spirulina platensis*) with 15 days (295.6 $\mu\text{g mL}^{-1}$) of incubation period.

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