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Molecular Characterization Using *gtf* Gene Detection and Medium Optimization of *Beta-D-Glucan* Production from *Pediococcus parvulus F1030* Isolated from Local Egyptian Boza

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Authors' contributions

This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.

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Study Protocol

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ABSTRACT

Background: Lactic acid bacteria (LAB) have great benefits which can improve the nutritional value of food, reduces some infections, immune modulator effect, curbs some types of cancer and restricts glucose level of serum.
Aims: This study was carried out to detect glycosyltransferase gene (*gtf*) encoding glucosyltransferase enzyme responsible for beta-D-glucan production in *Pediococcus parvulus F1030* which is isolated by authors from local Egyptian Boza (local alcoholic beverage), and enhancing its production by medium optimization.
Materials and Methods: Molecular identification and DNA sequencing were performed by using specific primers and the medium optimization was enhanced using Plackett-Burman Design.
Results: The isolate detected as *Pediococcus parvulus* by the molecular identification with using 16S rDNA and submitted in GenBank as *Pediococcus parvulus F1030* with accession number

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ky942131 by the authors. The study revealed the presence of 97% identity with similar to *Pediococcus parvulus* accession number *ky425809*. The *gtf* gene was detected in the isolate by 16S rRNA gene using specific primers. The medium optimization showed enhancement of beta-D-glucan production. The medium number 2 yielded 11 g l^{-1} from it by using concentration 1.5 g l^{-1} from sucrose, 0.5 g l^{-1} from $CaCl_2$, 0.75 g l^{-1} from tween80, 3 g l^{-1} from $KHPO_4$ and 1.5 g l^{-1} from glutamic acid.

Conclusion: Molecular characterization of isolated *Pediococcus parvulus* showed 97% identity with a similar organism in NCBI site, the detection of *gtf* gene carried out by using PCR technique with specific primers and beta-d-glucan production was enhanced with medium optimization using Plackett-Burman Design.

Keywords: Pediococcus parvulus; lactic acid bacteria (LAB); gtf gene; beta-d-glucan production; medium optimization.

1. INTRODUCTION

Beta-Glucan (β-glucan) comprises a group of β-d-glucose polysaccharides naturally occurring in the cell wall of cereals, bacteria and fungi with significantly different physicochemical properties depending on the source. Typically, β-glucans form a linear backbone with 1-3 β-glycosidic bonds but vary in respect to molecular mass, solubility, viscosity, branching structure, and gelation properties, causing diverse physiological effects in animals [1]. *Pediococcus parvulus* is effective as anti-oxidant. It counteract the free radicals which cause damage and premature ageing, Beta Glucan has an effect against cancer, with antimicrobial, and a cholesterol-lowering effect [2]. Beta-Glucan enhances the action of white blood cells (WBC's) in the body which exist to fight infection. They comprise macrophages, neutrophils, natural killer cells (NK) T cells and others. Their roles are to fight and devour invading unnatural cells, such as those typically found in cancer. Beta-glucan also has anti hyperglycemic effect [3]. *Pediococcus parvulus* can induce the production of inflammation-related cytokines by polarized macrophages provoked changes in short-chain fatty acid formation in the cecum, distal colon, and feces of rats, which may be beneficial to colonic health [4]. Two substituted (1,3)Beta-D-glucan is synthesized by *P. parvulus* 2.6 by the glycosyltransferase (GTF) which is encoded by the *gtf* gene [5].

2. MATERIALS AND METHODS

2.1 Molecular Identification of *Pediococcus parvulus*

Bacterial DNA isolation and PCR amplification of the 16S rDNA gene. Test isolate was cultured to exponential phase in 10 ml of Lactobacillus-MRS broth. DNA was extracted from the cells. The pellet was dried and then resuspended in buffer solution. The 16S rDNA gene was PCR amplified from the isolated DNA using the following primers (based on the *E. coli* 16S rDNA rrnB sequence; GenBank accession no. U00096): forward primer (beginning at base 5), TGAGAGAGTTTGATCCTGGCTCAG; and reverse primer (beginning with base 1541), AAGGAGGTGATCCAGCCGCA. PCR was accomplished using reagents from a Qiagen Taq PCR Core Kit (Qiagen, Inc., Valencia, Calif.). Thermocycling conditions were as follow: 3 min at 94°C; followed by 30 cycles of 30 s at 94°C, 30 s at 45°C, and 1.5 min at 72°C; followed by 10 s at 72°C and hold at 4°C. PCR products were analyzed by 1% agarose electrophoresis separation and ethidium bromide staining and comparing the bands to a 100-bp ladder molecular weight standard [6].

2.2 Detection of the *gtf* Gene in Genomic DNA of Isolated *Pediococcus parvulus*

By using specific primers according to (Werning et al., 2008).

Primers: Primers of *gtf* gene detection used were F-(5´ATGTTAAATGATAATGATTCAGAAC TAAAAAAATTTC-3) and R-(5´-TTAATCATTCCA ATC AACTGTTTCCGTGTT-3´).

Total DNA for PCR detection of the gene (*gtf*) encoding the glycosyltransferase (GTF) was used for characterization of the *gtf* gene. DNA sample was extracted and stored at -20ºC until used. For detection of the *gtf* gene encoding glycosyltransferase enzyme in the exopolysaccharides (EPSs) producing strain, specific primers GTFF and GTFO were used for PCR amplification Each 50-ml PCR reaction was

carried out with 1.25 U of BIOTAQ DNA polymerase (Bioline, Luckenwalde Germany) and contained 1 mM of each primer, 0.75 mM of $MgCl_2$, 20 mM Tris-HCl pH 8.4, 50 mM KCl, 25 mM of each incubated at 56ºC for 30 min, and centrifuged at 11,000 xg for 5 min. The supernatant containing the DNA was mixed with 5 mg of polyvinylpyrolidone, vortex then centrifuge at 13,000 xg for 10 min to remove inhibitors of DNA polymerases present in Boza by sedimentation. The supernatant was kept frozen at -20ºC before use in PCR reaction dNTP, and 5 ml of DNA template. Cycling conditions were 13(95ºC, 5 min); 35 (95ºC, 1min; 50ºC, 1 min; 72ºC, 30s), and13 (72ºC, 10 min). The PCR products were separated on a 0.8%(wt/vol) agarose gel, and the amplicons were purified with the QIAquick Gel extraction kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. The DNA sequences of the amplicons of approximately 493 bp were determined and identified by BLAST searching of the GenBank DNA database [5]. The DNA sequencing has been deposited in GenBank (*Pediococcus parvulus F1030* accession number ky942131). Sequences were submitted to NCBI GenBank using BankIt (http://www.ncbi.nlm.nih.gov/WebSub/?tool=genbank).

2.3 Medium Optimization for Beta-D-Glucan Production

The Plackett-Burman experimental design Plackett and Burman (1946) is an efficient technique for optimization of the factors affecting active compounds production and was used to reflect the relative importance of various chemical factors.

Five medium components were tested in thirteen runs (Table 1) according to the Plackett-Burman experimental design matrix as cleared in a table (2).The studied factors were Sucrose, Glutamic acid, K_2HPO_4, Tween 80 and $CaCl_2$.Sucrose was tested as carbon source which can induce production of β-D-Glucan [7]. The glutamic acid powder was chosen as the nitrogen source [8]. K_2HPO_4 was chosen to acts as a buffering agent in the medium to maintain its pH for a long duration [9]. The surfactant Tween 80 was tested as it alters the membrane permeability and enhances the release of the extracellular

Table 1. Factors and coded levels examined independent variables affecting the β-D-Glucan produced by *Pediococcus parvulus* F1030 and their levels in the Plackett-Burman design experiment

Factor (g l^{-1})	Symbol	Low level (-1)	High level (+1)
Sucrose	A	0.5	1.5
Glutamic acid	B	1.5	4.5
K_2HPO_4	C	1	3
Tween 80	D	0.25	0.75
$CaCl_2$	E	0.5	1.5

Table 2. Plackett-Burman Design for two levels of 5 variables in uncoded values along with the observed β-D-glucan concentrations

Run order	Sucrose	Glutamic acid	K_2HPO_4	Tween 80	$CaCl_2$
1	0.5	1.5	1	0.25	0.5
2	1.5	1.5	3	0.75	0.5
3	1.5	1.5	3	0.25	0.5
4	1.5	1.5	1	0.25	1.5
5	1	3	3	0.50	1
6	1.5	4.5	1	0.75	0.5
7	0.5	4.5	3	0.75	0.5
8	0.5	4.5	1	0.25	0.5
9	1.5	4.5	3	0.25	1.5
10	0.5	1.5	3	0.75	1.5
11	0.5	4.5	3	0.25	1.5
12	0.5	1.5	1	0.75	1.5
13	1.5	4.5	1	0.75	1.5

glucosyltransferase, which is expected to increase β-D-Glucan biosynthesis [9]. The $CaCl_2$ was considered for its stimulatory effect on glucosyltransferase production [10]. Each of the five factors were examined in two levels: low level (-1) and high level (+1) [11]. Bacteria were grown at pH 6 and temperature 37°C.

The experimental data were analyzed by Minitab statistical software [8].

2.4 Extraction and Estimation of Yielded β -D-glucan

For extraction and purification of (EPSs), bacterial cells were removed from fermented media by centrifugation (16,000 ×g, 4ºC, for 30 min). The EPSs present in the supernatant was precipitated by addition of two volumes of cold acetone and incubation overnight at 4ºC. After centrifugation at 14,000 ×g for 10 min at 4ºC, the precipitate was washed 3 times with 70% acetone and sedimented by centrifugation and the estimation was detected according to [2]. The sedimented β-D-glucan was filtered and weighed by (g l^{-1}).

3. RESULTS AND DISCUSSION

3.1 Molecular Identification

The isolate detected as *Pediococcus parvulus* and submitted in GenBank by the authors as *Pediococcus parvulus* F1030 with accession number ky942131 the analysis revealed the presence of 97% homology from tested bacteria with the 16S rDNA gene of *Pediococcus parvulus* accession number ky425809 in NCBI database by using basic local alignment search tool for nucleotide sequence (BLASTn). The *gtf* gene was detected with 16S rRNA gene by using specific primers at 420 bp at the left side in the Fig. 1 while *Lactobacillus plantarum* and *Lactobacillus casei* at the middle and the right side respectively. PCR assay for 16S rRNA.*gtf* gene detected which was done to identify the isolate. As showed in Fig. 1. The PCR size of the amplicon obtained from the tested isolate corresponded to the predicted size of about 474 bp. As showed in Fig. 2. The tree was constructed by using the neighbor-joining method with online BLAST n. The result showed 97% similarity to (*Pediococcus parvulus* mf 221accession number kj994427), which represent identification of LAB isolated from traditional pickles of a cubuk region in Turkey. Fig. 5 representing that the interactions among the production of β-D-Glucan and two variable factor K_2HPO_4 and glutamic. The results showed increase of β-D-Glucan with increase of K_2HPO_4 and decrease of glutamic acid, this indicated the presences of indirect relationship between these factors.

3.2 Medium Optimization for β-D-glucan Production

3.2.1 Estimation of β -D-glucan production by *Pediococcus parvulus F1030*

The medium optimization in Table 4 showed that the highest yield of β-D-glucan, was11g l^{-1} with trial number 2 whereas the control was 4.5 g l^{-1} (trial 13) at fixed pH 6 and temperature 37°C.

The data in Table 4 revealed that there was an extreme variation in the β-d-glucan concentration in the 13 trials ranging from 1g l^{-1} to 11g l^{-1}.The results with medium optimization showed to higher yield when compared with yielded β-D-glucan from 4 kinds of *Paenibacillus polymyxa* isolated from Korean soil. The results showed that a novel strain JB115 had the highest yield β-D-glucan 10.6 ± 2.3 g l^{-1} the β-D-glucan

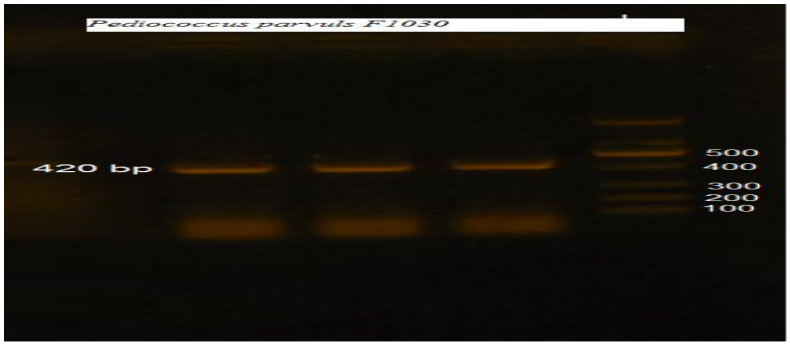

Fig. 1. PCR product electrophoresis on agarose gel 1% (30 min /70 vol.)

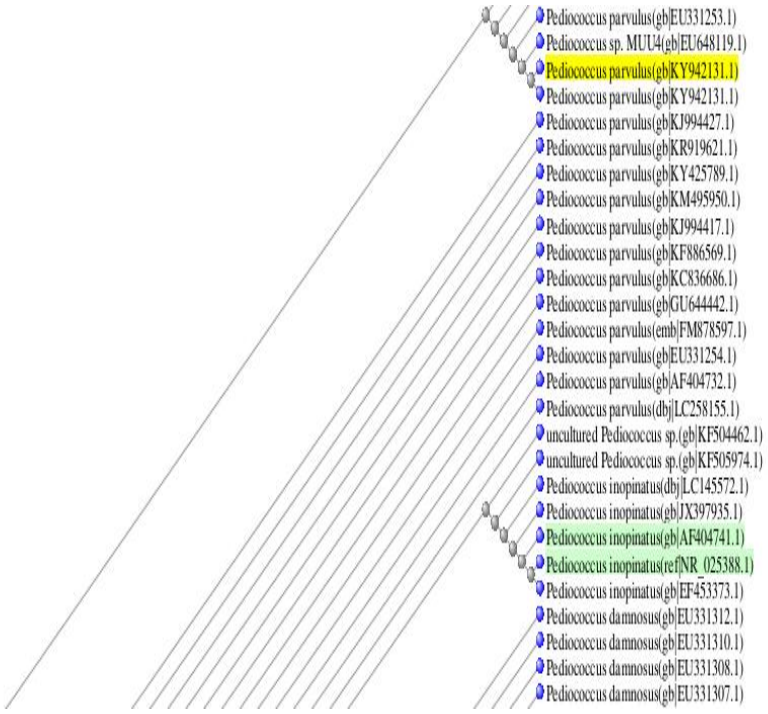

Fig. 2. Dendrogram and rectangular phylogenetic relationships for *Pediococcus parvulus*

Table 3. 16S rRNA sequence similarity for *Pediococcus parvulus* with some isolate presented in NCBI database

Sequences producing significant alignments:

Select: All None Selected:0

Alignments Download GenBank Graphics Distance tree of results

Description	Max score	Total score	Query cover	E value	Ident	Accession
Pediococcus parvulus strain F1030 16S ribosomal RNA gene, partial sequence	876	1004	100%	0.0	100%	KY942131.1
Pediococcus parvulus strain MA53 16S ribosomal RNA gene, partial sequence	752	812	100%	0.0	97%	KY425809.1
Pediococcus parvulus strain MA55 16S ribosomal RNA gene, partial sequence	752	812	100%	0.0	97%	KY425789.1
Pediococcus parvulus strain NF10 16S ribosomal RNA gene, partial sequence	752	812	100%	0.0	97%	KX010105.1

was proved to be b-(1 / 3) e and b-(1 / 6) e linked glucan structure using FT-IR, 1 H NMR and 13C NMR spectra [12]. Additionally, this variation can be attributed to the variable medium composition Fig. 3. The results in Figs. 4 and 5 revealed that the sucrose and K_2HPO_4 had highly significant positive effect while glutamic acid had the negative effect on the β-D-glucan production.

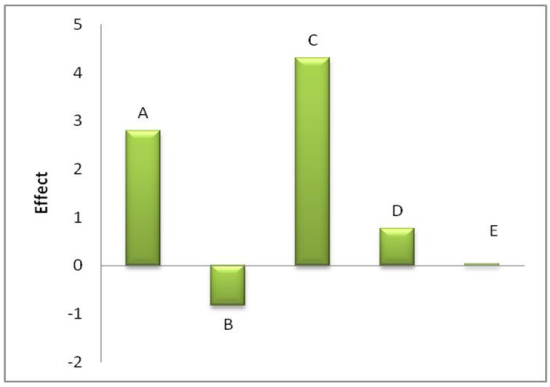

Fig. 3. Two Main effects of different medium components on the β-D-Glucan production by *Pediococcus parvulus F1030* while A (sucrose), C (K_2HPO_4), B (glutamic), D ($CaCl_2$) and E (Tween 80)

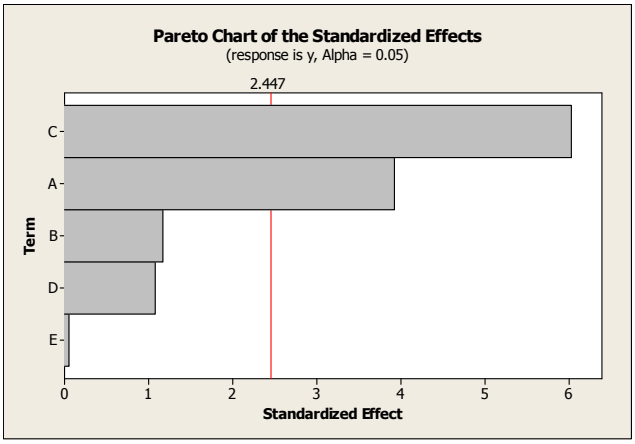

Fig. 4. Pareto chart shows the amount of effect of each medium components on the β-D-Glucan production by *Pediococcus parvulus F1030* while A (sucrose), B (glutamic) C (K_2HPO_4), D ($CaCl_2$) and E (Tween 80)

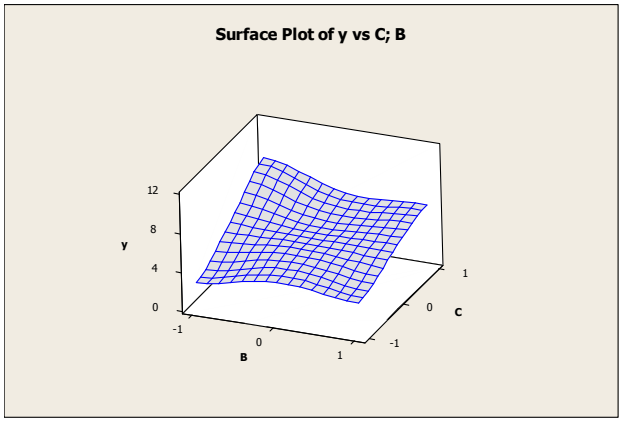

Fig. 5. Three-dimensional response surface plot for production of β-D-Glucan by *Pediococcus parvulus F1030* showing the interactive effects of medium components

Table 4. Randomized Plackett-Burman experimental design of the two levels of the five tested factors of media affecting β-D-glucan production by *Pediococcus parvulus F1030*

Run order	Sucrose	Glutamic acid	K_2HPO_4	Tween 80	$CaCl_2$	β -D-Glucan g l^{-1}
1	-1	-1	-1	-1	-1	1.2
2	1	-1	1	1	-1	11
3	1	-1	1	-1	-1	8.5
4	1	-1	-1	-1	1	4.0
5	0	0	0	0	0	5.0
6	1	1	-1	1	-1	4.2
7	-1	1	1	1	-1	4.8
8	-1	1	-1	-1	-1	1.0
9	1	1	1	-1	1	7.0
10	-1	-1	1	1	1	5.6
11	-1	1	1	-1	1	6.8
12	-1	-1	-1	1	1	3.0
13	-1	1	-1	1	1	4.5

4. CONCLUSION

Molecular characterization of isolated *Pediococcus parvulus* using 16S rDNA showed 97% identity with a similar organism in NCBI site, *gtf* gene encoding glucosyltransferase enzyme was detected by using 16S rRNA gene PCR technique with specific primers. Production of β -D-glucan from the isolate was enhanced with the positive addition of Sucrose and K_2HPO_4 and a negative addition of glutamic acid in an application of experimental design with two levels of the five tested factors of media using Plackett-Burman Design.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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