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Genetic characterization of acetic acid bacteria producing bacterial cellulose

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Abstract
The aim of the study was to determine genetic characterization of acetic acid bacteria producing the bacterial cellulose (BC) and increase the amount of production by genetic modification to be applied. Acetic acid bacteria were isolated from home-made vinegar and examined cellulose production yields. Acetic acid bacteria which product high yields of cellulose were determined as *Gluconacetobacter hansenii*. DNA isolation, plasmid extraction and amplification of DNA fragments were carried out for gene scans of these bacteria. All of genome sequence was made by Macrogen Inc. (Korea). But, the whole genome sequence wasn't obtained in the analysis performed and instead, sequences of DNA fragments on various sizes (20 bp - 150 kb) called contig could be identified. Mutant strains were obtained by UV application. It was confirmed that mutant strains produced high yield cellulose.

Keywords: cellulose synthase, gene expression, mutation, bacterial cellulose

1. Introduction
Bacterial cellulose is exopolysaccharide that it was producted by a broad group of bacteria as *Gluconacetobacter* (*Acetobacter*), *Agrobacterium*, *Aerobacter*, *Achromobacter*, *Azotobacter*, *Rhizobium*, *Sarcina*ve *Salmonella* [1]. Exopolisaccharides are branched long chain polysaccharides containingsugar derivatives as glucose, galactose, and sucrose [2]. They have many industrial applications as textile, paper, food, cosmetic, pharmacology and medical. Cellulose is the major polisaccharide among the exopolysaccharides [3]. The most important sources of cellulose are plant cellulose in the world. However, cellulose production decreases day by day because forested lands destroy with fires and unconscious logging. Therefore, bacterial cellulose has become crucial with biotechnological researches as an alternative to plant cellulose. In recent years, cellulose production has been carried out from acetic acid bacteria as *Gluconacetobacter* (*Acetobacter*) [3].
The molecular formula of bacterial cellulose is the same as that of plant cellulose, but their physical and chemical features are different [4]. Bacterial cellulose has higher purity, tensile strength and water holding capacity and degree of polymerization and crystallinity. Therefore, in industrial applications (acoustic speakers, high quality paper and dessert foods) it more suitable raw material than plant cellulose [1]. It was used as porous material which allows transfer of antibiotics or other medicines into the wound since fibrils of bacterial cellulose are about 100 times thinner than that of plant cellulose. In addition, it created efficient physical barrier against any external infection in wound area [5].
A model organism for the cellulose production is *Acetobacterxylinum* which rod-shaped, aerobic, Gram-negative bacterium [6]. Synthesis of bacterial cellulose is specifically regulated multi-step process, involving a large number of both individual enzymes and complexes of catalytic and regulatory proteins. The process includes the formation of UDPGlc, which is the precursor in the formation of cellulose, followed by glucose polymerization into the β 1-4 glucan chain [7].
The direct cellulose precursor is UDPGlc, which is a product of a conventional pathway, common in many organisms. UDPGlcpyrophosphorylase enzyme seems to be the crucial one in cellulose synthesis. Some phenotypic cellulose-negative mutants (Cel–) are deficient in this enzyme. The pyrophosphorylase activity is different among some *A.xylinum* strains [8].
Cellulose biosynthesis in higher plants or in the prokaryotes is catalyzed by UDPGlc-forming cellulose synthase and this is basically a processing of 4-β -glucosyltransferase. Cellulose synthase of *A. xylinum* has molecular mass of 400–500 kDa [9]. Lin and Brown [9] showed that the purified cellulose synthase preparations contain three different types of subunits, having molecular mass of 90, 67 and 54 kDa. However, Saxena *et al.* [10] revealed that it has only two polypeptides as 83 and 93 kDa. Saxena *et al.* [10, 11] appointed two genes as cesA and cesB in cellulose synthase operon. Wong *et al.* [12] and Ben-Bassat *et al.* [13] confirmed that these genes are BSsA and BSsB in the same operon. There are many genetic modification researches related to increase cellulose production from *A. xylinum*. This organism has cellulose synthase operon (*AxCes*operon) containing 3 or 4 genes [12, 14, 15]. Furthermore, *cmcax*v*eccpax*genes find out in the upper of the operon [16]. In this study, increasing of cellulose production and production yield as biotechnologic genetic modifications made on production from acetic acid bacteria of bacterial cellulose used industrial application as paper, textile, and food, cosmetic, medical will be achieved.

2. Material and Methods
2.1 Material
Acetic acid bacteria produced cellulose found in Adnan Menderes University Biology Department Microbiology Laboratory stocks were used as material.

2.2 The selection of the cellulose-producing bacteria
Hestrin-Schramm medium (HS) was used as basic medium. The HS medium for growth and cellulose production

comprised 20 g/l glucose, 5 g/l yeast extract, 5 g/l polypeptone, 2.7 g/lNa_2HPO_4 and 1.15 g/lcitric acid. The pH of the HS medium was adjusted to 6.0 with 1.0 M HCl. For preculture, stock culture was inoculated into 100 ml of the basal medium in a 250 ml Erlenmeyer flask, and culture was incubated at 30ºC for 48 h under static conditions. The culture was started by inoculating 1% of the culture supernatant from preculture. For static-flask fermentation, cultivations were performed at 30ºC for 7 days under static conditions. After cultivation, cultures observed formation of cellulose were selected [18]. Strainproducted the highest cellulose was determinated as *Gluconacetobacterhansenii* HE1 and carried on with this strain to research.

2.3 DNA and plasmid isolation

DNA isolation was carried out with classical phenol-chloroform method according to Gonza´lez *et al*. [19]. Gene Mark Pasmid Miniprep Purification Kit was used for plasmis isolation [20, 21].

2.4 Amplification of DNA fragments with PCR

The regions encoding cellulose synthase a gene characterizated from *Gluconacetobacterxylinus*strains were compare with Clustal W and primers were designed from homologous regions. Selected fragments of 512 bases were researched by PCR in strains inferedcellulaseactivity [12, 15].

Primers used to scan the bcsA gene were as follows:

bcsAF 5'ctatatagttggagcgctg 3'

bcsAR 5' acaaggatatagacattcac 3'

2.5 Mutant isolation

Mutant isolation was realised according to De Wulf [22]. The medium containing D-glucose (%2), yeast extract (%1), KH_2P04, (%2.5), K_2HP0_4 (%2.5), $MgSO_4.7H_2O$ (%0.5) was prepared for mutant isolation and bacteria was inoculated to medium. The cultures were incubated at 30ºC for 12 days under static conditions. After, the formed cellulose pellicle was aseptically transferred to sterile petri dish. The petri dish was placed on a rotary shaker at 30ºC under 200 rpm overnight. The cells removed from cellulose fibre and washed twice. The cell suspansion was transferred to a petri dish which was placed on a magnetic stirrer in a laminar flow cabinet. UV lamp (254 nm) was placed 10 cm above the medium surface and the mixing of the solution was performed homogeneously by stirring with a magnetic bar at 2 hours. After, a solution sample was taken and serially diluted in sterile 0.85% NaCl. From the dilution series was plated on agar based mutation medium and incubated at 30ºC for 8 days. At the end of time, the numbers of forming colonies werecounted [22].

2.6 Qualitative detection of mutant isolates

Congo red (25mg/l) and fluorescent brightener (Calcofluor White M2R) (%0, 02) HS agar medium on plates were prepared for qualitative detection of mutant isolates. Selected colonies were plated on Petries and incubated at 30ºC for 72 h. After, the forming colonies in HS Agar plates containing Calcofluor White M2R were examined under UV light (254 nm) [23].

2.7 Production and purification of cellulose from selected colonies

Production of cellulose from selected colonies was carried out according to Son *et al*. [18].The forming cellulose pellics on HS medium was separated from supernatant and was treated with 4% (w/v) NaOH solution at 80ºC for 1 h to eliminate bacterial cells or medium components. Pellicle was rinsed with 6% acetic acid until the pH of water became neutral. The purified cellulose was dried at 80ºC and then weighed [24].

3. Result and Discussion

3.1 Genome sequence analysis

DNA samples isolated for whole genome sequence was send to Macrogen Inc. (Korea). However, the whole genome sequence wasn't obtained in the analysis performed and instead, sequences of DNA fragments on various sizes (20 bp - 150 kb) calledconting could be identified. The contig-46 data was given in below as cellulose synthase operon.

Cellulose synthase operon protein D

>atgacaatttttgagaaaaaaccggatttcaccctgtttcttcagaccctgtcatgggaa
attgatgatcaggtcgggatcgaggtcaggaacgagctcctgcgtgaggtcggacgg
ggcatgggcacgcgcatcatgccgccgccgtgccagaccgtggacaagctgcagat
cgaactgaacgcgcttctggccctgatcggctggggcaccgttacgctcgaactcctca
gcgaggaccagtccctgcgcatcgtgcatgaaaacctgccgcaggttggcagcgcgg
gcgaaccttcgggcacgtggctggctccggtgctggaggggctgtatggccgctggg
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gatctgaatgctgttccgcgtcagaccatcatcatgtacatgcgcgtgcgcagttccgcg
acctga

MTIFEKKPDFTLFLQTLSWEIDDQVGIEVRNELLREVGR
GMGTRIMPPPCQTVDKLQIELNALLALIGWGTVTLELL
SEDQSLRIVHENLPQVGSAGEPSGTWLAPVLEGLYGRW
VTSQAGAFGDYVVTRDVDAEDLNAVPRQTIIMYMRVR
SSAT

Cellulose synthase operon protein C

>atgctgcatgcgcatatggcgctgcgcatcgcctccacgcgcaatattgacctgacga
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TEVEGGLGANTLVYRNRKHEVRVGVNLTYFGYKHNE
DFYTYGQGGYFSPQSYFAATVPVRYSGHSGLFDWDVT
GSIGYQLFHEHSSAFFPTNPVYQALANGLAGVSTAELSL
ESARYPGDDVGSLVGGFDGRVGYRVSHSLRLDLSGRF
QKAGNWDEGGAMISAHYLIMDQ

Cellulose synthase operon protein C

>gtgacccataaacgatatgcttcgtccctgtccgccggtcttctcgcaacgacctgcgt
cgcaggtctgttgctgcaggcgaacggcgcacgggcacagcaggcggcagaggcg
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RAILARNGDNPIALMGLARVLMGEGQENEANALLSRL
GGRYSDQVQQIEVSGIMAEAARTSDSAQKVSLLRQAM
TKAPDDPWLRINLANALQQQGDSAEAANVMRPLLTSP
RTPADYQAAILYASGNGNDTLARRLLAGLSPDDYSPAI
RTIADEMAIKADLASRLSMVSNPTPLVREX

Cellulose synthase catalytic subunit [UDP-forming]

atggggaaaattctttccattcgcggtgctgggctgattattggtgtttttggcctgtgtgcg
ctgattgcggctacgtccgtgaccctgccgccagaacagcagttgattgtggcatttgta
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VCVVIFFIVGHKPSRRSQIFLEVLSGLVSLRYLTWRLTET
LSFDTWLQGLLGTMLLVAELYALMMLFLSYFQTIAPLH
RAPLPLPPNPDEWPTVDIFVPTYNEELSIVRLTVLGSLGI
DWPPEKVRVHILDDGRRPEFAAFAAECGANYIARPTNE
HAKAGNLNYAIGHTDGDYILIFDCDHVPTRAFLQLTMG
WMVEDPKIALMQTPHHFYSPDPFQRNLSAGYRTPPEGN
LFYGVVQDGNDFWDATFFCGSCAILRRTAIEQIGGFAT
QTVTEDAHTALKMQRLGWSTAYLRIPLAGGLATERLIL
HIGQRVRWARGMLQIFRIDNPLFGRGLSWGQRLCYLSA
MTSFLFAVPRVIFLSSPLAFLFFGQNIIAASPLALLAYAIP
HMFHAVGTASKINKGWRYSFWSEVYETTMALFLVRVT
IVTLLSPSRGKFNVTDKGGLLEKGYFDLGAVYPNIILGLI
MFGGLARGVYELSFGHLDQIAERAYLLNSAWAMLSLII
ILAAIAVGRETQQKRNSHRIPATIPVEVANADGSIIVTGV
TEDLSMGGAAVKMSWPAKLSGPTPVYIRTVLDGEELIL
PARIIRAGNGRGIFIWTIDNLQQEFSVIRLVFGRADAWV
DWGNYKADRPLLSLMDMVLSVKGLFRSSGDIVHRSSP
TKPSAGNALSDDTNNPSRKERVLKGTVKMVSLLALLTF
ASSAQAASAPRAVAAKAPAHQPESSDLPPLPALLPATS
GAAQAGAGDAGANGPGSPTGQPLAADSADALVENAE
NTSDTATVHNYTLKDLGAAGSITMRGLAPLQGIEFGIPS
DQLVTSARLVLSGSMSPNLRPETNSVTMTLNEQYIGTL
RPDPAHPTFGPMSFEINPIFFVSGNRLNFNFASGSKGCSD
ITNDTLWATISQNSQLQITTIALPPRRLLSRLPQPFYDKN
VRQHVTVPMVLAQTYDPQILKSAGILASWFGKQTDFL
GVTFPVSSTIPQSGNAILIGVADELPTSLGRPQVNGPAVL
ELPNPSDANATILVVTGRDRDEVITASKGIAFASAPLPT
DSHMDVAPVDIAPRKPNDAPSFIAMDHPVRFGDLVTAS
KLQGTGFTSGVLSVPFRIPPDLYTWRNRPYKMQVRFRS
PAGEAKDVEKSRLDVGINEVYLHSYPLRETHGLIGAVL
QGVGLARPASGMQVHDLDVPPWTVFGQDQLNFYFDA
MPLARGICQSGAANNAFHLGLDPDSTIDFSRAHHIAQM
PNLAYMATVGFPFTTYADLSQTAVVLPEHPNAATVGA
YLDLMGFMGAATWYPVAGVDIVSADHVSDVADRNLL
VISTLATSGEIAPLLSRSSYEVADGHLRTVSHASALDNAI
KAVDDPLTAFRDRDSKPQDVDTPLTGGVGAMIEAESPL
TAGRTVLALLSSDGAGLNNLLQMLGERKKQANIQGDL
VVAHGEDLSSYRTSPVYTIGTLPLWLWPDWYMHNRPV
RVLLVGLLGCILIVSVLARALARHATRRFKQLEDERRK
S

3.2 Isolation of mutant strains

The *Gluconacetobacter hansenii* HE1 by exposure to UV for to increase cellulose production the mutant strains were obtained. Selected colonies were plated on HS Agar plates containing Congo red and Calcofluor White M2R and incubated at 30ºC for 72 h. After incubation, the plates were examined and the numbers of colonies given in Table 1. While cellulose producing mutants were isolated as dark red on HS agar plates containing Congo red, cellulose-negative mutants were isolated as white colonieson HS-Congo red plates (Fig. 1). In addition, cellulose producing colonies were fluoresced when observed under UV-light (Fig. 2).

Table 1: The numbers of colonies of mutant strains.

Mutant Isolates	HS Agar Plates Containing Congo Red (25mg/l)	HS Agar Plates Containing Calcoflour (%0.02)
Red Colonies	90	-
White Colonies	210	-
Fluoresce	-	36
Non-Fluoresce	-	180

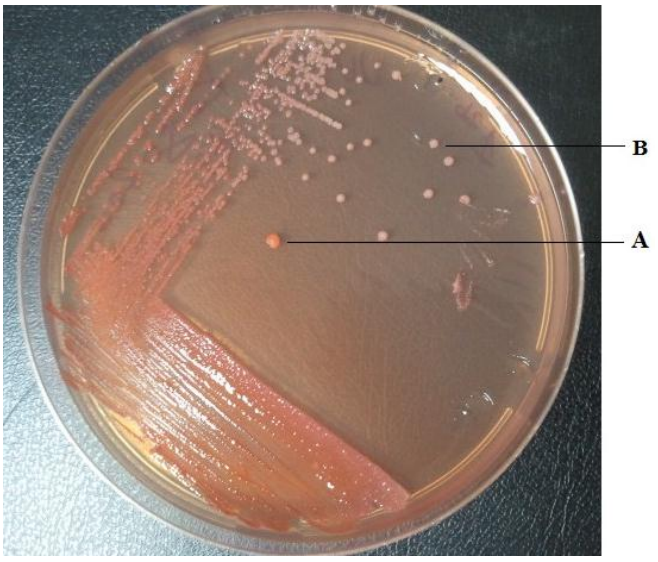

Fig 1: Image of colonies on HS Agar plates containing congored (25mg/l). A. Cellulose producing mutants as dark red B. Cellulose-negative mutants as white colonies.

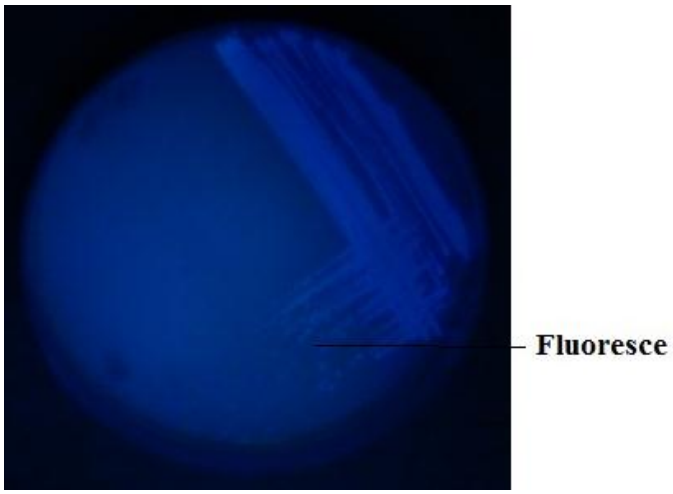

Fig 2: Image of colonies on HS Agar plates containing Calcoflour (%0.02). Cellulose producing mutants were fluoresced.

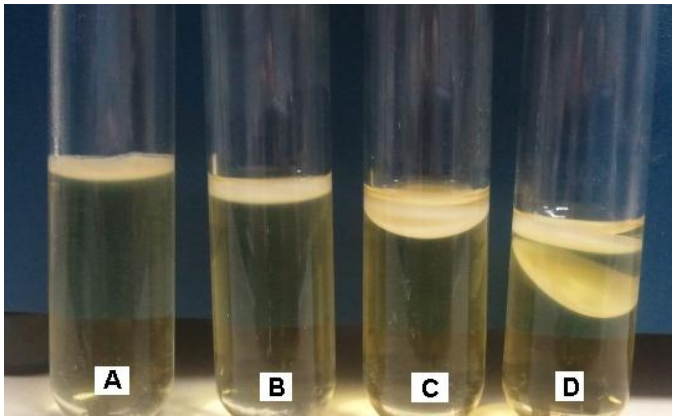

Fig 3: Image of cellulose production yield of mutant strains and wild strain. A. Control group, B. Mutant strain 90, C. Mutant strain 97, D. Mutant strain 95

Cellulose production yield of 126 mutant strains were investigated and compared with wild strain as control group. Among these strains, 13 mutant strains showed high cellulose yield (Fig. 3). It was showed that the mutant strains produced more cellulose than the control group as Figure 3.

3.3 Genome sequencing analysis all of mutant strain

Genome sequencing analysis all of mutant strainwas carried out by DONE Genetik & Biyoinformatik A.Ş. Estimated genome size of mutant *Gluconacetobacterhansenii* HE1 strain was 3.6 Mb. In the library preparation step illumine Nextera XT Library Preparation Kit (Cat no: FC-131-1024) was used in this analysis. DNA samples were sequenced in illumine MiSeq ® platform. Ultimately, 719.898 fragment reading and 65x reading deep were obtained in this analysis. The obtained data were seperated to gene regions and aligned as conting. The hypothetical feature genes were excluded to analysis and residual 3129 geneswas examined. Total length of the aligned region is 2.8 Mb. Alignment CLC Genomics Workbench is carried out with the program and the following parameters are used.

Mismatch cost = 4
Cost of indels = Linear gap cost
Insertion cost = 3
Deletion cost = 3
Insertion open cost = 6
Insertion extend cost = 1
Deletion open cost = 6
Deletion extend cost = 1
Length fraction = 0.7
Similarity fraction = 0.8
Global alignment = Yes

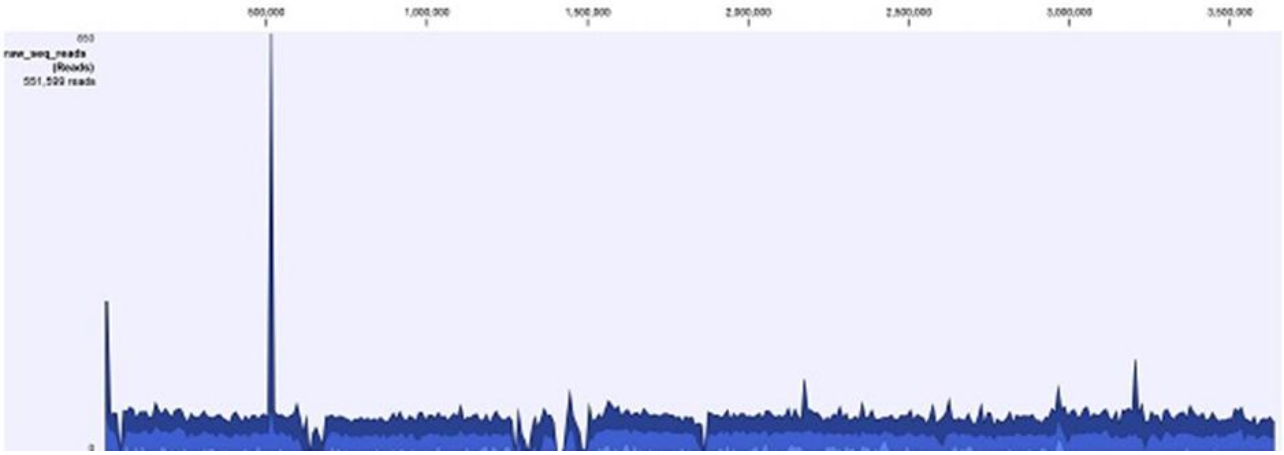

Fig 4: Diagram the depth of the alignment after align.

After align, variant analysis among obtained readings with reference contins was carried out by CLC Genomics Workbench programand the following parameters are used. The obtained variants were given Table 2.

Required variant probability= %90.0
Minimum Coverage = 10
Minimum Frequency = %70
Insertion cost = 3
Deletion cost = 3
Neighborhood radius = 5
Minimum central quality = 20
Minimum neighborhood Q = 15
Read direction filter = Yes
Direction frequency (%) = 5.0
Required variant probability= %90.0

Table 2: List of determined variants in DNA samples.

Target Contig	Reference Location	Type	Lenght	Reference	Allel
contig_32_3905_1284_diguanylate_cyclase/phosphodiesterase_(GGDEF_&_EAL_domains)_with_PAS/PAC_sensor(s) mapping	691	SNV	1	A	C
contig_39_25013_27109_Hemeregulated_cyclic_AMP_phosphodiesterase_(EC_3.1.4.-) mapping	2053	Insertion	4	-	CGCA
contig_39_63511_61448_COG0464:_ATPases_of_the_AAA+_class mapping	870	Insertion	1	-	G
contig_26_10029_12077_ClpB_protein mapping	1328	Insertion	4	-	TCAA
contig_26_10029_12077_ClpB_protein mapping	1329	Insertion	3	-	CTT
contig_30_59258_57588_Hemeregulated_cyclic_AMP_phosphodiesterase_(EC_3.1.4.-) mapping	1537	Insertion	4	-	ATCT
contig_54_1594_353_Arabinose_efflux_permeasemapping	1055	Deletion	1	N	-

In studies conducted in recent year's mutation and genetic engineering applications were used to increase cellulose production yield. De Wulf *et al*. [22] showed that obtained mutant strain by UV was producted more cellulose (3,3 g/l) than wild strain. Mutant sucrose synthase gene expression was incresed twice cellulose production [25]. Dgc1 gene was sequenced and obtained mutant strain was producted %36 more cellulose than wild strain [26]. DNA fragments in 14,5 kb lenght of strains as *A. xylinum* ATCC 23769 and ATCC 53582 were cloned and determined nucleotitesequance. DNA fragments sequancedcontain genes as endo-ß-1, 4-gluconaz, cellulose complement protein, cellulose synthase subunits (A, B, C, D) and ß-glucosidaz. At the end of 7 days of incubation, A. *xylinum* ATCC 53582 producted more fivefold cellulose than *A. xylinum* ATCC 23769 [27]. CMC*ax* protein to understand the structural and functional relationship of which endoglukonaz (CMC*ax*) gene is responsable from cellulose synthase and hydrolysis was researched by Kawano *et al*. [28]. The addition of CMC*ax* protein to the culture medium, cellulose production was increased by *A. xylinum*53582 [28]. Gene fragment encoding pyrroloquinolinequinone glucose dehydrogenase from strain *Gluconacetobacterxylinus* BPR2001was cloned by Shigematsu *et al*. [29]. Mutant of this strain without glutamate dehydrogenase was called as GD-I. Mutant GD-I strain producted 1,7 fold more cellulose than wild strain [6, 29].

4. Conclusion

Cellulose production capacities of acetic acid bacteria isolated from wine and vinegar fermentation are different. In vitro factors as pH and temperature with the carbon and nitrogen sources used in the development of bacteria effects cellulose production yield. However, the modifications in responsable genes from cellulose production increase production yield. Due to increase in the amount of product with these genetic modifications, production of cellulose used in many industrial applications has great importance in terms of biotechnological studies.

In this study, genome sequence of *Gluconacetobacter hansenii* HE1isolated from home-made vinegar was analysed. In additional mutant strains were obtained UV exposured to bacteria to ensure an increase in the cellulose production. As a result, used genetic modifications increased the cellulose production.

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References

1. Shoda M, Sugano Y. Recent advances in bacterial cellulose production. Biotechnology and Bioprocess Engineering. 2005; 10:1-8.
2. Sutherland IW. Structure-functionrelationship in microbialexopolysaccharides. Biotechnology Advances, 1994; 12:393-448.
3. Sutherland IW. Novel and established applications of microbial polysaccharides, Trends in Biotechnology. 1998; 16:41-46.
4. Yoshinaga F, Tonouchi N, Watanabe K. Research progress in production of bacterial cellulose by aeration and agitation culture and its application as a new industrial material. Bioscience, Biotechnology, and Biochemistry. 1997; 61:219-224.
5. Czaja W, Krystynowicz A, Bielecki S, Brown RMJR. Microbial cellulose – The natural power to heal wounds. Biomaterials. 2006; 27:145-151.
6. Chawla PR, Bajaj IB, Survase SA, Singhal RS. Fermentative production of microbial cellulose. Food Technology and Biotechnology. 2009; 47:107-124.
7. Saxena IM, Brown RMJR, Fevre M, Geremia RA, Henrissat B. Multi domain architecture of beta glycosyltransferases: implications for mechanism of action. Journal of Bacteriology. 1995; 177:1419-1424.
8. Valla S, Coucheron DH, Fjaervik E, Kjosbakken J, Weinhouse H, Ross P, *et al*. Cloning of a gene involved in cellulose biosynthesis in *Acetobacter xylinus*: complementation of cellulose negative mutant by UDPG pyrophosphorylase structure gene. Molecular Genetics and Genomics. 1989; 217:26-30.
9. Lin FC, Brown RMJR. Purification of cellulose synthase from *Acetobacter xylinum*, in: Cellulose and Wood Chemistry and Technology, Schmerck C., Ed., New York: John Wiley & Sons, 1989; 473-492.
10. Saxena IM, Roberts EM, Brown RM JR. Modification of cellulose normally synthesised by cellulose-producing microorganisms. U.S. Patent 4950597, 1990b.
11. Saxena IM, Lin FC, Brown RM JR. Identification of a new gene in an operon for cellulose biosynthesis in *Acetobacterxylinum*. Plant Molecular Biology. 1991; 16:947-954.
12. Wong HC, Fear AL, Calhoon RD, Eichinger GH, Mayer R, Amikam D, *et al*. Genetic organization of the cellulose

synthase operon in *Acetobacter xylinum*. Proceedings of the National Academy of Sciences of the United States of America. 1990; 87:8130-8134.
13. Ben-Bassat A, Calhoon RD, Fear AL, Gelfand DH, Meade JH, Tal R, *et al*. Methods and nucleic acid sequences for the expression of the cellulose synthase operon, U.S. Patent 5268274; 1993.
14. Saxena IM, Kudlicka K, Okuda K, Brown RMJR. Characterization of genes in the cellulose-synthesizing operon (acsoperon) of *Acetobacter xylinum*: Implications for cellulose crystallization. Journal of Bacteriology, 1994; 176:5735-5752.
15. Nakai T, Moriya A, Tonouchi N, Tsuchida T, Yoshinaga F, Horinouchi S, *et al*. Control of expression by the cellulose synthase (bcsA) promoter region from *Acetobacter xylinum* BPR 2001. Gene, 1998; 213:93-100.
16. Standal R, Iversen TG, Coucheron DH, Fjaervik E, Blatny JM, Valla S. A new gene required for cellulose production and a gene encoding cellulolytic activity in *Acetobacter xylinum* are colocalized with the bcs operon, Journal of Bacteriology. 1994; 176:665-672.
17. Hestrin S, Schramm M. Synthesis of cellulosby*Acetobacter xylinum*: preparation of freze dried cells capable of polymerizing glucose to cellulose. Biochemical Journal. 1954; 58:345.
18. Son HJ, Heo MS, Kim YG, Lee SJ. Optimization of fermentation conditions for the production production of bacterial cellulose by a newly isolated *Acetobacter* sp. A9 in shaking cultures. Biotechnology and Applied Biochemistry. 2001; 33:1-5.
19. Gonza´lez A, Hierro N, Poblet M, Mas A, Guillamon JM. Application of molecular methods to demonstrate species and strain evolution of aceticacid bacteria population during wine production. International Journal of Food Microbiology. 2005; 102:295-304.
20. Grones J, Turňa I. Transformation of microorganisms with the plasmid vector with the replicon from pAC1 from *Acetobacter pasteurianus*. Biochemical and Biophysical Research Communications. 1995; 206:942-947.
21. Winfrey MR, Rott MA, Wortman AT. Unraveling DNA: MolecularBiologyforthe Laboratory, Prentice Hall, 1997.
22. De Wulf P, Joris K, Vandamme EJ. Improved cellulose formation by an *Acetobacter xylinum* mutant limited in(keto)gluconate synthesis. Journal of Chemical Technology and Biotechnology. 1996; 67:376-380.
23. Ross P, Mayer R, Benziman M. Cellulosebiosynthesisandfuction in bacteria. Microbiology Reviews. 1991; 55:35-58.
24. Ishihara M, Matsunaga M, Hayashi N, Tisler V. Utilization of D-xylose as carbon source for production of bacterial cellulose. Enzyme and Microbial Technology. 2002; 31: 986-991.
25. Nakai T, Tonouchi N, Konishi T, Kojima Y, Tsuchida T, Yoshinaga F, *et al*. Enhancement of cellulose production by expression of sucrose synthase in *Acetobacter xylinum*. Proceedings of the National Academy of Sciences. 1999; 96:14-18.
26. Bae S, Sugano Y, Shoda M. Improvement of bacterial cellulose production by addition of agar in a jarfermentor. Journal of Bioscience and Bioengineering. 2004; 97:33-38.
27. Kawano S, Tajima K, Uemori Y, Yamashita H, Erata T, Munekata M, *et al*. Cloning of cellulose synthesis related genes from *Acetobacter xylinum* ATCC 23769 and ATCC 53582: Comparison of cellulose synthetic ability between strains. DNA Research. 2002; 9:149-156.
28. Kawano S, Yasutake Y, Tajima K, Satoh Y, Yao M, Tanaka I, *et al*. Crystallization and preliminary crystallographic analysis of the cellulose biosynthesis-related protein CMCax from *Acetobacter xylinum*, Acta Crystallographica Section F Structural Biology and Crystallization Communications. 2005; 61:252-254.
29. Shigematsu T, Takamine K, Kitazato M, Morita T, Naritomi T, Morimura S, *et al*. Cellulose production from glucose using a glucose dehydrogenase gene (gdh)-deficient mutant of *Gluconacetobacter xylinus* and its use for bioconversion of sweet potato pulp. Journal of Bioscience and Bioengineering. 2005; 99:415-422.