Anbar Sciences Journal (ASJ)

P-ISSN: 3105-4498 , E-ISSN: 3105-448X

Research Article
Open Access

CLONING AND EXPRESSION OFBSUBUNIT GENE OF PHYCOCYANINFROM SPIRULINALAXA IN E. COLI BL21(DE3)

Noor K. Shaker [1]and Harith K. Buniya*[2]

[1]College of Education for pure Sciences, University of Anbar, Ramadi, Iraq.
[2] College of Education for pure Sciences, University of Anbar, Ramadi, Iraq.
***Corresponding author:** Dr. Harith K. Buniya, College of Education for pure Sciences, University of Anbar, Ramadi, Iraq. Email: hkbuniya@uoanbar.edu.iq

Article Info:

Received: Oct.19, 2024
Revised: Nov.08, 2024
Accepted: Jun. 05, 2025
Published: Feb. 15, 2025

DOI:
10.63298/asj.2025.184949

How to Cite:
Shaker, N., &Buniya, H. (2024). Cloning And Expression Ofβsubunit Gene of Phycocyaninfrom Spirulinalaxa in E. Coli Bl21(De3). Anbar Sciences Journal, 1(1), 16-21.

Available From:https://asj.uoanbar.edu.iq

Abstract: The blue-green algae pigment C-phycocyanin (C-PC) has many pharmacological properties, such as antibacterial and antitumor activities. In this study, we expressed the gene β-subunit of Spirulina laxa (1119 bp) in E. coli BL21(DE3). Use specific primers to amplify the β-subunit gene of Spirulina, digest it with EcoRI and BamHI and connect it to the pGEM®-3Zf(+) linear cloning vector, and introduce the recombinant DNA (cpcB-pGEM®-3Zf) into the expression host BL21 (DE3). Then induced with 1 mM IPTG. Gene expression was analyzed by 12% SDS-PAGE. Recombinant CPC/β has a molecular weight of approximately 37 kDa. The recombinant protein has high antifungal properties. In an antimicrobial test, Candida albicans and Aspergillus niger were evaluated for resistance to the antifungal effects of CPC/β.

Keywords: Spirulina, antifungal ,Recombinant Protein, Cyanophyta.

1. Introduction

Spirulina is a multicellular cyanobacteria that has a spiral shape. It contains minerals, vitamins, protein and unsaturated fatty acids. This algae is used as a dietary supplement for both humans and animals [1]. *Spirulina* is a cyanobacteria that contains many important nutrients and benefits. It is often used as a dietary supplement because it is rich in protein[60 to 70%] and other nutrients. It has high economic value as well as nutritional potential [2].

Phycobiliproteins (PBPs) are a class of light-harvesting proteins that account for 50% of the total protein in Spirulina. In *Spirulina*, C-phycocyanin (C-PC), a major light-harvesting biliary protein, is synthesized in large quantities in Spirulina cells, up to 20% of the total protein [3]. Due to its numerous biological and pharmacological characteristics, phycocyanin (C-PC), one of the principal light-harvesting biliproteins of cyanobacteria, are particularly important [4[. C-PC can be found in cosmetics and food as well as being an indicator of nutrients, coloring agent and natural preservative. It has a wide range of benefits including antioxidant, anti-arthritic, anti-inflammatory, anti-cancer, immune-boosting and hepato protective properties [5].

C-Phycocyanin is a blue luminous phycobiliprotein with a molecular weight between 20,000 and 23, 000 Dalton [6]. The originality and novelty of the fluorescent phycobiliprotein molecule were demonstrated by the C-phycocyanin extract's broad spectrum antibacterial efficacy, which was derived from a *Spirulina* spp. [7]. Therefore, the purpose of this study was to produce CPC/β protein in Escherichia coli as an expression host and evaluate its antifungal activity against pathogenic fungi.

2. Materials and Methods

Spirulina Isolation and Growth

Spirulina samples were taken from a hot spring in the Kubaisa district of Anbar province. Algae were grown in BG11 medium at 30 °C under sterile conditions with manual agitation for 5 min twice a day to prevent cushion formation. After 16 days, biomass was harvested and identified by morphological microscopy [8].

Genomic DNA extraction

Genomic DNA was extracted from algae samples according to the Geneaid Presto Mini gDNA Bacteria Kit (Geneaid Kit Taiwan).

Amplification of the *cpcB* gene

The designed primer gene *cpcB*size is 1119 bp: forward primer 5'-GGGCGAATTCGGAGATAAGTCCATGTTTG - 3' and reverse primer: 5'-CGCGGATCCCATGCTTAGGGCGTTGATCGC - 3' [9]. Both *Eco*RI and *Bam*HI restriction sites are found in the forward and reverse primers, respectively. Forward and reverse primers also contain restriction sites . They can be found in the Amplicycle reagent used by thermocycler (Biosystems, Singapore). Additionally, thechain reaction PCR contain 1.5 mM magnesium chloride, 2.5 μl 10x PCR buffer and 1 U of Taq DNA polymerase . These were created by Bioneer, Korea. PCR reactions required 35 cycles of denaturing at 94°C for 2 minutes. Next, they annealed at 58°C for 30 seconds before extending the reaction for 1 more minutes at 72°C. Next, these product were analyzed on a 1% agarose gel with 0.5 μg ethidium bromide per gram of DNA solution [10].

Construction of expression vector

According to the manufacturer's instructions, *Eco*RI and *Bam*HI restriction enzymes were used to digest pGEM®-3Zf(+) (Promega, USA)with ampicillin resistance. After being purified from the gel using a Gel Extraction Kit (BIONEER), the products were ligated with*cpcB* gene by T4 DNA Ligase (Promega, USA) [10]. The ligation mixture transformed into *E. coli* BL21(DE3) (a strain from NEB, USA) through CaCl2. This was accomplished by the addition of 100 μg/ml ampicillin-laced LB medium to plates with colonies formed from ligation experiments.

3. Expression of recombinant protein

The *Escherichia coli* BL21 (DE3) were shaken at 37°C and 250 rpm. After creating an induction media, 1 mM Isopropyl-β-D-thiogalactopyranoside (IPTG) was added to the culture. SDS-PAGE was performed using the Laemmli protocol [11] after protein bands were visualized by Coomassie blue R250 staining.

Biological activity

The microorganisms used in this study were the pathogenic fungi *Candida albicans* and *Aspergillus niger*, provided by the Research Laboratory of the Faculty of Pure Science Education, Anbar University. Antifungal tests were performed according to [12] Agar well diffusion assays are used to assess the sensitivity of test microorganisms to CPC/β. In this assay, fungal cultures were maintained at a pH of 6.8 on potato dextrose agar (PDA) medium. The spread plate method is used to create plates with agar on selected pathogens. Make 5 mm holes in the agar matrix using a cork borer and (50 μl) CPC/μl is added to each well. Thereafter, the plate was incubated at 37°C for 24 hours, the antifungal activity of CPC/β was recorded as the zone of inhibition, and the diameter was measured using a transparent ruler. Treatment using uninduced cells as a negative control.

4. Results and Discussion

Genomic DNA Extraction and amplified

Genomic DNA was extracted (figure1). A gene-specific primer containing *Eco*RI and *Bam*HI restriction sites was used in the amplify (figure2) .

The digested PCR product then ligated with digestedpGEM®-3Zf (+) vector (digested by thsame restriction enzymes)

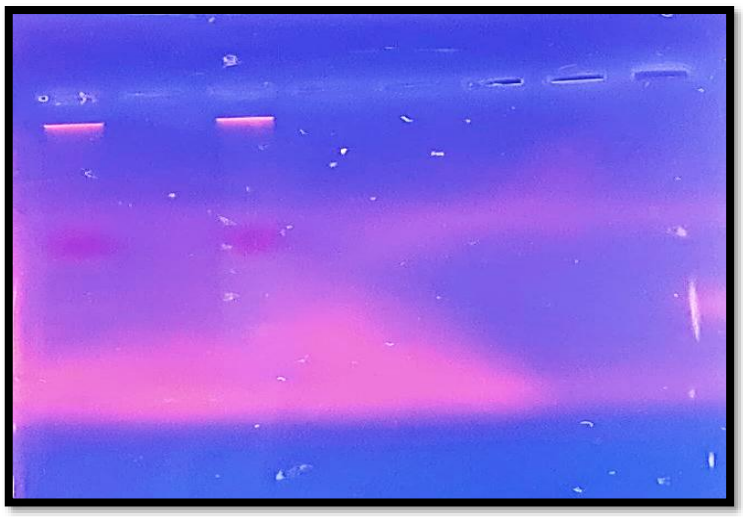


Figure 1: Agarose Gel Electrophoresis of Extracted Genomic DNA (1%, 90 v/cm for 45 min).

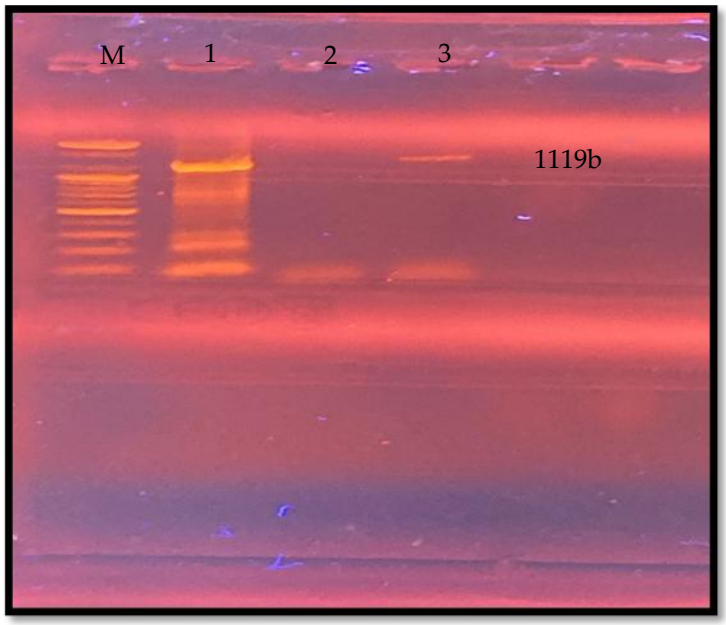


Figure 2: Agarose gel electrophoresis (1%, 90 V/cm) of cpcB gene PCR products. M: 100 bp DNA ladder. Lane 1, 3PCR products, lane 2 negative control.

SDS PAGE analysis of *cpcB*gene

The ligation product was then transformed into *E. coli* BL21(DE3). Plating 100 µl of cells transformed with *E. coli* BL21 (DE3) resulted in an overpopulated bacterial colony (Figure 3). Induce expression in transformed cells with IPTG for 3 hours at 37°C. On 12% SDS-PAGE, the expression profile of CPC/β (37 kDa) was shown to be overexpressed in *E. coli* BL21 (DE3), as shown in Figure (4).

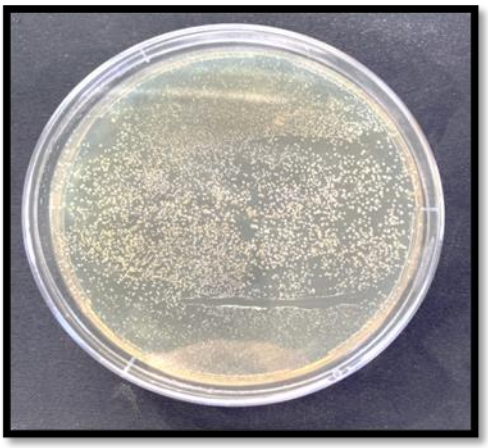


Figure 3 : growth of recombinant E. coli BL21(DE3) on LB agar

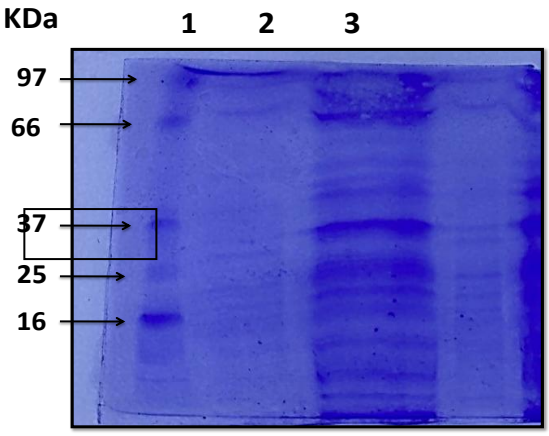


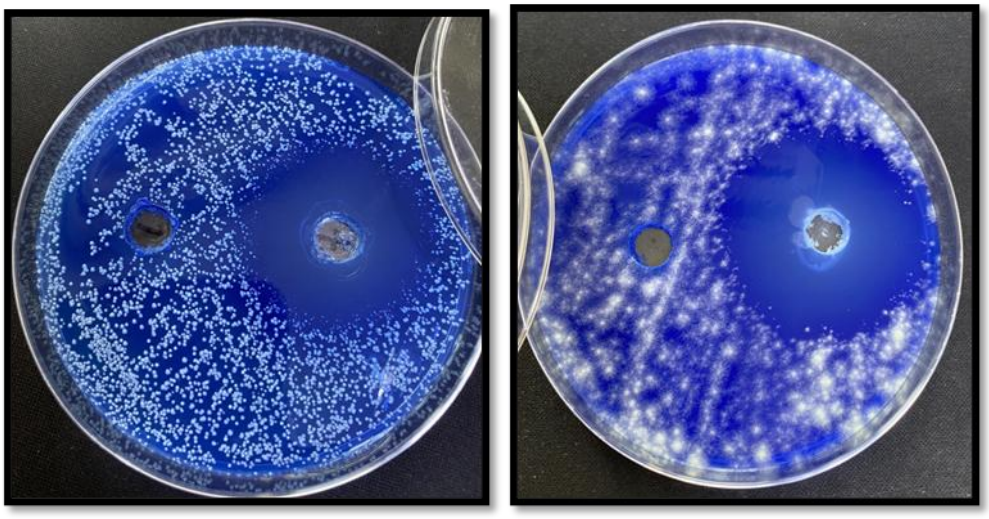
Figure 4: Investigation of CPC/β protein abundance in E. coli BL21 (DE3). Cell lysate samples

The samples were analyzed using 12% SDS-PAGE and stained with Coomassie Brilliant Blue G-250. Lane 1 contains the low molecular weight protein marker, lane 2 represents the uninduced culture, and lane 3 corresponds to the induced culture.Antifungal activityof the CPC/β

The antifungal activity of phycocyanin against two fungal pathogen strains, *Candida albicans* and *Aspergillus niger*, was tested on potato dextrose agar (PDA) medium. By measuring the area of the zone of inhibition. The growth of all the fungi is inhibited by the *Spirulina* phycocyanin.*Candida albicans* had the highest zone of inhibition (45 mm clear zone) (Table 1 and Figure 5), while *Aspergillusniger* had an inhibition diameter of 43 mm.

Table 1 . Agar well diffusion assay of the antifungal effect of *Spirulina* phycocyanin against ; *Candida albicans, Aspergillus niger*. Inhibition zone diameter (mm)

Strain	Control	*Spirulina* phycocyanin
Candida albicans	0	45
Aspergillus niger	0	43



Candida albicans *Aspergillus niger*

Figure 5. Inhibition zone of Candida albicans, Aspergillusniger induced by Spirulina phycocyanin and uninduced cells of Spirulina phycocyanin as a negative control.

The main purpose of this study is to clone the phycocyanin β subunit gene of *Spirulina laxa* into the expression vector of *Escherichia coli* BL21(DE3). In addition, SDS-PAGE was used to detect the expression of C-PC recombinant β subunit and evaluate its utility in inhibiting the growth of pathogenic fungi. *Spirulina* has been used by humans for a long time. Native isolates of *Spirulina* are the most important photosynthetic microorganisms due to their nutritional value [13], The examination of a few components in *Spirulinalaxa* revealed that the biomass contains (59.2%) protein, (14.8%) carbohydrate, and (410.6 mg/100 mg) chlorophyll a . Phenols and flavonoids together accounted for 94.5 mg and 56.9 mg, respectively. According to the findings, *S. laxa*has a lot of antioxidants [14].

The antibacterial effects of *Spirulina* phycocyanin have not been extensively studied [15]. Abedin and Taha (2008) [16] Results of a study on the antifungal activity of *Spirulina* extracts against the fungal strain *Candida sp* and *Aspergillus sp* Similar to this study. Souza et al., [17] reported the inhibitory effect of *Spirulina* extract on *Aspergillus flavus*. These results are consistent with the current study. Phycocyanin can target fungal biomass content of glucosamine C6H13NO5. This compound is involved in the formation of fungal cell walls [18].

5. Acknowledgments

The authors thank the Department of Biology, College of Education for Pure Science, University of Anbar, for providing the laboratory equipment needed to carry out this study.

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