
**Identification of native cyanobacteria from Iran and Investigation of phycocyanin production by spectrophotometer**

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**Introduction**:

Cyanobacteria have diverse morphologies and spread at difference habitats of terrestrial and aquatic environments [1]. Cyanobacteria identified based on morphological, ecological and molecular criteria [2], morphological characters are so important and molecular analysis help us under sequencing of 16S rRNA gene [3]. The phycobiliproteins are pigments exist in cyanobacteria, red algae and cryptomonads. They are located at photosystem II light-harvesting apparatus [4]. On the basis of their visible absorption properties, the phycobiliproteins have been categorized to four spectroscopic classes: phycoerythrocyanin (PEC, $\lambda_{Amax}$ = 575 nm), phycoerythrins (PE, $\lambda_{Amax}$ = 565- 575 nm), phycocyanins (PC, $\lambda_{Amax}$ = 640 nm) and allophycocyanin (APC, $\lambda_{Amax}$ = 650- 655 nm) [5]. Some characteristics of phycobiliproteins make them well-suited for fluorescence analyses as fluorescent tags with numerous applications in flow cytometry, histochemistry, immunoassay and detection of reactive oxygen species. They can also be used as natural dyes and anti-tumoral agents [4].

**Materials and Methods**:

First, strains were isolated from different samples in BG11 medium, under 1-2 Klux, 12/12 light/hour condition, and then morphological characters were studied with light microscopy and key reference book [1]. DNA extracted with modified method based on SDS shock and freeze-thaw [6]. 1000 base-pair fragment of 16S rRNA gene amplified with especially primers for cyanobacteria (Cya359f and PcR) [7]. Strains were described and named truly and phylogenetic tree was constructed by Mega7 with neighbor joining method and 1000 bootstraping. Strains were cultured on liquid BG11, in logarithmic phase biomasses collected and dried at 40℃. Dry biomass was dissolved in phosphate buffer, cells breaked with sonication in ice. Then, existence and amount of phycocyanin were gained with optical density based on Bernnett & Bogorad equation [8].

$$\text{C-PC} = \frac{E_{615} - 0.474\,(E_{652})}{5.34} \quad \text{mg}\cdot\text{l}^{-1}$$

$$\text{APC} = \frac{E_{652} - 0.208\,(E_{615})}{5.09} \quad \text{mg}\cdot\text{l}^{-1}$$

$$\text{C-PE} = \frac{E_{562} - 2.41\,(\text{PC}) - 0.849\,(\text{APC})}{9.62} \quad \text{mg}\cdot\text{l}^{-1}$$

**Results**:

Strains which identified with polyphasic approach were belonged to different clades including single cell cyanobacteria (*Synechosystis saline* strain nish-2: IBRC-M 50009), heterocytous cyanobacteria (*Nosoc* sp. strain can-15: IBRC-M 5064) and filamentous cyanobacteria (*Spirulina*


*subsalsa* strain Nish-1: IBRC-M 50004, *Arthrospira bakrishnanii* strain Pol-1: IBRC-M 5183, *Oscillatoria neglecta* strain srt-2: IBRC-M 5165) (Fig.1, Fig.2, Tab.1). Strain Pol-1 strain Nish-2 produced higher phycocyanin comparison to other strains (Tab.1)

Table 1: Strain information include: nearest taxa sample site and phycocyanin production.

| Strain name | Nearest approved taxon (% similarity) | | Sample Source | Phycocyanin (µg/ml) |
|---|---|---|---|---|
| **Pol-1** | *Arthrospira balkrishnanii*[#] | <95 | Oroomie Lake, polluted site | 16 |
| **Nish-1** | *Sprulina subsalsa* | 100 | Spring in Neishaboor | 11 |
| **Nish-2** | *Synechocystis salina* | 98 | Spring in Neishaboor | 15 |
| **Can-15** | *Nostoc* sp. | 99 | Kanni berazan wetland | 9 |
| **Srt-2** | *Oscillatoria* sp. | 99 | Badab-e surt spring | 6 |

[#] **This strain show low similarity to defined taxa and approved with morphological characters.**

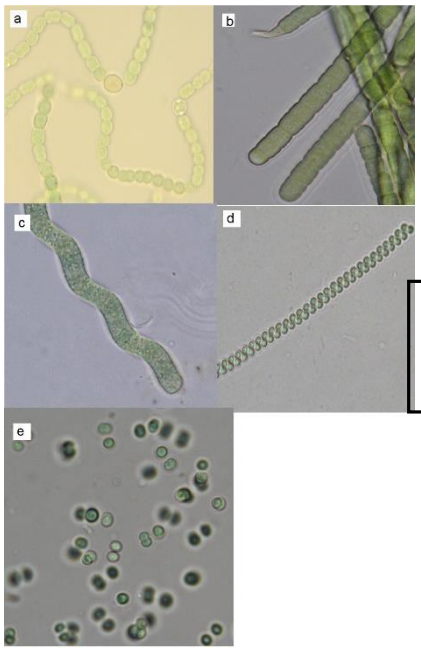


**Figure 1: Light microscopy of the strain (Scale 10µm )**

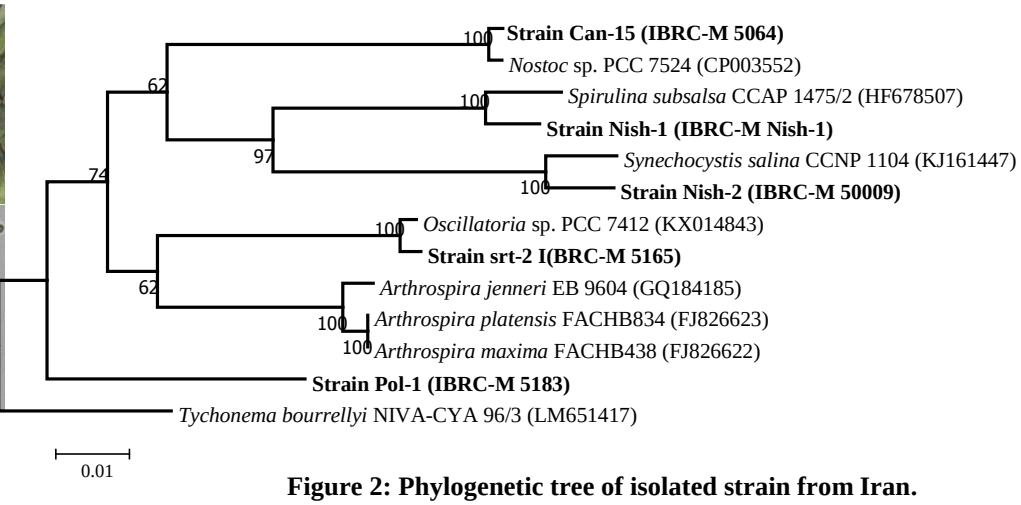


**Figure 2: Phylogenetic tree of isolated strain from Iran.**

**Discussion:**


Cyanobacteria are the main source of phycocyanin and can be used in biotechnological studies. In this study showed cyanobacteria existed in Iran with diverse morphology and based on site, sampling time and type of samples many of the biotechnological strains could be isolated. Between our strain, strain Pol-1 and Nish-2 produced high phycocyanin, *Arthrospira* have large width and *Synechocystis* have high growth rate, so these strains could produce higher phycocyanin than others. Now, *Spirulina platensis* (*Arthrospira fusiformis*) and *Synechocystis* sp. PCC 6803 used as the main source in industry and studies for phycocyanin production. Native strain doesn't have high productivity, we should optimize medium culture and growth rate to gain higher productivity.

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