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Genetic Fingerprinting and Plasmid Content of *Acetobacter xylinum* Strains Producing Bio-Cellulose

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Abstract: Cellulose is the earth's major biopolymer and becoming tremendous economic importance globally. The bacterial cellulose has significant advantages over natural cellulose. Bacterial cellulose fibers can be oriented in regular or randomly depending on the type of incubation period for *Acetobacter* sp. and other bacterial genera synthesize cellulose. *Acetobacter xylinum* produces sufficient amount of cellulose to warrant commercial interest. Most scientists focus in its production because it produces ultrafine cellulose fibers. In this study our experiments were carried out to compare and screen five isolated and identified *Acetobacter xylinum* strains for cellulose yields. Some of strains showed the highest productivity, plasmid isolation indicated the *Acetobacter xylinum* under study contain four different pattern of plasmid DNA molecules. Molecular fingerprinting using RAPD-PCR was done and showed differences in RAPD patterns produced by our used strains.

Key words: *Acetobacter xylinum* · Bacterial Cellulose · Plasmids · RAPD-PCR

INTRODUCTION

Different genera of bacteria produce cellulose but the most efficient cellulose producing bacteria are *Acetobacter xylinum* [1]. The investigation of bacterial cellulose encourages scientists long time ago to keep pace for commercial applications [2-6]. Several researchers found that *A. xylinum* produces cellulose microfiber formation when the culture conditions are optimized [2, 7, 8]. *A. xylinum* for hundred years produces cellulose and receive attention from researchers and scientists to limit the commercial application [2, 9].

Mutation plays an important role in different microbial productivities, several investigators attempt to induce variants of mutation with different mutagens in *A. xylinum* and isolate different mutants with high rate of cellulose production [10-13].

Isolation of plasmids from *A. xylinum* has also been done. Valla *et al*. [12], Valla *et al*. [14] and Rezaee *et al*. [15] found that the plasmids harboring *A. xylinum* are playing a role in cellulose biosynthesis, they found also no differences between the number of plasmids and cellulose production. The plasmid genes probably have little involvement in cellulose synthesis in *A.* xylinum. The conjugation between two strains leads to transfer of a broad range of plasmids, making them capable of being genetically transformed [16, 17].

The Random Amplified Polymorphic DNA (RAPD) method is fast and sensitive method detect polymorphism within various genomes [18, 19]. This technique is used to amplify random segment of DNA genome with random primers of arbitrary nucleotide by PCR. The produced banding patterns mean alteration of DNA [20-23].

The objective of the present study was to compare cellulose production yield by some *Acetobacter xylinum* strains and identify the plasmid patterns harboring strains. We also aimed to determine the genetic variation among used strains using RAPD-PCR. The promising

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strains in this study may be used in various economic industrial applications and for successive genetic improvement as well.

MATERIALS AND METHODS

Bacterial Strains and Culture Media: Five *Acetobacter xylinum* were previously isolated and identified kindly obtained from Genetics laboratory, Faculty of Science, King Abdul Aziz University was used in this study, cultured on Luria-Bertani agar plates (LB) containing (in g/L): peptone, 10.0; yeast extract, 5.0; NaCl, 5.0; agar 20 with pH adjusted to 7.2.

Cultivation of the bacterium for cellulose production was done in the 150ml liquid culture of HS described by Hestrin and Schramm [24] and modified by Hungund and Gupta [25] for 10 days.

Harvesting and Weighing of BC: The cell mass was estimated by measuring the optical density at 600 and 650 nm as indicated in Table (1). The BC membranes pellicles produced in the surface of the cultivation medium were harvested by centrifugation of the cell culture at 8000rpm for 10 min. After harvesting, the BC were washed with distilled water and soaked in 0.1 M NaOH to remove bacterial cells possibly attached to the BC pellicles. Then, the pellicles were washed again with deionized water several times to warrant the complete remove of the alkali, leaving the pellicle at neutral pH. The purified cellulose was dried at 80°C for 12 h. in empty petri dish until reaching a constant mass [26]. The mass of the BC pellicles were determined using a precision balance to compare the quantity of the obtained cellulose with the all used strains. The cellulose mass were quantified after subtraction the weight of the empty petri dish.

DNA and plasmid isolation from *Acetobacter xylinum* strains under study according to Rezaee *et al*., [15].

RAPD-PCR Analysis: A set of three random primers was used to detect the fingerprinting of *Acetobacter xylinum* under study and illustrated in Table (3). The amplification reaction was carried out in 25 µL reaction volume containing 1×PCR buffer, 1.5 mM MgCl2, 2 mM dNTPs, 1 U Taq DNA polymerase, 25 mg templates DNA and 1µM primer from each of used random primers. The PCR amplification was performed in a thermal cycles programmed to fulfill 40 cycles after an initial denaturation cycle for 5 min at 94°C. Each cycle consisted of a denaturation step at 94°C for 1 min, an annealing step at 36°C for 1 min and an elongation step at 72°C for 1.5 min. The primer extension segment was extended to 7 min at 72°C in the final cycle (Ref.??). The amplification products were resolved by electrophoresis in a 1.5% agarose gel containing ethidium bromide (0.5 µg) in 1×TBE buffer at 90 volts. Gel was photographed under UV light with gel documentation system.

RESULTS

The cell mass was estimated after growth the *Acetobacter xylinum* for 10 days by measuring the optical density at 600 and 650 nm. as indicated in Table 1. Results showed that no significance differences between used strains except the *Acetobacter xylinum* 4which was the best growing strain it reached OD of 1.227 at 600 nm. and 1.195 at 650nm.

Our experiment was carried out to compare the used *Acetobacter xylinum* for cellulose yields. Our results showed that strains *Acetobacter xylinum* 2, *Acetobacter xylinum* 3, *Acetobacter xylinum* 4 and *Acetobacter xylinum* 5 yielded the highest BC, 1.36, 1.04, 1.16 and 1.24 gm respectively. The lowest cellulose production was by *Acetobacter xylinum* 1, it weighted 0.88 gm. as indicated in Table 2 and Fig. 1. This result provides that the used *Acetobacter xylinum* strains are more suitable for BC production and the higher production rates can be achieved from three of the used strains.

DNA and Plasmid Isolation from *Acetobacter xylinum* Strains: Genomic DNA and plasmid extraction was done and the yield of obtained DNA and plasmid with each *Acetobacter xylinum* are displayed in Fig. 2.

Results indicated that successful DNA and plasmid isolation, this result demonstrate that the band patterns observed in agarose gel electrophoresis are good and clear. All studies strains were harboring plasmids with different molecular weights. All used strains contain four different molecular weight plasmids with different intensity. This result of pending patterns explain that the used *Acetobacter xylinum* contain complex pattern of plasmid DNA molecules. Results also proved that the used extraction method was suitable for isolation of plasmids in *Acetobacter xylinum* strains.

Molecular Fingerprinting of the Five Used *A. xylinum.* *By* RAPD-PCR: In the present study, extracted DNA was used for RAPD-PCR based techniques. The isolated DNA was adopted for study the variation between the five used *A. xylinum*. The random primer used gave reproducible results, this results represented that the DNA template are pure and good for RAPD analysis and the used primers success to amplify extracted DNA as indicated in Fig. 3.

Table 1: Optical density of *Acetobacter xylinum* strains after growing in HS medium for 10 days

Strains	Absorbance at 600	Absorbance at 650
*Acetobacterxylinum*1	0.678	0.628
*Acetobacterxylinum*2	0.146	0.167
*Acetobacterxylinum*3	0.171	0.190
*Acetobacterxylinum*4	1.227	1.195
*Acetobacterxylinum*5	0.146	0.166

Table 2: Weight of cellulose produced by each of used *Acetobacter xylinum* strains

Bacterial strains	Weight of produced cellulose (gm)
*Acetobacter xylinum*1	0.88
*Acetobacter xylinum*2	1.36
*Acetobacter xylinum*3	1.04
*Acetobacter xylinum*4	1.16
*Acetobacter xylinum*5	1.24

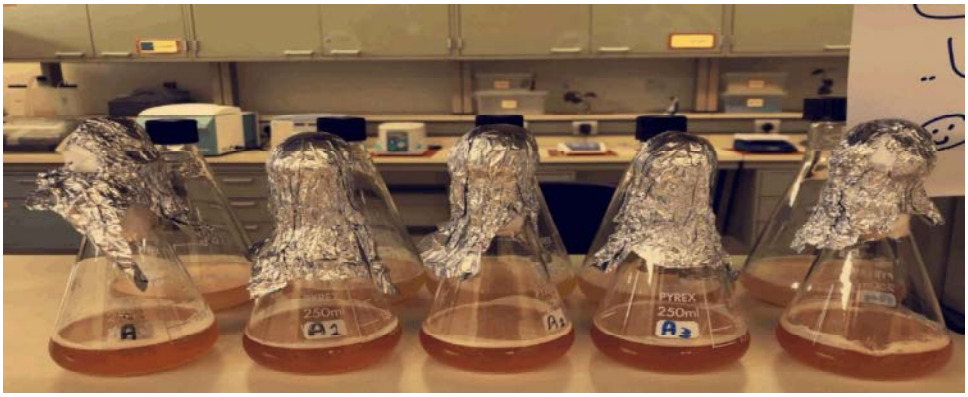

Fig. 1: Cellulose production by each *Acetobacter xylinum* strains after growing in HS medium for 10 days (A: Strain No.1, A1: Strain No.2, A2: Strain No.3, A3: Strain No.4 and A4: Strain No.5)

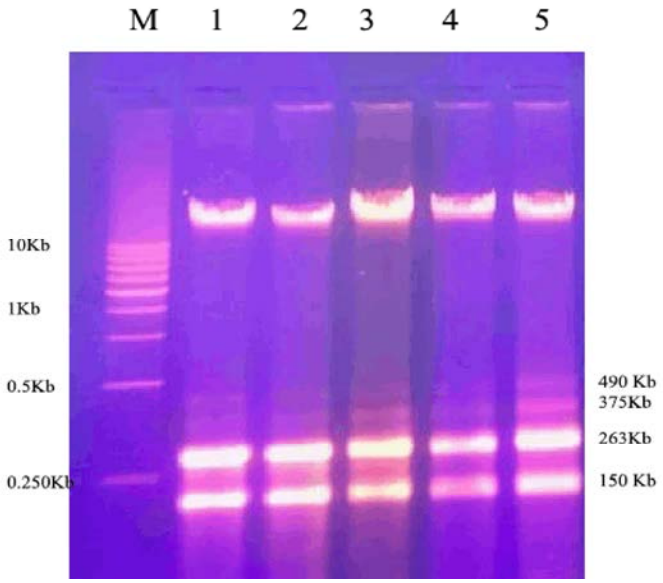

Fig. 2: DNA and plasmid patterns in five *Acetobacter xylinum* under study, (M: DNA molecular weight marker from 10 Kb to 250 bp, Lane 1, 2 , 3, 4 and 5 represents *Acetobacter xylinum* strains 1, 2, 3, 4 and 5 respectively. All studied strains contain 4 plasmid patterns with at the same molecular weight (150, 263, 375 and 490 Kb)

Table 3: Used primer sequences and number of bands produced by each *A. xylinum* strains under study

	`*A. xylinum* strains				
	No. of bands produced by each primer and its molecular weight by(bp)				
Primer sequence	Strain 1	Strain 2	Strain 3	Strain 4	Strain 5
OPA-09	3520.564	4282.228	4282.228	4282.228	3520.564
5' GGGTAACGCC3'	2778.126	3520.564	3520.564	3520.564	2778.126
	1929.769	2778.126	2778.126	2778.126	1929.769
	1358.918	1929.769	1929.769	1929.769	1358.918
	683.129	1358.918	1358.918	1358.918	1072.341
		683.129	683.129	683.129	683.129
OPA-10	2924.939	3645.466	3645.466	3645.466	3247.755
5'GTGATCGCAGG3'	2167.648	2924.939	2924.939	2924.939	2924.939
	1436.344	2167.648	2167.648	2167.648	2605.835
	829.758	1436.344	1436.344	1436.344	2167.648
	444.346	885.467	1060.627	829.758	
		444.346	829.758	444.346	
			444.346		
OPA-11	2066.063	2066.063	2066.063	2066.063	2066.063
5' CAATCGCCGT3'	1486.128	1486.128	1486.128	1486.128	1486.128
	757.227	757.227	757.227	757.227	757.227
OPA-13	4089.038	4089.038	4089.038	4089.038	5545.599
5' CAGCACCCAC3'	2755.793	2755.793	2755.793	2755.793	4089.038
	2011.781	2011.781	2011.781	2011.781	2868.144
	729.815	729.815	729.815	729.815	
OPA-18	2500.590	1078.117	3611.964	3611.964	3177.533
5' AGGTGACCGT3'	1610.220		2939.098	2939.098	887.107
	1078.117		2500.590	2500.590	
			1610.220	1610.220	
			1078.117	1078.117	

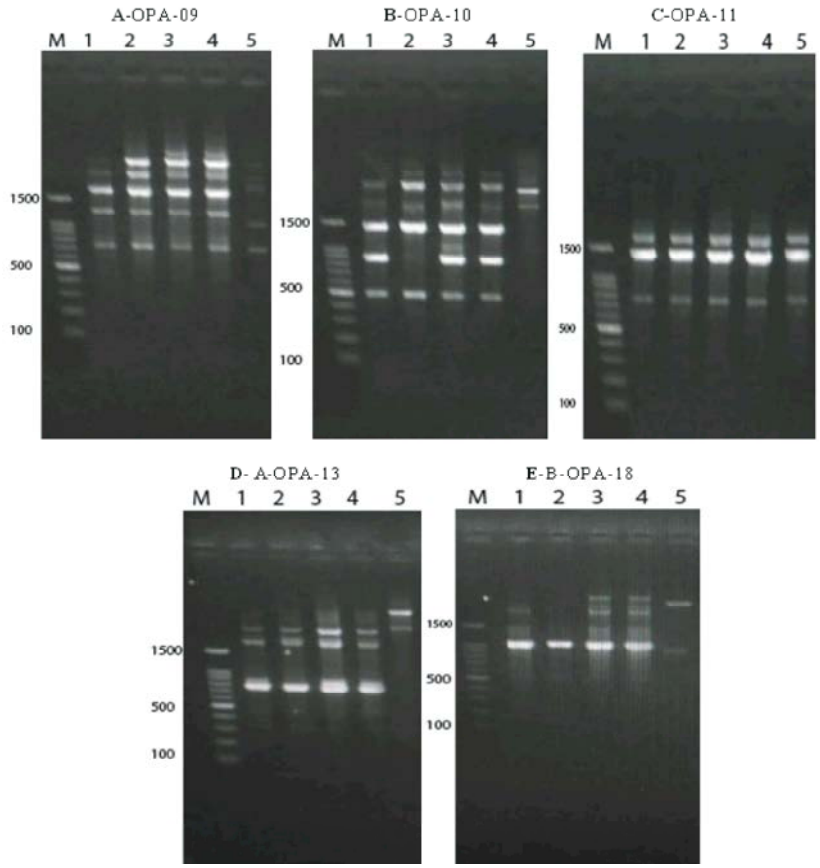

Fig. 3: Shows the RAPD-PCR products resulting from amplification with all used primers; A banding patterns produced with OPA-09 primer with the five used strains; B: banding patterns produced with OPA-10; C: banding patterns produced with OPA-11; D: banding patterns produced with OPA-13; E: banding patterns with OPA-18. The number of bands produced by each strain with each used primer are indicated in Table 3

The six RAPD primers amplified all used five *A. xylinum* strains and the number of produced bands by each primer is indicated also in Table 3. RAPD was carried out with the five used primers OPA-09, OPA-10, OPA-11, OPA-13 and OPA-18 generated different fragments at different molecular weight sizes. The molecular weight of each produced bands with each primer are indicated in Table 3. The primer OPA-11 generates fragments at the same molecular weight with all five *A. xylinum*. This result indicated that this primer will be used to fingerprint *A. xylinum* at the same molecular weight.

DISCUSSION

Our experiments revealed that our used *A. xylinum* strains are more suitable for commercial scale of cellulose production, as higher production rates can be achieved. Bacterial celluloses produced from all strains are suitable to apply to many applications. Therefore the bacterial *A. xylinum* production of cellulose is a suitable option to replace other cellulose producers such as plants, these results are in-agreement with [27-30].Isolation of plasmids has been done and the results demonstrated that the banding patterns are similar in all strains which suggested that these strains have the same genetic background. All studies strains harboring plasmids with different molecular weight. The plasmid content of used strains suggested that the *Acetobacter xylinum* strains in our study contain complex pattern of plasmid DNA molecules, the plasmids in *Acetobacter xylinum* play an important role in *Acetobacter xylinum* BC biofilm as indicated by Valla *et al*. [14], Coucheron [31] and Rezaee *et al*. [32]. In recent years the RAPD-PCR technique has been used for the molecular analysis of bacterial strains. Pattern produced by RAPD- PCR is used to detect DNA alterations within the DNA molecule. It can be used for estimation of genome variation. We have used the RAPD method to obtain genomic DNA fragments of the strains under study. The banding pattern obtained of amplified isolated DNA by the used random primers allowed us to detect the variation of these strains, the same results are supported by the work of Jamal *et al*. [23], Basavaraju *et al*. [33] and Abdalnabi and Essam [34].

CONCLUSIONS

This study showed that the bacterial cellulose derived from *Acetobacter xylinum* is suitable for a preliminary study and more studies for over production through molecular genetic tools are needed for production at commercial scale. The plasmid isolation protocol used is an effective for isolation of plasmid with *Acetobacter xylinum* strains. RAPD-PCR also proved that variation at molecular level among used strains.

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