Extraction, Purification and Spectroscopic Characterization of Phycobiliproteins Extracted from some Nostoc Spp.
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ABSTRACT
Phycocyanin and phycoerythrinn are considered the major phycobiliprotein in many cyanobacteria as well as being a secondary phycobiliprotein in some red algae. In this article, the biomass of Nostoc linckia was harvested at the 16th day, which approximately equaled 361 mg/L dry biomass for attaining the maximum content of phycocyanin, and the biomass of Nostoc carneum was collected at the 17th day that approximately equaled 326 mg/L dry biomass for attaining the maximum content of phycoerythrin. Phycocyanin and phycoerythrin were extracted from both Nostoc linckia and Nostoc carneum respectively. They were extracted by successive cycles of freezing and thawing using 50 mM phosphate buffer solution (pH 7) then purified by single step of precipitation in 65 % of ammonium sulphate. Phycocyanin and phycoerythrin achieved 2.29 and 3.02 of purity ratio, respectively. The purified phycocyanin and phycoerythrin exhibited maximum absorbance at 614 and 560 nm respectively.

Keywords: Nostoc spp. growth, phycobiliproteins, phycocyanin, phycoerythrin, extraction, purification ratio, characterization

INTRODUCTION
Cyanobacteria are a division descending from algae. Cyanobacteria are considered the only known oxygen photobacteria prokaryotes. Cyanobacteria are unicellular or multicellular oxygenic photoautotrophs prokaryotes that are found in almost every possible habitat on earth possess chlorophyll (a) and perform oxygenic photosynthesis associated with photosystems I and II (Castenholz and Waterbury, 1989; García-Pichel and Pringault, 2001).

In the last few years, the variety and physiology of cyanobacteria have acquired great interest as a rich source of bioactive compounds that serves as an excellent base for discovering their biotechnological applications. (Bhadury et al. 2004; Abed et al. 2009). Cyanobacterial genera as Microcystis, Anabaena, Nostoc and Oscillatoria provide a wide range of secondary metabolites, consequently they are considered as promising microalgae for production of bioactive natural products (Singh et al., 2017).

Phycobiliproteins are monophyletic family of homologous heterodimeric proteins which consist of a globin-type core that carries the chromophores (the light-capturing part) which are the most important constituents of the phycobilisomes, and an N-terminal extension that is mainly involved in oligomerization (Schmidt et al., 2007). Phycocyanin and allophycocyanin are two pigments-proteins which universally found in all cyanobacteria and red algae studied (Gantt et al., 1979), while phycoerythrin is a variable component and its presence in the phycobilisomes of certain organisms is depending on the light conditions, particularly the quality of light available (Tandeau de Marsac, 1977).

Aim: Extraction, purification and spectroscopic characterization of both phycocyanin and phycoerythrin from Nostoc linckia and Nostoc carrinea respectively.

MATERIALS AND METHODS
Isolation, purification and identification of cyanobacterial isolates
Nostoc carreum and Nostoc linckia were isolated from garden soil samples in Dakahlia, Egypt. Culture purification was according to Hoshaw & Rosowski (1973). Identification of Nostoc carreum and Nostoc linckia were approved with the standard ones according to Bornet & Flahaut (1886); Desikachary (1959). The two cyanobacteria were grown in axenic culture at 28± 2°C for 21 days incubation period under continuous illumination 3200 lux in 500 ml conical flasks containing 200 ml BG-11 medium (Stainer et al., 1971) & (Rippka et al., 1979), adjusting pH at 7.

Determination of growth (dry biomass)
From each cyanobacterium, biomass was harvested at 3 days intervals through incubation period (21 days) by self-sedimentation then filtered by a glass fiber filter paper. The biomass was washed once with dist. water and filtered again. This collected biomass was dried in an electric oven at about 60°C.

Extraction of phycobiliproteins
From each cyanobacterium, biomass was harvested by self-sedimentation then filtered by a glass fiber filter paper. The biomass was washed once with dist. water and filtered again. This collected biomass was referred to as the wet biomass which was kept in freezing at -20°C for extracting biliproteins. After the incubation at 28± 2°C for 21 days under continuous illumination 3200 lux, the fresh biomass was collected at the beginning of the stationary phase. Phycocyanin (PC) and phycoerythrin (PE) are pigment-protein complexes from the light-harvesting phycobiliprotein of cyanobacteria, which will be used in the biosynthesis of silver nanoparticles (AgNPs). They were extracted at the beginning of the stationary phase by taking the fresh biomass of 1 litre culture which corresponding to 361 mg/L dry biomass of Nostoc carreum and corresponding to 326 mg/L dry biomass of Nostoc linckia. Biliproteins containing cultures were harvested by centrifugation at 4000 rpm for 10 min. then the biomass pellets washed twice with distilled water. The washed biomass for each cyanobacterium added to 25 ml of 50 mM phosphate buffer (pH 7), then subjected to freezing (- 20°C) and thawing at room temperature. PC was extracted by 5 cycles of repeated freezing and thawing, however PE needed 6 cycles for complete diffusion of pigments. The biomass residue was discarded by centrifugation at 4000 rpm for 10 min. and supernatant containing biliproteins extract was collected and termed as crude extract.

Purification of phycobiliproteins
The crude extract was further purified by single step of precipitation using 65% (NH4)2SO4 after the method of
Chakdar and Pabbi (2012), where it was mixed thoroughly with the biomass extract and kept 12 h at 4°C. Biomass pellets were recovered by centrifugation (HERMEL Z32 HK) at 4000 rpm for 30 min. at 4°C and dissolved in 10 mL of (50 mM) phosphate buffer. The purity index of homogenate was checked by detecting the absorption spectra.

**Determination of phycobiliproteins**

PE and PC concentration and the purity ratio were estimated by (Bennett and Bogorad 1973) equations:

**RESULTS**

**Growth curve of Nostoc linckia**

The results showed that *Nostoc linckia* growth in BG%11 medium (photo 1) increased progressively with a lag phase of 3 days (Table 1) followed by the exponential phase till reached the stationary phase on 24th day.

**Table 1. Growth curve of Nostoc linckia**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Days</th>
<th>Dry weight (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>3</td>
<td>46 ± 8</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>91.66 ± 13</td>
</tr>
<tr>
<td></td>
<td>9</td>
<td>142 ± 13</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>227 ± 15</td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>325.8 ± 12</td>
</tr>
<tr>
<td></td>
<td>18</td>
<td>401 ± 15</td>
</tr>
<tr>
<td></td>
<td>21</td>
<td>425 ± 8</td>
</tr>
<tr>
<td></td>
<td>24</td>
<td>404 ± 9</td>
</tr>
</tbody>
</table>

**Growth curve of Nostoc carneum**

The results showed that *Nostoc carneum* growth in BG-11 medium increased steadily with a lag phase of 6 days (Table 2) followed by the logarithmic phase till attained the stationary phase on 24th day.

**Table 2. Growth curve of Nostoc carneum**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Days</th>
<th>Dry weight (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>3</td>
<td>56 ± 9</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>83 ± 11</td>
</tr>
<tr>
<td></td>
<td>9</td>
<td>122 ± 11</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>154 ± 12</td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>226 ± 9.5</td>
</tr>
<tr>
<td></td>
<td>18</td>
<td>349 ± 14</td>
</tr>
<tr>
<td></td>
<td>21</td>
<td>398 ± 6.9</td>
</tr>
<tr>
<td></td>
<td>24</td>
<td>404 ± 10</td>
</tr>
</tbody>
</table>

**DISCUSSION**

In cyanobacteria, light is harvested by phycobiliprotein. The synthesis of phycobiliprotein depends on the provided environmental nitrogen and these phycobiliproteins may act as a nitrogen store (Tandeau de Marsac and Houmard, 1993). Therefore, cyanobacteria need a nitrogen source for growth that presented in ammonium, nitrate and nitrite (Guerrero and Lara, 1987). In this study, the biomass of *Nostoc linckia* was harvested at the 16th day, which approximately equalled 361 mg/L dry biomass for attaining the maximum content of phycocyanin, and the biomass of *Nostoc carneum* was collected at the 17th day that approximately equalled 326 mg/L dry biomass for attaining the maximum content of phycoerythrin. Hussein et al. (2000) studied different species of cyanobacteria: *Calothrix marchica*, *Cylindrospermum muscicola* var. *longispora*, *Anabaena fertilissima*, *Tolipothrix bouteillei* and *Nostoc muscorum* and recorded their maximum content of total biliprotein, phycocyanin, allophycocyanin, phycoerythrin and total pigment. They documented that the phycobiliprotein is considered 50 % of the total protein at which C%phycocyanin level reaches to 17 % of the dry weight and allophycocyanin reaches to 11 % of dry weight. Phycocyanin in some *Anabaena* and *Nostoc* spp. is the main pigment while in other *Nostoc* spp. can only reach to 10 % of dry weight according to Moreno et al. (1995). Phycocyanin of various cyanobacteria reached their highest values at the 10th day of incubation of A. fertilissima and N. muscorum, 14th day for C. marchica and T. bouteillei while Cyl. muscicola var. *longispora* needed 16th day (Hussein, et al. 2000).

**Extraction, Purification, and Spectral analysis of the phycobiliproteins**

Freeze thaw method has been selected for extraction of phycobiliproteins, because it has many
advantages over other methods, which are not reductive, low yielding, and probably could destroy the characteristic fluorescence properties of the protein (Sonani et al., 2006). The extraction of the phycobiliproteins have been achieved with 0.05 mM of phosphate buffer at pH 7 at which phycobiliprotein are most stable near pH 7 according to Mishra et al. (2010). In this study, following 65% ammonium sulphate precipitation, we have achieved 2.29 purity ratio (Table 3) for PC which is greater than that reported by Kumar et al. (2014) and 3.02 purity ratio (Table 4) for PE which is greater than that reported by Chakdar and Pabbi (2012).

Table 3. Estimation of spectroscopic purity and concentration of PC

<table>
<thead>
<tr>
<th>PC crude extract</th>
<th>65 % ammonium sulphate precipitation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Volume (mL)</td>
<td>OD_{280}</td>
</tr>
<tr>
<td>30</td>
<td>0.805</td>
</tr>
<tr>
<td>10</td>
<td></td>
</tr>
</tbody>
</table>

Table 4. Estimation of spectroscopic purity and concentration of PE

<table>
<thead>
<tr>
<th>PE crude extract</th>
<th>65 % ammonium sulphate precipitation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Volume (mL)</td>
<td>OD_{280}</td>
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<tr>
<td>30</td>
<td>0.799</td>
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<tr>
<td>10</td>
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The photosynthesis in cyanobacteria can dominate wide region ranged from 450 to 650 nm of solar spectrum due to the presence of colored protein-based pigments called phycobiliproteins, which are the family of the colored water soluble photosynthetic proteinaceous pigments (Sonani et al., 2016). Phycobiliproteins are classifying according to their spectral properties. In this research, PC extracted from Nostoc linckia has $\lambda_{\text{max}} = 614$ nm as illustrated in Figure 1 and PE extracted from Nostoc caremum has $\lambda_{\text{max}} = 560$ nm as illustrated in Figure 2. The characteristic maximum absorbance wavelength of (PC) $\lambda_{\text{max}} = 610-620$ nm, allophycocyanin (APC) $\lambda_{\text{max}} = 650-655$ nm and (PE) $\lambda_{\text{max}} = 540-570$ nm which are the majorly found phycobiliproteins (Singh et al., 2015).

REFERENCES


Mervat H. Hussein et al.


استخلاص وتنقية والتوصيف الطيفي للفيوكوبيلينوتين المستخلص من بعض أنواع جنس نوستوك
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قسم النباتات - كلية العلوم - جامعة المنصورة - مصر
قسم تطوير صناعات التكنولوجيا الحيوية - معهد بحوث الهندسة الوراثية والتكنولوجيا الحيوية - مدينة الأبحاث العلمية والتطبيقات
التكنيكولوجية - جمهورية الجزائر - الأسكندرية - مصر.

يتناول هذا البحث عزل نوعين من النسيجيات Nostoc carneum و Nostoc linckia، والفيوكوبيلينوتين من ألسمة جنوب صين وجنوب كوريا، حيث تم استخلاص صبغ فين من N. linckia في اليوم 16 من بداية نمو النبات (361 mg/L) من الوزن الجاف، والذين يعادل (326 mg/L) من الوزن الجاف في اليوم 17 من N. carneum. وأيضا تم استخلاص صبغ الفيوكوبيلينوتين من طحالب N. linckia في اليوم 17 عن طريق حفظ وزن الحالة وكمية (0.132 mg/mL). وحصلت على نسبة بنسبة تسمح بعدة أيام من منتجة بنسبة 141.29% على التوالي.