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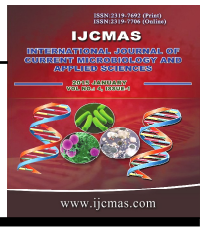

Original Research Article

Investigation of capability to clone β-glucosidase producing gene isolated from *Bifidobacterium breve* into *E. coli* and its expression

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A B S T R A C T

Keywords

Bifidobacteria breve, *E. coli* DH5 alpha, β-Glucosidase, p-Nitrophenyl-β-D-glucopyranoside, etc.

Bifidobacteria breve is industrially important bacterium. In the present study the samples of Infant fecal and Yakult were collected for the isolation of bacterial species using spreading and streaking techniques. The isolated bacterial species were identified by performing various biochemical tests. Genomic DNA from *B. breve* was cleaved with the restriction enzyme Sma1 and ligated to pBR322 for transformation into *E. coli DH5 alpha.* β-Glucosidase gene was functionally expressed in *E. coli DH5 alpha*; the transformants were able to grow on p-Nitrophenyl-β-D-glucopyranoside and ampicillin. B-glucosidase-positive clone was demonstrated in the results by presence of its colonies on a medium supplemented with p-Nitrophenyl-β-D-glucopyranoside.

Introduction

The significance of a healthy lifestyle has created a great interest in probiotics. Many species of lactic acid bacteria (LAB), *Bacillus*, and fungi such as *Saccharomyces* and *Aspergillus* have been used over the years in the food industry. A few have gained the probiotic status and most of this belongs to *Lactobacillus, Streptococcus and Bifidobacterium* genera. In the human gastrointestinal tract, *Bifidobacterium* is most important group of bacteria present and numerous strains of it have gained recognition as probiotics. Pathogenicity of *Bifidobacteria* for humans is rare, although *Bifidobacterium* has been found in the feces and alimentary tract of infants, older people, and animals. *Bifidobacteria* are gram-positive pleomorphic rods, ranging from uniform to branched, bifurcated Y and V forms, spatulate or club shaped.

They are non- motile, non-spore forming and are strictly anaerobic (although some strains can tolerate oxygen in the presence of carbon dioxide) that can be isolated from a variety of materials such as human and animal feces, sewage, fermented milk product and from the oral cavity (Kleerebezem and Vaughan, 2009; Ravula and Shah, 1998).

Apart from the properties mentioned above,

the main phenotypic characteristics of *Bifidobacterium* are producing lactic acid and acetic acid as the main products of glucose utilization. *Bifidobacterium* was first isolated in 1899 from a healthy breast-fed infant by Tissier of the Pasteur Institute in France (Salazar *et al.*, 2009).

Since their first isolation from human breast-fed infants' faeces (Leahy *et al.*, 2005; Sambrook *et al.*, 1989), they have been the object of numerous nutritional, biochemicals, ecological, taxonomical and genetic studies.

It has been demonstrated that *Bifidobacteria* have several health-promoting effects, including immunomodulation, elimination of procarcinogens, production of vitamin, prevention of diarrhoea and intestinal infections, alleviation of constipation, production of antimicrobials against harmful intestinal bacteria, and protection of the mucosal epithelium against invasion by pathogenic bacteria (Tochikura *et al.*, 1986; Vankerckhoven *et al.*, 2008; Ventura *et al.*, 2004; Verdenelli *et al.*, 2009; Wei *et al.*, 2007).

B. breve thought to exhibit β-glucosidase activity and *Escherichia coli DH5 alpha* (Stratagen, Germany) cannot synthesize the beta-glucosidase and was used as a host. In this study, we cloned the β-gal gene from *B.breve* to *E. coli DH5 alpha* by using pBR322 vector.

Preliminary characterizations of the enzymes were taken into consideration for construction of potential cloning vectors. β-Glucosidase gene was functionally expressed in *E. coli DH5 alpha*; the transformants were able to grow on media supplemented with p-Nitrophenyl-β-D-glucopyranoside and ampicillin.

Materials and Methods

Bacterial species and plasmids

Bifidobacterium breve was isolated from faeces of 28-day-old healthy breast-fed infant and Yakult. *Escherichia coli DH5α* (Stratagen, Germany) thought not to synthesize the β-glucosidase and was used as a recipient for all transforming experiments. β-Glucosidase gene from *B. breve* was cloned into *E. coli DH5 alpha* by using pBR322 vector.

Media used

Reinforce clostridial prussian blue medium (1 % Peptone from casein, 0.3 % Yeast extract, 1% Meat extract, 0.5 % D(+) Glucose, 0.5% Sodium Chloride, 0.3 % Sodium acetate, 0.1 % Starch, 0.05 % Cysteine, Prussian blue 0.001%, distilled water, pH 6.8) and BD *Bifidobacterium* Broth, Modified (4.25 % Columbia agar, Glucose, Lactulose, Cystein-HCl, distilled water, pH 5.5) were used for cultivation of *Bifidobacteria.*

LB medium (1 % Bacto-tryptone, 0.5 % Bacto-yeast extract, 1 % NaCl, distilled water, pH 7.0) was used for preparation of E. coli competent cells. The cultivation of *E. coli* and *Bifidobactria* was performed at 37°C and 30°C respectively.

Genus and species identification

Bacteria isolated were gram-positive, catalase negative, non-motile, unable to produce gas from glucose and indole from tryptophan and as well as to reduce nitrate. But possessed fructose-6-phosphate phosphoketolase (EC 4.1.2.22) activity with L, V and Y shaped cellular morphology. And they were identified as the *Bifidobacterium* genus. The F6PPK activity was detected. Species identification was

done by using 16S rRNA sequencing method.

Preparation of genomic DNA

Genomic DNA was prepared by the alkaline lysis method. Quantitative analysis of DNA was done by using agarose gel electrophoresis and photographed by Gel documentation system.

Construction of recombinant plasmid and transformation

The Genomic DNA from *B. breve* was cleaved with the restriction enzyme Sma1 and ligated to pBR322 for transformation into *E. coli DH5 alpha*. The genomic DNA was combined with vector DNA in ligation buffer (7µl) with 2µl of T4 DNA ligase enzyme in a final volume of 50ul and incubated at 4^{o}C for 16 hrs.

E. coli DH5 α was grown on LB media overnight at 37^{o}C for the preparation of competent cells. 100 µl of competent cells were mixed in ligation mixture of bacterial genomic DNA and vector DNA, kept it on varying temperature from ice cold to 42^{o}C for approx. 37 minutes. Then this mixture was added into LB broth and incubated for one hour. After centrifugation LB broth was added to pellet.

Detection of β-glucosidase positive clones

β- Glucosidase positive clones were detected on LB agar media supplemented with ampicillin and p-Nitrophenyl-β-D-glucopyranosidase (substrate for β-D-glucosidase gene).

Sequencing of the fragments from *Bifidobacterium*

The amplified fragments from *Bifidobacterium* were sequenced. The sequenced gene was aligned using multiple sequence alignment tool CLUSTAL W. Then phylogenic tree for the target gene was obtained.

Results and Discussion

As mentioned above *Bifidobacteria* were gram-positive, catalase negative, non-motile, unable to produce gas from glucose and indole from tryptophan and as well as to reduce nitrate but possessed fructose-6-phosphate phosphoketolase (EC 4.1.2.22) activity with L, V and Y shaped cellular morphology.

Gene cloning and expression

To clone the β-glicosidase gene, genomic DNA was extracted from *B. breve* (Figure 1) and cleaved with SmaI. *Escherichia coli* DH5 alpha (Stratagen, Germany) cannot synthesize the β-glucosidase and was used as a recipient. The number of fragments generated by cleavage with restriction enzyme SmaI was large and we were unable to determine the total number of fragments generated. The SmaI fragments were ligated into pBR322.

For ligation, a standard amount of genomic DNA was combined with vector DNA in ligation buffer with of T4 DNA ligase enzyme. These ligation mixtures were used to transform *E. coli DH5 alpha*. β-Glucosidase gene was functionally expressed in *E. coli DH5 alpha*. β-glucosidase-positive clone was demonstrated in the result by presence of its colonies on a LB medium supplemented with p-Nitrophenyl-β-D-glucopyranoside (Figure 2 & 3).

Molecular studies revealed its characterization as 16S rDNA amplification

has 927bp nucleotides in length identified as *Bifidobacterium breve* and this 16S rDNA nucleotide sequence has been deposited in Genbank and assigned accession number LC003041.Comparison of 16S rDNA sequences with sequences deposited in NCBI showed that isolate strain was most closely related to bacterial *Bifidobacteriumspp*. Partial sequence of 16S ribosomal RNA gene shown sequence similarity of 98%. The phylogenetic tree showed the grouping of *Bifidobacterium spp*. (Figure 4).

In conclusion, *Bifidobacterium breve* with several important industrial features was selected. Lactose is digested with difficulty by a large proportion of the world population. Also, lactose has low solubility in water, which leads to problems in the concentration of whey and in the preparation of certain food items. Additionally, lactose has a relatively low level of sweetness. These problems can be overcome to a large extent by hydrolysis of lactose to its monosaccharides (glucose and galactose) which are sweeter, more soluble, and more digestible than lactose. *B. breve* is a promising source for the production of β-glucosidase enzyme because it is a food-approved organism. After SmaI digestion, bacterial DNA was ligated to pBR322 for transformation into *Escherichia coli DH5 alpha* and screened for results. The results in this study showed that DNA extracted from *B. breve* was cloned into the *E. coli* host DH5 alpha and screened on a media supplemented with p-Nitrophenyl-β-D-glucopyranoside, resulting in a β-glucosidase positive clone.

Further studies will be required to determine the exact location of the β-glucosidase gene. Moreover, studies on the properties of this recombinant *E. coli* DH5 alpha will be required to find out its similarities with *B. breve* and its industrial importance.

Figure.1 0.8% Gel of agarose. Lane 1–6 shows DNA isolated from *Bifidobacterium breve*

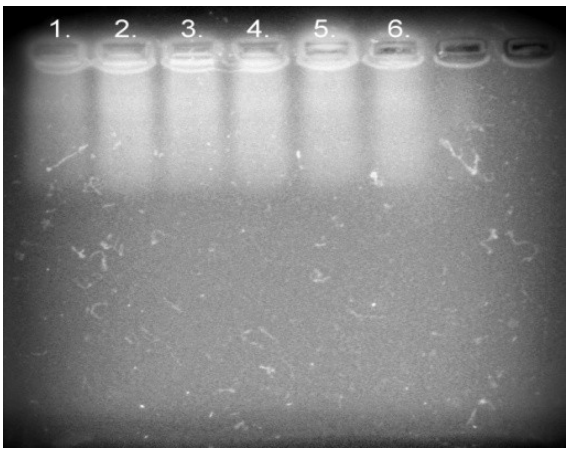


Figure.2 β-glucosidase positive clones of *E. coli* DH5 alpha that had taken gene from *Bifidobacterium breve* showing growth on LB agar plates supplemented with ampicillin and p-Nitrophenyl-β-D-glucoranosidase

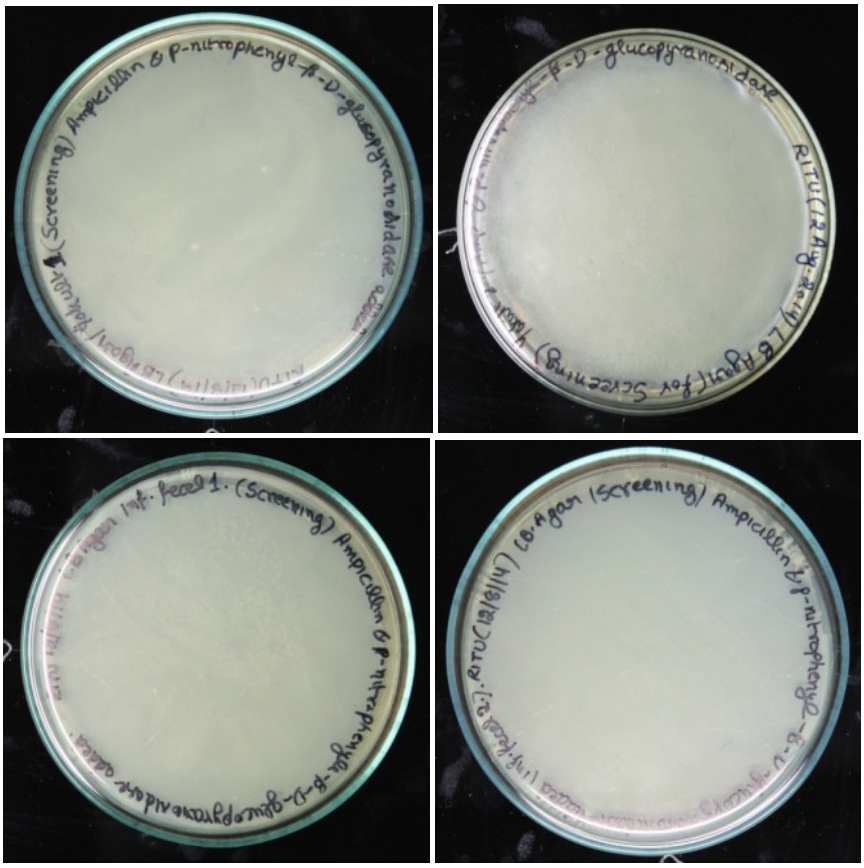


Figure.3 0.8% Gel of agarose. Lane 1–4 shows DNA isolated from β-glucosidase positive clone of *E. coli* DH5 α

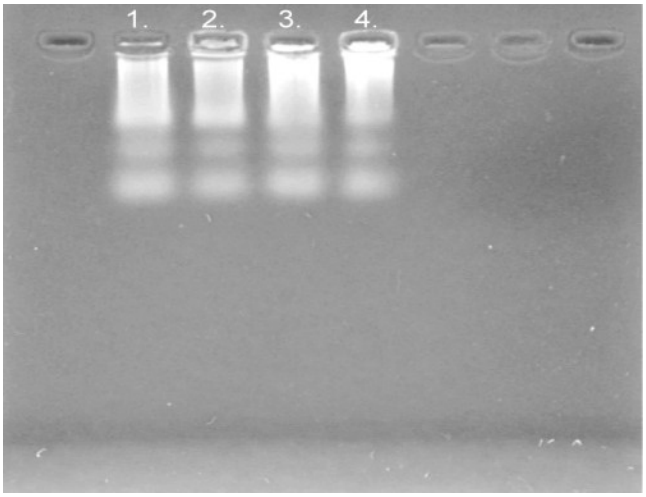
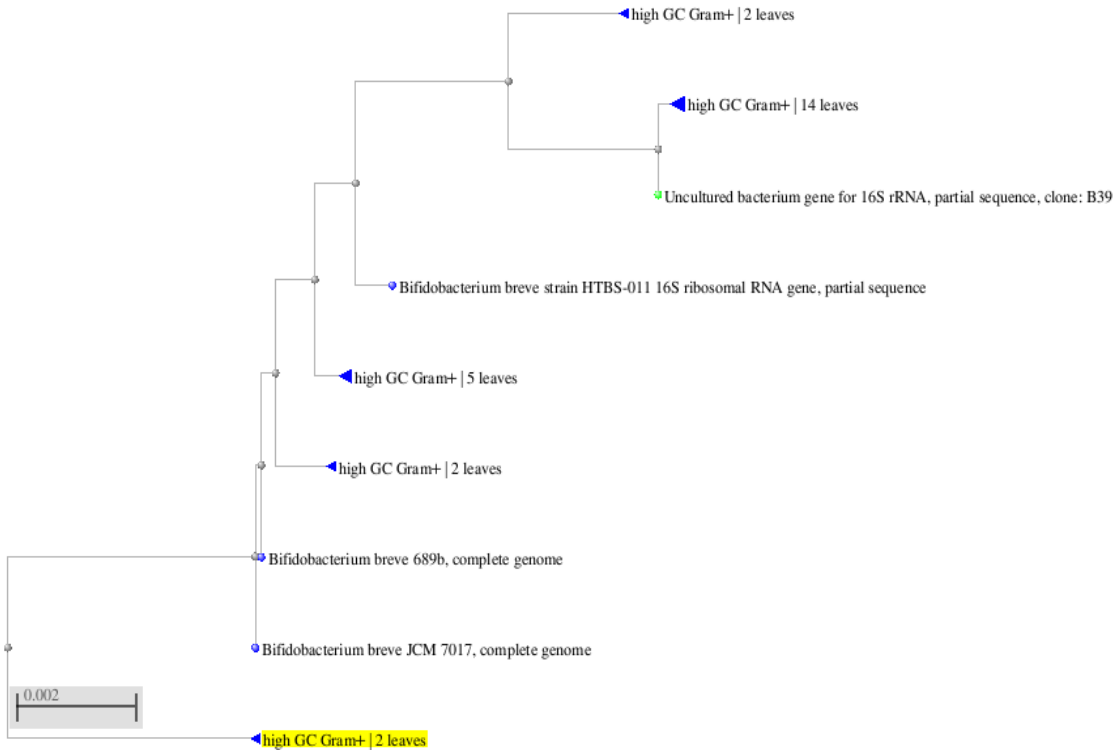


Figure.4 Phylogenetic analysis of sequenced gene



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