NESTED-PCR ISOLATION OF *BAR* GENE FROM A RESISTANT-BASTA HERBICIDE *STREPTOMYCES COELICOLOR* EGY

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ABSTRACT

Resistance of *Streptomyces* isolate against Basta (Bialophos) herbicide was tested on three doses of Basta (2, 4 and 6g/L) in starch nitrate agar medium (SNAM). The stressed cultures were studied to find gene confers resistance for herbicide. The *bar* gene was isolated *via* polymerase chain reaction (PCR) from the culture. This local isolate was identified as *Streptomyces coelicolor* Egy. Almost 450pb amplified DNA fragment was cloned at ELORI and Hind III sites in plasmid pAHC25 with *gus* genes. The construct was transformed into *coli* strain DH5α.

INTRODUCTION

Members of genus *Streptomyces* (Actinobacteria:ctinomycetales) produce hundreds of antibiotics, one of which is bialaphos. It consists of a glutamic acid analogue moiety, called phosphino-thricin [PTC or glufosinate = 2-amino-4-(hydroxymethylphosphinyl) butanoate] and two alanine residues.

Some microorganisms can detoxify glufosinate by producing an enzyme that causes acetylation of the amino group. Genes encoding the acetylating enzyme were isolated from *S. hygroscopicus* (Thompson *et al*.,1987) and from *S. viridochromogenes* (Wohlleben *et al.*, 1988).

A *bar* (bialaphos resistance) gene encodes a phosphinothricin acetyl transferase (PAT) enzyme. Importance of phosphinothricin (PPT) herbicide-resistance (*bar*) gene lies on its use as a selection marker gene for producing transgenic plants (Park and Smith, 1995; Li *et al*., 2003).

El-Domyati and Mohamed (2004) has reported the presence of *bar* gene in the genome of a set of eight *Strepto-myces* isolates varies in their ability to grow on selective medium supplemented with different concentration (2, 4 and 6g/L) of basta (herbicide). This is a preliminary step towards the isolation of bar gene for herbicide resistance.

Polymerase chain reaction (PCR) has made it possible to detect and amplify a specific locus genome (Olsen, 1992). The main objective of this work was detection and isolation of *bar* gene from a Basta (herbicide) resistant *Streptomyces* isolate by using the nested-PCR technique.

MATERIALS AND METHODS

Source of bacterium isolate: The Streptomycete isolate under investigation was kindly provided by Dept. Agricultural Microbiol., Institute of Soil, Water and Environment Research

(ISWER), Agricultural Research Center (ARC), Giza, Egypt.

Selected herbicide: The Basta (glufosinate) herbicide was kindly provided by AGERI, ARC, Giza.

Herbicide tolerance of streptomycetes: The selected *Streptomyces* isolate was tested for its ability to grow in the presence of Basta herbicide in starch nitrate agar medium (SNAM) as reported by Waksman and Lechevalier, (1961). Three concentrations of Basta were maintained (i.e., 2, 4 and 6 g/L) and inoculated plates were incubated at 28°C±2 for 15 days. Similarly, SNAM culture without herbicide was used as control.The growth rate of *Streptomyces* isolate among the herbicide stressed cultue was determined and recorded as no growth (-), weak (+), moderate (++) and abundant (+++) growth.

DNA isolation: DNA was extracted from the *Streptomyces* strain and was quantified according to Mohamed *et al.,* (2001).

Analysis PCR: PCR was carried out according to the procedure given by El-Domyati and Mohamed (2004) on a volume of 25µl. Two oligonucleotide primers (bar-3: 5' TAC ATC GAG ACA AGC ACG GTC ACC T 3' and bar-4: 5'ACG TCA TGC CAG TTC CCG TG 3') were used for PCR isolation of bar gene of the DNA of the selected isolate.

Gel electrophoresis analysis: The electrophoresis of PCR product was carried out as described by Sealey and Southern (1982) (using a Pharmacia GN-100 submarine gel electrophoresis apparatus). The gel (1.2%) were electrophoresed at 65 V for 2.5 h with TBE buffer (Sambrook *et al*., 1989) and then stained with ethidium bromide solution (10mg/ml) for 10-15min Photo-graphs were taken under UV light using a Polaroid camera.

Cloning of full-length *bar* gene: The amplified *bar* gene from *Streptomyces* strain was cloned into the plasmid pAHC25 (Kindly provided by Dr. Ahmed Bahieldin, AGERI, ARC, Giza, Egypt). This was followed to transform into *Escherichia coli* strain DH5α (Hanahan, 1983) by using PCR-Script (Amp Cloning Kit, Stratagene®, cat. No. 211190).

Minipreparation and digestion: The plasmid DNA was prepared as described by Sambrook *et al*. (1989) and subjected to digestion with *Eco*R1/*Hind*III as mentioned by El-Domyati and Mohamed (2004) for the confirmation of the presence of insert.

RESULTS AND DISCUSSION

The *bar* gene, isolated from different *Streptomyces* species,encode a *Phosphinothricin acetyltransferase* (PAT) and is widely applied in plant genetic engineering. The gene was expressed in *E. coli* and the corresponding protein was purified and used for functional and structural studies (Wehrmann *et al*., 1996). Treatment of genetically modified plants carrying bar gene with bialaphos provides a very efficient means of selection in genetic transformation protocols. Although this use of the bar gene was popular in many laboratories.

In this study,a gene confers resistance to the herbicide bialaphos (bar) was studied. The selected *Streptomyces* isolate was *in vitro* tested for its ability to grow in the presence of Basta herbicide in SNAM using three concentrations, i.e., 2 (Recommended dose), 4 and 6 g/L. Data in Table 1 show that the tested Streptomycete isolate was herbicide- resistant isolate as it showed abundant growth in the presence of three folds of the recommended dose of Basta (2g/L). Similar results were obtained by El-Domyati and Mohamed (2004) who reported that three out of eight *Strepto-*

myces isolates were Basta-resistant up to three folds of the RD.

Table-1: The toxicity of Basta herbicide on the growth of *S. coelicolor* Egy isolate.

Treatments	Concentrations of Basta herbicide (g/L) in SNAM		
	2 (RD)	4	6
S. coelicolor Egy	+++	+++	+++
Control*	+++	+++	+++

*: SNAM with no herbicide inoculated with the same *Streptomyces* strain.
+++:Abundant growth. RD: recommendded dose.

Based on the above findings the DNA of resistant *Streptomyces* strain was used for PCR isolation of *bar* gene and a PCR product with a size of about 450pb was amplified (Figure-1). This followed by subcloning into the plasmid pAHC25 with *gus* genes. The recom-binant plasmid was transferred into *Escherichia coli* strain DH5α for DNA multiplication of storage (Figure-2).

In conclusion, we have isolated and studied the organization of *S. coelicolor* Egy genes responsible for the bio-synthesis of the antibiotic herbicide bialaphos. Bialaphos production genes were PCR isolated and cloned from genomic DNA using a plasmid vector (pAHC25).This will be further continued by determination of its nulcleotide sequence as well as its expression into putative transgenic plants.

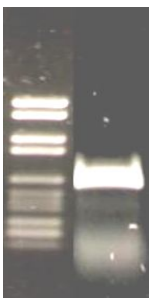

Figure-1: Agarose gel (1.2%) electro-phoresis shows nested-PCR isolation of bar gene of the DNA extract of *S. coelicolor* Egy.

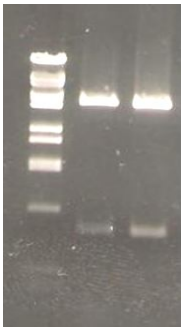

Figure-2: Agarose gel (1.2%) electrophoresis shows DNA *Eco*R1/*Hind*III digestion confirmation of subcloning the PCR product of *bar* gene into pAHC25 plasmid.

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