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Research Article

Bioinformatics, Identification and Cloning of β-galactosidase from *Lactobacillus plantarum*

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Abstract: *Lactobacillus plantarum* is a lactic acid bacterium, mostly found in fruits and vegetables. It has been used in a variety of food fermentations. It is reported that strains from this species have probiotic activity. In this study, pUC19 was chosen as a vector to clone β-galactosidase gene. To clone this gene we used *Eco*RI and *Kpn*I restriction enzymes that give a DNA fragment of 5000 bp in length which contains β-galactosidase gene. The same endonuclease enzymes were used to cut the vector (pUC19). To know the length of isolated DNA fragment as well as digested pUC19, agarose gel electrophoresis was used along with Lambda HindIII and Hyperladder I respectively. QIAGEN kit was used to extract DNA fragments from the agarose gel. Then it utilised for ligation in further processes. Furthermore, the DNA fragments were transformed into host cells (*E. coli*) and they were spread on LB agar plates containing X-gal and IPTG to confirm the presence of inserted DNA.

Keywords: *Lactobacillus plantarum*, β-galactosidase, Probiotics, Cloning.

1. Introduction

Lactobacillus spp. are members of the lactic acid bacteria (LAB) group, are commonly found in environmental niches and raw foods (Kim *et al.*, 2017). They are key components in a variety of fermentation processes, enhances quality and safety of foods by producing organic acids and other antimicrobial compounds (Zhai *et al.*, 2019). *Lactobacillus plantarum* (LP) is one species of special interest as it is used in vegetable, dairy and meat fermentations (Dong *et al.*, 2017). It is also found in the human gastrointestinal (Aimutis, 2014). It has been observed, it is safe to use as probiotic (Dong *et al.*, 2017), as it survives on passing throughout gastrointestinal tract at low pH and contacts with bile, show adhesion to the intestinal epithelial cells, stabilizes intestinal microflora (Zeng *et al.*, 2016; Imran *et al.*, 2016). This is one of the most excellent probiotics to deal with digestive distress problems (Aimutis, 2014). Genome of LpWCFS1 was sequenced with whole genome sequencing and assembly approach. The chromosome of LpWCFS1 is single, circular having 3,308,274 bp. It possesses 3052 protein encoded genes of which 2120 have biological functions. The genome encodes all the enzymes needed for the phosphoketolase and glycolysis pathways (Kleerebezem *et al.*, 2003). β-galactosidase is the lactose-hydrolysing enzyme, has been reported as an important enzyme for dairy industry (Kim *et al.*, 2017). β-galactosidase enzyme posses two catalytic activities (Nguyen *et al.*, 2007):

a) Hydrolysis of disaccharide lactose into galactose and glucose,
b) Conversion of lactose into other disaccharides, allolactose which is a natural inducer of lac operon.

The aim of this study is using of Bioinformatics to identify β-galactosidase from LP and development of a strategy for cloning the β-galactosidase gene.

2. Materials and Methods

2.1 Genomic DNA and plasmid

Lactobacillus plantarum and *E. coli* species were kindly provided by Erbil Teaching Hospital. Firstly, *Lactobacillus plantarum* was grown in rich MRS broth for 1-2 days. Then Sigma's GenElute Bacterial Genomic Kit was used for *Lactobacillus plantarum* genomic DNA extraction. Finally, the genomic DNA suspended in 100µl of TE buffer, then stored at -20°C for further analysis. 0.5µg/µl concentration of pUC19 plasmid DNA was used for cloning β-galactosidase gene.

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2.2 Gel electrophoresis and DNA Purification

Mini agarose gel electrophoresis prepared at 0.5gms of powdered agarose were mixed into 100ml of 1X TBE buffer. The mixture was then heated in microwave until it becomes clear. 1µL of Web Green was then added into the solution. After the gel prepared for LP genomic DNA and pUC19 plasmid, a voltage of 80v/cm² was allowed to pass through the gel till the trapping dye nearly reached the edge of the gel (nearly 45 minutes), then examined and photographed under UV light source. Then the purification of bacterial DNA and plasmid from the agarose gel was carried out using the QIAquick Gel extraction kit protocol.

2.3 Ligation and transformation

Insert ratio used for ligation is 1:3 (vector). After purifying genomic DNA and plasmid from gel, components mentioned in the table below (Table 1) were added in a sterile microcentrifuge tube. Incubate at room temperature for about 3 hours. Then we added 1µl of 0.5M EDTA to inactivate the enzyme. This sample was further used for transformation (Untergasser, 2008). In a suitable sterile 1.5ml of Eppendorf tube, mixed 5µl of interested plasmid DNA (recombinant plasmid) with 100µl of competent cells (*E. coli*), then placed the tube on ice for an hour. Then the cells are heat shocked at 42°C for 2 minutes in an Eppendorf heating block. Further, 1ml of LB medium is added and allowed to grow at 37°C for 30 minutes with shaking, which helps gene in its expression. The cells are then spread over LB agar plates containing ampicillin, X-gal and Isopropyl β-D-1-thiogalactopyranoside (IPTG). After spreading, the plates were labelled clearly and kept in incubator for overnight at 37°C in an inverted manner.

Table 1: Components for Ligation.

Components	Vector	Insert	Buffer	Ligase	Water
Volume (µl)	10	30	3	1	30

2.4 Bioinformatics analysis of β-galactosidase gene and protein

All of nucleotide and amino acid sequences in this study were obtained from the National Centre for Biotechnology Information (NCBI). Retrieval of the general information we used Gene bank (Sayers *et al.*, 2009). General protein information on β-galactosidase enzyme of LP carried out by using database Benson *et al.*, 2009. For sequence analysis, we used nucleotide-nucleotide BLAST (BLASTn) and for analysis of the protein, we used protein-protein BLAST (BLASTp).

3. Results

3.1 General Characteristics of β-galactosidase I gene from bioinformatics analysis

General information on the enzyme β-galactosidase I have been obtained using NCBI database from the Gene bank. Protein consists of 683 amino acids with its accession number NP_786678.1. Its nucleotide sequence from the accession number NC_004567.1 shows that the genome of LpWCFS1 consists of 3308274 base pairs in its circular DNA which has been published in the NCBI REFSEQ database on 17 March 2017. It has been found that the genome of this organism consists of 3135 genes, one of which called β-galactosidase I with 2052 nucleotides and it has been given with the gene ID: 1063513, updated on 17 March 2017. The Gene record shows that the symbol of the gene is given as Lac A with its description β-galactosidase I and its locus has been tagged as lp_3469 as protein-coding gene. The protein, β-galactosidase I coded by lac A gene has been assigned its enzyme classification (EC) number as 3.2.1.23.

3.2 NEBcutter result

In this study, we used two different restriction enzymes (*Eco*RI and *Kpn*I) segregate β-galactosidase gene from genomic DNA. These two enzymes *Eco*RI and *Kpn*I were under consideration with the help of NEBcutter program which is shown below in Fig. 2. The figure shows two ORFS, but ORF with 683 amino acids belongs to β-galactosidase gene, which we have seen in the previous figure. As *Eco*RI and *Kpn*I enzymes were chosen it was important to select fragment of DNA having β-galactosidase gene and having restriction sites of *Eco*RI and *Kpn*I enzymes on either side of gene. Fortunately, these two enzymes gave a long fragment of DNA with 5000bp and two ORFs. So, we decided to go with these two enzymes to separate β-galactosidase gene from genomic DNA.

3.3 Separation of LP genomic DNA

As we can see in Fig. 3 lane one consists of Lambda HindIII that gives different band sizes in kb from larger to smaller 23.1, 9.4, 6.5 and 4.3 respectively, and lane 2 consists of LP genomic DNA with one band 23 kb which is nearly similar to the size of genomic DNA of LP.

Fig. 1: The genomic location of the β-galactosidase I gene. The red arrow shows its location in the genome and its distance with the other (Sayers *et al.*, 2009).

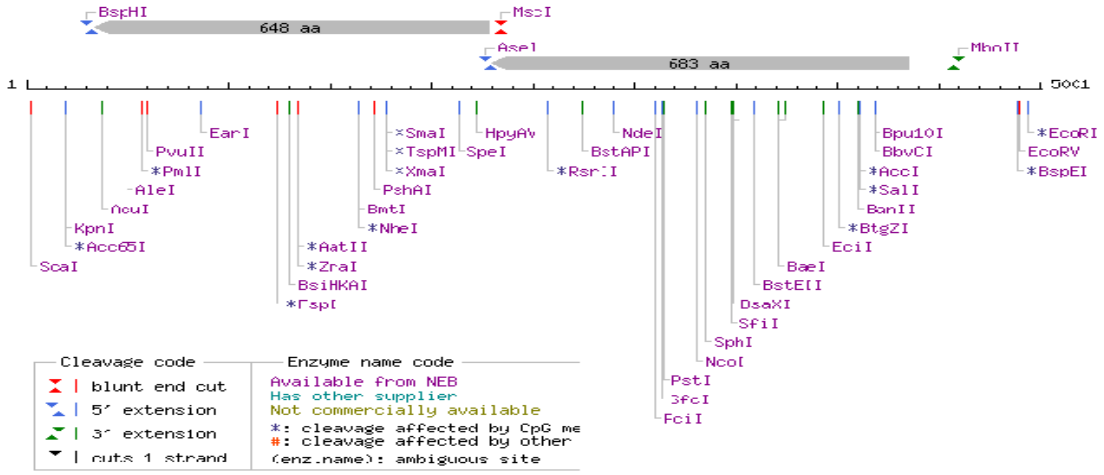

Fig. 2: NEBcutter output (Vincze *et al.*, 2003).

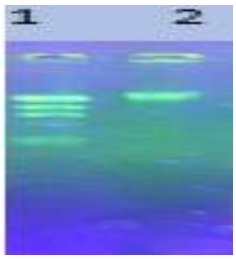

Fig. 3: Agarose gel electrophoresis of LP genomic DNA.

3.4 Gel electrophoresis of LP genomic DNA and pUC19 plasmid after restriction digestion

Fig. 4A shows distinguished bands of genomic DNA after restriction digestion. The fragment of DNA with β-galactosidase gene after restriction digestion with *Eco*RI and *Kpn*I is 5000 bp by restriction enzyme tool NEBcutter. The 3rd fragment of Lambda HindIII is 6.5 bp and 4th is of 4.3 bp. So, the band appeared in the gel between these two bands may be our gene of interest which can be seen in lane 4. Lane 2 which has genomic DNA, which treated with *Eco*RI shows a smear along with lane 4 which have genomic DNA that treated with *Eco*RI + *Kpn*I. Fig. 4B shows distinguished bands of pUC19 after restriction digestion. This plasmid DNA was run along with Hyperladder I which is used as control, while Lane II consists of circular pUC19, which was used to differentiate between linear and circular bands. Lane 3 and 4 consists of pUC19 DNA digested with *Eco*RI and *Kpn*I which clearly show distinguished linear bands respectively. Lane 5 consists of double digestion of pUC19 with *Eco*RI and *Kpn*I which clearly shows a linear band which results that double digestion successfully occurred. The sites of *Eco*RI and *Kpn*I on pUC19 are present at a difference of 12-15 bp. So the band of double digestion obtained is similar to the size of other two previous bands. Our attempts to screen or to see recombinants and non-recombinants colonies after ligation and transformation, the sample was spread on LB agar plate containing ampicillin, X-gal and IPTG. After incubation (24 hours) blue and white colonies of *E. coli* were present on the LB agar plate.

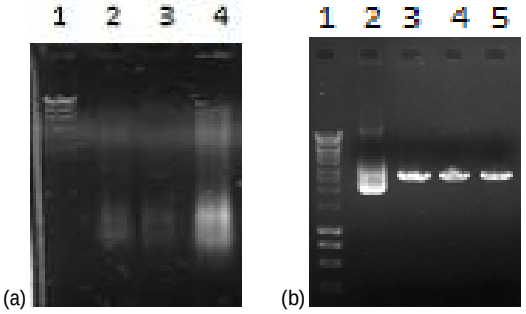

Fig. 4: Agarose gel electrophoresis analysis of LP genomic DNA and pUC19 plasmid.

(A) Lane 1 consists of Lambda HindIII, lane 2 consists of genomic DNA + *Eco*RI, lane 3 consists of genomic DNA + *Kpn*I, and lane 4 consists of double digestion of genomic DNA with *Eco*RI + *Kpn*I.

(B) Lane 1 consists of Hyperladder I; Lane 2 consists of pUC19 (Circular DNA); Lane 3 consists of pUC19 + *Eco*RI; Lane 4 consists of pUC19 + *Kpn*I and Lane 5 consists of pUC19 + *Eco*RI + *Kpn*I.

3.5 Characteristics and structures of β-galactosidase protein from bioinformatics analysis

β-galactosidase I protein from LP is composed of 683 amino acids with its accession number NP_786678.1 and with an estimated molecular mass of 464 kDa. The amino acid sequence of β-galactosidase I protein from LpWCFS1 had the highest genetic relationship and showed 99% homology with other β-galactosidase protein from LP with the E value 0, but lower homology with β-galactosidase *Lactobacillus spicheri* with 73% and lowest homology with other bacteria <70% (Fig. 5). Secondary structure analysis revealed that the protein contained helix (32.06%) extended strand (21.67%) and random coil (46.27%) (Fig. 6).

Fig. 5: Evolution tree according to β-galactosidase I from other species (Sayers *et al.*, 2009).

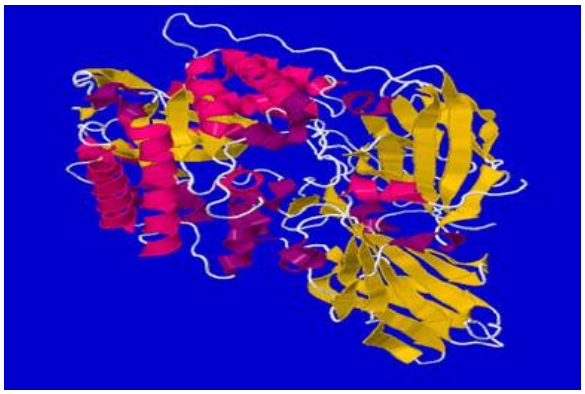

Fig. 6: The 3D structure of β-galactosidase I.

4. Discussion and Conclusion

The gene of interest of our study was β-galactosidase from LP as it has many applications in food industries as well as it is a probiotic strain, safe to use and different enzymes can be used without broad purification (Yadav, 2017; Jiang & Yang, 2018). Recent studies have shown that certain strains of lactobacilli appear to have protective immunomodulation properties and are able to induce a systemic immune response. In addition, lactobacilli have the ability to inhibit the adhesion of pathogenic bacteria to the intestinal wall. Taken together, these results show that probiotic bacteria may be effective in the treatment of Crohn's disease (Madsen *et al.*, 2001). pUC19 was used as a vector to clone this gene because it possesses multiple cloning sites and many genes for screening between recombinants and non-recombinants. A restriction enzyme tool known as NEBcutter was used to generate restriction sites which would give a DNA fragment with β-galactosidase gene. Accordingly, we got *Eco*RI and *Kpn*I enzymes that give fragment of DNA with 5000 bp having β-galactosidase gene. Also, these enzymes produce cohesive ends, which increase efficiency of ligation. *Eco*RI and *Kpn*I have six nucleotide restriction sites GAATTC and GGTACC respectively (Raven *et al.*, 1993; Hammond *et al.*, 1991). Bioinformatics analysis is a powerful tool for protein identification, study of its localization, function, modification and possible interaction. Both sequences and structures of β-galactosidase are conservative and special. The presence or absence of β-galactosidase activity is screened by using X-gal and IPTG. According to Brown (2006), the non-recombinant colonies will appear blue in colour, whereas recombinant colonies will be white in colour. So, on the basis of this concept, we can conclude that recombinant colonies were formed. β-galactosidase breaks X-gal to deep blue coloured product and IPTG acts as an inducer. From previous three decades, biotechnology is largely noticed only due to gene cloning. The ability to clone genes means that gene significant for plant or animal protein can now be isolated from normal host, inserted into cloning vector and introduced into bacterium. The gene will be expressed and recombinant protein can be synthesized by bacterial cells. So, protein can be obtained in large amount which can further use in medicine, agriculture, etc.

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