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Bacterial Cellulose Production as Affected by Bacterial Strains and Some Fermentation Conditions

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Abstract: Thirty nine of cellulose producing bacteria were isolated from some rotten fruits and tested for cellulose production. One isolate was selected as high effect cellulose producing bacteria and completely identified as *Komagataeibactersaccharivorans* PE 5 through 16S ribosomal DNA sequencing and morphological, culture and biological characteristics. This isolate in addition to *Acetobacterxylinum* ATCC 10245(as a reference strain) were tested for cellulose production using static culture technique. In a series of experiments, two modifications in GAM medium were recommended to obtain the maximum growth and cellulose production by*Komagataeibactersaccharivorans* PE 5 and*Acetobacterxylinum* ATCC 10245 on the first and second modification media, respectively. Modified GAM medium No.1 contains mannitol, 15; trypton,6; ethanol 0.7% (v/v); acetic acid 0.2% (v/v); nicotinic acid, 0.0002 and$CaCl_2.2H_2O$, 0.1gl^{-1}.Whereas the contains of modified GAM medium No.2 was glucose, 15; yeast extract, 3;peptone,3; ethanol 0.5%(v/v); acetic acid 0.3% (v/v); folic acid, 0.0004 and NaCl, 0.1gl^{-1}. After optimization of culture conditionsincluding pH (3.5) and incubated temperature (30 ºC),the cell growth and cellulose yield of *Komagataeibactersaccharivorans* PE 5 and *AcetobacterXylinum* ATCC 10245 on modified GMA media increased about 4.08 & 4.86 fold and 1.75 &1.39 fold, respectively than that recorded on GAM medium after 7 days, understatic culture. Using agitated culture at 150 rpm led to decrease of cellulose production by these strains about 64% and 53% respectively. Modified GAM media supplemented with molasses as sole carbon source enhanced the cellulose yield which increased about 76.94% for *Komagataeibactersaccharovorans* PE 5 and about 28.41 for*AcetobacterXylinum* ATCC 10245 as compared to control treatment (modified GAM medium).

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Keywords; Bacterial cellulose, *A.xylinum, K. Saccharivorans,* molasses, Process optimization.

1. Introduction

Microbial cellulose is an exopolysaccharide produced by various species of bacteria, such as those of the genera *Gluconacetobacter, Agrobacterium, Aerobacter, Achromobacter, Azotobacter*, *Rhizobium, sarcina*and *salmonella* (**Shoda and Sugano, 2005**). Production of cellulose from *Acetobacterxylinum* was first reported by **Brown (1886).** Also some acetic acid bacteria in genus *komagataeibacter* (formerly *Gluconacetobacter)* such as *K. xylinus*, *K. nataicola, K. hansenii*and *K. swingsii*were used for bacterial cellulose or biocellulose production**(Castro *et al*., 2011 and Suwanposri*et al*., 2014).**

The microbial productivity of bacterial cellulose (BC) depends on culture conditions such as the composition of the medium, pH, temperature and cultivation method **(Chawla *et al*., 2009).**

Jahan *et al*. (2012) reported that the BC production by *Gluconacetobacter* sp. increased from 0.52 to 4.5 gl^{-1} (8.65 fold increase) in medium containing 1% glucose, 1.5% yeast extract, 5% peptone, 0.27% disodium hydrogen phosphate, 0.115% citric acid and 0.4% (w/v) ethanol. In general, glucose has been used as a carbon source for cellulose production from *Gluconacetobacterxylinus*. It has been reported that cellulose was also synthesized from monosaccharides **(Masaoka*et al*.,1993). Son *et al*. (2003)** stated that BC production was dependent on the presence of $MgSO_4.7H_2O$ and co-substrates such as ethanol and lactic acid in medium. The optimal conditions for BC production by *komagataeibacter* sp. on soya bean whey (SBW) were pH 6.21, 1.61%ethanol concentration(v/v) and 28ºC**(Suwanposri*et al*. 2014)**. The use of optimized medium of SBW increase BC production 3.6 fold compared to standard Hestrin -Schramm (HS) medium.

Lin *et al*. (2014) found that a sugar concentration of 3%waste beer yeast hydrolysates (WBY) treated by ultrasonication gave the highest BC yield (7.02 gl^{-1}), almost 6 times as that from untreated WBY (1.21 gl^{-1}). Also, **Keshk*et al*. (2006)** stated that the yield of the bacterial cellulose (BC) produced by *G. xylinus* ATCC 10245 from beet molasses was higher than that using glucose as a sole carbon source. IR spectra studies indicated that BC produced from glucose has a relatively higher degree of polymerization. **Zeng *et al*. (2011)** used maple syrup 30g carbohydrates/L as a sole carbon source on synthetic medium for optimal production of BC.

Thus the present work was carried out to detect the optimum nutritionaland environmental factors affected on Bacterial cellulose (B.C) production by *AcetobacterXylinum* ATCC 102456 and local cellulose producing bacteria isolated from rotten fruit in order to optimize their production. Also, molasses was used as a low cost substrate for the production of B.C.

2. Material and Methods

1. Isolation of acetic acid bacteria and reference **bacteria**

Twenty one rottenfruit samples of apple, guava, peach, pineapple, pomegranate, strawberry, cantaloupe, orange, yousefi, date, gaga, kiwi, mango, banana, grape, apricot, figs, melon, plum, grapefruit and pear were collected from local markets in Benha city to isolate cellulose producing bacteriaondifferent media which form pellicle at the air liquid interface under static culture.

The collected isolates were subjected to preliminary identification according to **De ley *et al*. (1984)**. Besides, one strain was obtained from international culture collections, namely *Acetobacterxylinum ATCC* 10245 (as a reference strain).All tested bacteria were subculture on GAM medium slants, maintained at 4°C after incubation at 30°C for 7 days and transferred every 14 days.

2. Media used.

HS medium **(Hestrin and Schramm, 1954),** complex medium(COM) **(Kamide*et al*.,1990),** Gluconobacteroxydansmedium(DSM) (**Timke***et al.,*2005), sterile distilled water supplemented with ethanol |(4%), SEED medium **(Sudsakda*et al*.,2007**), and glucose-ethanol acetic acid medium(GAM) **(Hanmoungjai*et al*, 2007)**, were used throughout this investigation for bacterial cellulose production.

3. Standard inoculum

Standard inoculum was prepared by inoculation of one test tube containing 5 ml of GAM medium with 1 ml of tested culture, then incubated at 28- 30°C for 3 days. The contained of this tube was used as a standard inoculum ($O.D_{620nm}$from 0.21 to 0.44) understatic culture.

4. Genetic identification of cellulose producing bacteria

The most efficient cellulose producing isolate was selected and completely identified using the 16S rDNA sequences analysis.

a- Sequence analysis of 16S rRNA Gene

Isolation of cellular DNA was performed as described by **Ausubell *et al*. (1987)** and amplification of 16S rDNA according to **Lane (1991)** using the universal 16S primers (F1 5' AGAGTTT(G/C)ATCCTGGCTCAG 3'R1 5' ACGG(A/C)TACCTTGTTACGACTT 3').The sequencing was performed in two directions using the previously described primers **(Lane, 1991)** in GATC company (Germany). Sequencing data was analyzed by two different computer alignment programs, DNA Star (DNASTAR, Inc., USA) and Sequence Navigator (Perkin, Corp., USA).

b- Phylogenetic relationships

The BLAST database **(Altschul *et al.,* 1997)** of National Center for Biotechnology Information was used to compare resolved sequence of the PE 5 isolate with known 16S rDNA sequences. Determination of phylogenetic relationships was analyzed by the program Phylogenetic Analysis CLC free workbench version 4.5.1.

5. Cellulose production by static culture experiments.

a) Selection of suitable medium

Five media for cellulose production being HS, COM, DSM, SEED and GAM were used in this experiment for cellulose production in order to select the most suitable medium for securing high cellulose production. The fermentation process was carried out in 100 ml Erlenmyer flasks containing 50 ml sterile medium, then inoculated with the content of one tube standard inoculum and incubated at 28- 30°C for 7 days as a static culture. At the end of incubation period, the wet BC pellicle was placed between two sheets of filter paper to remove excessive water on it. Then it was weighted and calculated as gl^{-1} fresh weight (F. Wt).The optical density of bacterial growth in fermented culture was determined at 620nm.

b) Factors affecting cellulose production

These experiments were carried out to obtain maximum BC production by selected bacteria; different nutritional and environmental factorswere optimized using one variable at a time approach. The following factors were optimized: carbon, nitrogen, vitamins (0.0002g/l) & mineral saltssources. Effect of ethanol and acetic acid on cellulose production were investigatedas well as initial pH, incubated temperature and agitationculture(at 150rpm).All the optimization experiments were carried out in replicates and the data are presented as the means of replicates. The inoculation and propagation were carried out as mentioned before. The optical density of cell growth(O.D/620nm) was measured andthe pellicle formed at the air-liquid interface of the production medium was collected and rinsed with water for two to three times. It was then treated with 1 N NaOH at 80°C for 20 min. to neutralize NaOH, the pellicle was treated with 5% acetic acid solution. It was again washed with water for three times. The purified pellicle obtained was dried at 60°C until a constant weight and expressed as gl^{-1} dry BC weight, then cellulose yield|(%) was calculated, according to **Gamal *et al*. (1991).**

$$\text{Yield}(\%) = \frac{\text{Dry cellulose production } (gl^{-1})}{\text{Original Sugar } (gl^{-1})} \times 100$$

c) **Molasses uses for bacterial cellulose production:**

Black strap molassewas used as a carbon source at 1.5 % total sugar either alone or in combination with other constituents of medium. The propagation was carried out using static culture as mentioned before, cellulose produced were determined as dry weight after 7 days of incubation period.

6. Statistical analysis

Thecollected data were statistically analyzed using SPSS computer analysis program (**Forster, 2001).**

3. Result and Discussion

1-Isolation and selection of cellulose producing bacteria

In this study thirty nine cellulose producing bacteria were isolated from different twenty five fruit samples on HS, DSM, COM, SEED media and sterile distilled water supplemented with ethanol |(4%). The cells were Gram-negative, rod –shaped straight or slightly curved occurring singly, in pairs or chains. Single pure colony produced clear zone when cultured on GAM medium containing nystatin &cycloheximide and supplement with calcium carbonate. They grew aerobically and showed good growth in the presence of 0.3% acetic acid at pH 3.5.

In liquid media, produced a ring, film of surface pellicle at the air liquid interface, uniform turbidity of medium. Only three isolates, namely PE 5, AP 4 and PI 4(which were isolated from peach, apple and pineapple) gave the highest pellicle fresh weight being 14.56, 13.03 and 13.43 gl^{-1} respectively (data not show). Therefore, these isolates were selected as efficient cellulose producing bacteria along with reference strain (Acetobacterxylinum ATCC 10245).

2- Selection of suitable medium for cellulose production

Result in Table (1) clearly show that the amount of growth and cellulose fresh weight were higher in different tested cultures grown on GAM medium than HS, DSM, COM or SEED medium. Therefore, this medium was the preferable medium for the propagation and cellulose production by the tested bacteria. The highest figure of growth as O.D and cellulose fresh weight (F.Wt) on GAM medium were obtained by *Acetobacterxylinum* ATCC 10245 (0.63&115 gl^{-1}), followed by isolate No. PE 5 (0.49& 58.6 gl^{-1}). So, this isolate was selected for complete identification by 16S rDNA sequence analysis.

Table (1): Effect of different media on bacterial cellulose production by *Acetobacterxylinum*ATCC 10245 and different bacterial cellulose isolates after 7 days at 30° C using static culture.

Cellulose producing bacteria	DSM medium		HS medium		COM medium		SEED medium		GAM medium	
	Fresh wt.* gl^{-1}	O.D/620nm**	Fresh wt. gl^{-1}	O.D/620nm	Fresh wt. gl^{-1}	O.D/620nm	Fresh wt. gl^{-1}	O.D/ 620nm	Fresh wt gl^{-1}	O.D/ 620nm
A.xylnium ATCC 10245	4.7±0.25	0.01±0.001	17.02±0.2	0.44±0.01	18.7±05	0.12±0.01	28.4±0.12	0.12±0.01	115±1.4	0.63±0.01
PE 5	24.6±1.04	0.29±0.02	14.36±0.1	0.32±0.01	53.5±03	0.45±0.01	50.7±0.46	0.42±0.01	58.6±1.13	0.49±0.01
AP 4	22.92±0.5	0.12±0.02	12.5±0.42	0.33±0.01	79.7±0.33	0.44±0.01	40.8±0.45	0.22±0.002	45.6±1.06	0.48±0.01
PI 4	23.4±0.23	0.2 ±0.02	12.5±0.43	0.26±0.03	28.8±0.26	0.16±0.01	46.83±0.2	0.18±0.004	50.63±1.04	0.411±0.01

Results are expressed as the means± standard error of three replicates . *.fresh wt: fresh weight **. O.D/ 620nm: optical density at 620nm

3- Identification of the most potent isolate:

Comparison of the nucleotide sequences of 16S rRNA genes of the most efficient isolate (PE 5 isolate) with sequences available from Gen Bank, performed with the use of the BLASTN 2.2.25 software, indicated that the bacterial isolate PE 5 showed 97% homology with *Komgataeibacter saccharovorans* LMG 1582. Construction of a phylogenetic tree based on comparative analysis of the 16S rRNA genes was performed with the use of various algorithms implemented in CLC free workbench, version 4.5.1. The phylogenetic analysis based on 16S rRNA gene sequences showed that strain PE 5 formed a phyletic lineage, within the genus *Komagataeibacter* and *Gluconacetobacter* (Fig.1).The bacterial isolate was identified to*Komgataeibacter saccharovorans* PE5.

4- Factors affecting bacterial cellulose production

A. Effect of nutritional factors:

1. Carbon source:

Data presented in Table (2) show that the highest figure of growth was obtained by *Acetobacter xylinum* ATCC 10245 in GAM medium supplemented with starch as sole carbon source followed bysupplemented with glucose being 0.50 and 0.46, respectively whereas the medium containing glucoserecorded the highest cellulose dry weight and yield. Fig (2)illustrated that glucose at 15gl^{-1} gave the highest dry weight and yield(4.62gl^{-1}&30.83%)by *Acetobacter*

xylinum ATCC 1024 whereas increasing glucose concentration to 20 gl^{-1} lead to decrease the cellulose production about 14.71%. This result was agreement with that obtained by **Jahan *et al*. (2012)** for cellulose production by *Gluconacetobacter* sp. F6. On other hand, mannitol was the best carbon source for the medium growth of *Komgataeibacter saccharovorans* PE 5.The cellulose dry weight (gl^{-1}) and yield (%) increased about 98% and 97.7 % than glucose medium. In similar studies **Suwanposri *et al.* (2014)** found that *Komgataeibacter*spPAP, produced 3.5 gl^{-1} cellulose on HS medium containing 2% (w/v) mannitol after 2-3 days at 25-30°C.There was a gradual increase in cellulose production by *Komagataeibacter saccharivorans* PE 5 with increase of mannitol concentrations reaching a maximum at 15gl^{-1}(Fig.2).

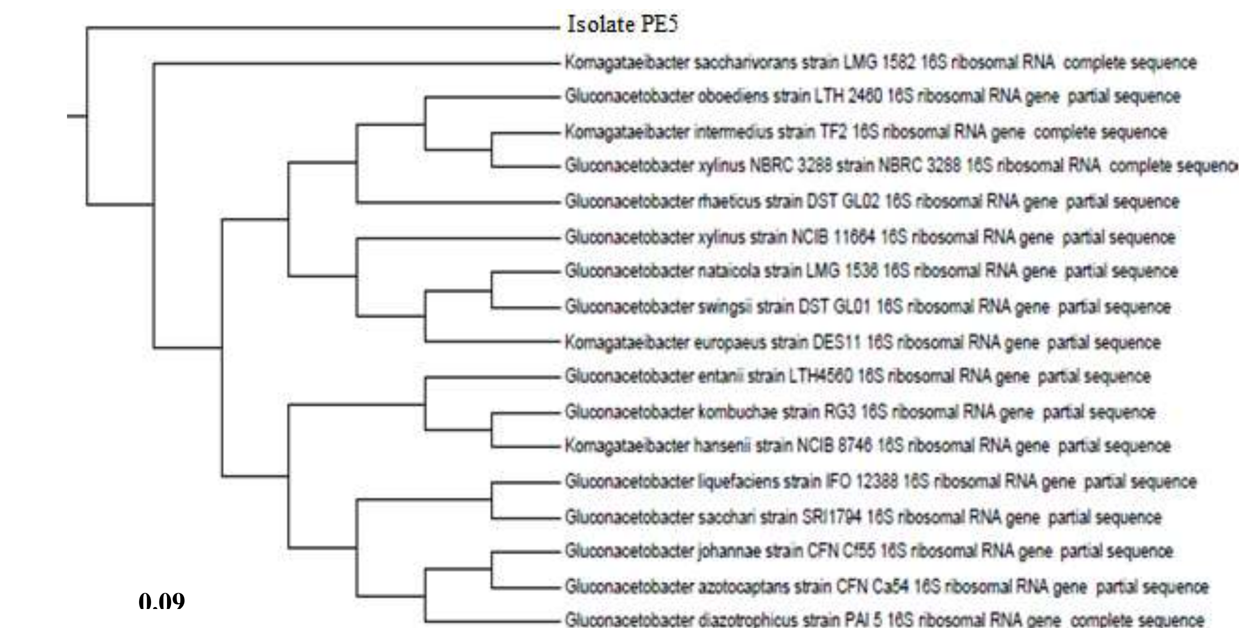

Fig. (1): Neighbour-joining phylogenetic tree, based on 16S rRNA gene sequences, showing the relationships between isolated bacterium PE5and related taxa. Bar, 0.09 changes per nucleotide position.

Table (2): Effect of different carbon sources on bacterial cellulose production by *Acetobacter xylinum* ATCC 10245 and *Komagataeibacter saccharivorans*PE 5 grown on GAM medium after 7 days at 30°C using static culture.

Carbon Source	***Acetobacter xylinum* ATCC 10245**			***Komagataeibacter saccharivorans* PE 5**		
	O.D/ 620nm**	**Dry wt.(g/l)***	**Yield(%)**	**O.D/620nm**	**Dry wt.(g/l)**	**Yield(%)**
Glucose (control)	0.46	4.6	30.68	0.209	1.45	9.66
Maltose	0.42	3.64	24.26	0.11	1.55	10.34
Sucrose	0.34	3.53	23.55	0.24	1.73	11.54
Mannose	0.14	2.30	15.38	0.17	0.75	5.00
Fructose	0.35	3.65	24.36	0.12	1.41	9.40
Galactose	0.09	2.56	17.07	0.15	0.52	3.45
Starch	0.50	3.63	24.20	0.26	1.89	12.60
Mannitol	0.25	3.52	23.46	0.32	2.87	19.14
Glycerol	0.34	2.80	18.69	0.14	1.08	7.20

*.Dry.wt : dry weight **. O.D/ 620nm:optical density at 620nm

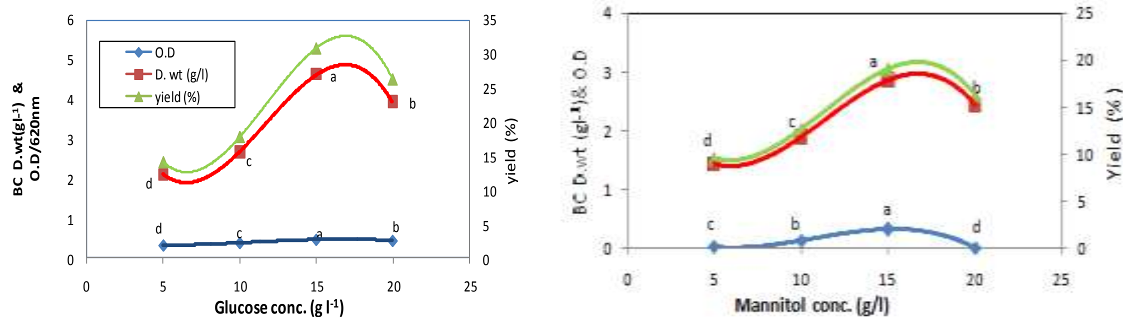

Fig.(2): Effect of glucose and mannitol concentrations on the growth and bacterial cellulose production by *Acetobact xylinum* ATCC 10245 and *Komagataeibacter saccharivorans* PE 5, respectively after 7 days at 30ºC using static culture. Values in the same line sharing the same letter do not differ significantly, according to Duncan's at 5 % level.

Therefore, the glucose and mannitol as carbonsources at 15gl^{-1}were chosenfor the further studiesduring the cellulose production by *Acetobacterxylinum* ATCC 10245 and *Komgataeibacter saccharivorans* PE 5, respectively.

2. Nitrogen sources

The tabulated data in table (3) show the organic nitrogen sources gave the bacterial growth and cellulose production by both tested strains higher than inorganic nitrogen sources. The highest figures of growth, cellulose dry weight and yield being 0.45, 4.59 gl^{-1}and 30.62 % were obtained by *Acetobacter xylinum* ATCC 10245 on GAM medium containing mixture of yeast extract and peptone (3:3 g/l) as a nitrogen source. Different ratios of this mixture as nitrogen source were used. Increasing the ratio of yeast extract: peptone to 2:1 (4:2 g/l)or 5:1(5:1 g/l) led to decrease the cell growth and culture yield to 0.34& 20.13% or to 0.31&17.05% respectively (Fig 3). Using the mixture of yeast extract and peptone was agreement with the finding of **Jahan *et al*. (2012).** Various researches have also reported yeast extract to support maximum BC production **(Kim *et al*., 2006 and Pourramezen*et al*., 2009).** Also, it could be noticed that the replacement the mixture of yeast extract and peptone with tryptone led to increase the cell growth, cellulose concentration and yield of *Komagataeibacter saccharivorans* PE 5 about 2.8,1.67 and 1.67fold, respectively. Using6gl^{-1} tryptone gave maximum values of growth (OD), cellulose dry weight (gl^{-1}) and yield (%) comparingother concentrations (Fig.3).

Table (3): Effect of different Nitrogen sources on bacterial cellulose production by *Acetobacter xylinum* ATCC 10245 and *Komagataeibacter saccharivorans* PE 5 strain grown on GAM medium after 7 days at 30ºC using static culture.

Nitrogen Source	***Acetobacterxylinum*ATCC 10245**			***Komagataeibactersaccharivorans* PE5**		
	O.D/ 620nm	**Dry.wt (gl^{-1})**	**Yield(%)**	**O.D/ 620nm**	**Dry.wt (gl^{-1})**	**Yield(%)**
Beef extract	0.16	0.25	1.66	0.29	1.62	10.81
Yeast extract	0.43	3.23	21.54	0.23	3.22	21.48
Peptone	0.11	1.29	8.60	0.16	1.04	6.93
Tryptone	0.34	2.45	16.34	0.39	4.05	27.01
Casien	0.32	2.35	15.6	0.29	3.33	22.2
Urea	0.16	0.66	4.40	0.03	0.39	2.6
Ammonium sulphate	0.03	0.17	1.13	0.18	1.46	9.73
Ammonium chloride	0.13	0.21	1.40	0.08	0.79	5.26
Potassium nitrate	0.11	0.27	1.80	0.05	0.24	1.60
Ammonium nitrate	0.12	0.12	0.77	0.10	0.26	1.73
Yeast extract&Peptone(control)	0.45	4.59	30.62	0.14	2.42	16.14
Yeast extract&tryptone	0.43	4.09	27.28	0.22	2.91	19.41
Yeast extractt&casien	0.29	1.83	12.21	0.28	3.31	22.08

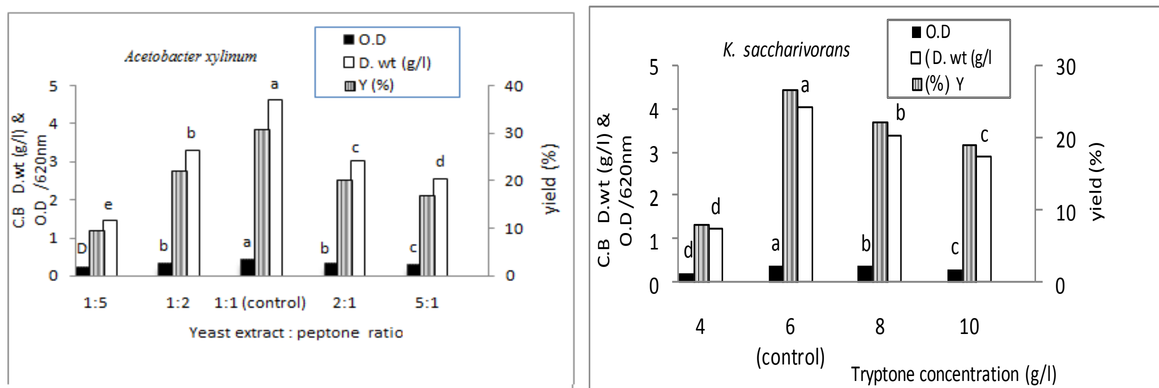

Fig.(3): Effect of different yeast extract & peptone ratios and different tryptone concentrations on the growth and bacterial cellulose production by *Acetobacter xylinum* ATCC 10245 and *Komagataeibacter saccharivorans* PE 5, respectively after 7 days at 30ºC using static culture.

Columns in the same parameters followed by the same letter don't significantly differ from each other, according to Duncan'sat 5%level.

3. Ethanol and acetic acid concentration

Data illustrated in Fig (4) show that increasing the ethanol concentration (v/v) led to increase the bacterial growth, cellulose production and yield by tested strains to record the maximum values at 5 and 7mll^{-1} for *AcetobacterXylinum*ATCC 10245 and *Komgataeibacter saccharivorans* PE 5, respectively. At these concentrations of ethanol, the highest figures of cell growth and cellulose production of*Acetobacter xylinum*ATCC 10245 and *Komgataeibacter saccharivorans* PE 5, were attained at acetic acid concentration of 3 and 2 mll^{-1}, respectively. Also, it could be noticed that increasing the ethanol concentration to 7 ml^{-1} and decreasing the acetic acid concentration to 2 mll^{-1}increased the cellulose yield by *Komgataeibacter saccharivorans* PE 5 about 1.16 fold as compared to control. **Zeng *et al*. (2011)** added both ethanol and acetic acid with 0.5 % (v/v) concentration to the optimum medium for BC production by *A.xylinum* BPR 2001. Also, **Son *et al*. (2003)** noticed that in medium containing 0.6 % ethanol, BC production by *Acetobacter* sp v6 was 4.02gl^{-1}which was about 3.1 times higher than that without ethanol (1.32 gl^{-1}).

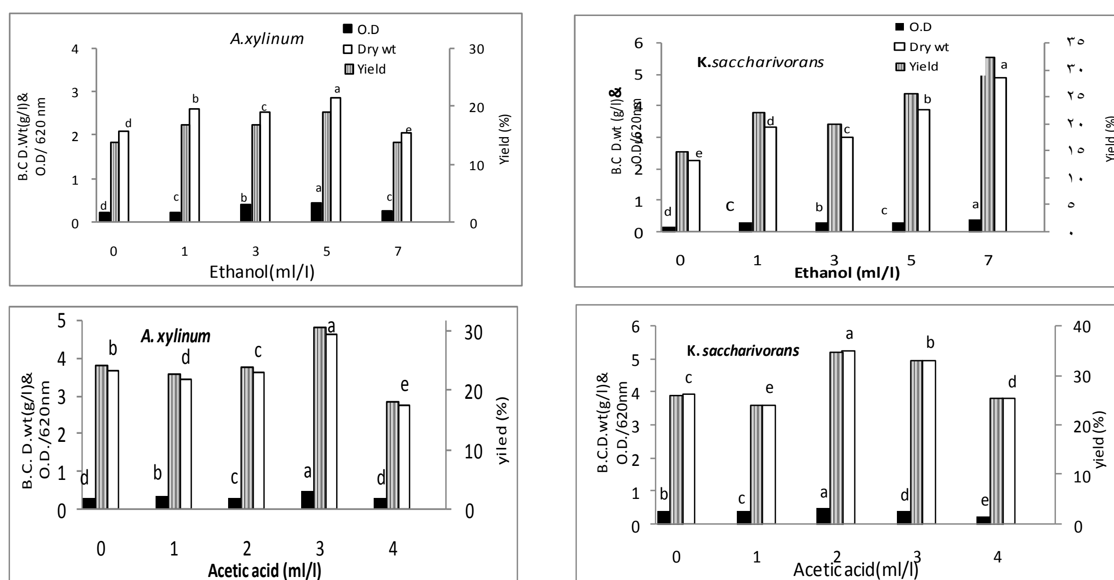

Fig. (4): Effect of different ethanol and acetic acid concentrations on bacterial cellulose production by *Acetobacter xylinum* ATCC 10245 and *Komagataeibacter saccharivorans* PE 5 grown on GAM medium after 7 days at 30 ºC using static culture.

Columns in the same parameters followed bythesame letterdon't significantly differ from each other, according to Duncan's at 5% level.

4. Different vitamin sources.

Data illustrated by Fig (5) indicated that some vitamins (i.e. calcium pantothenate, P-aminobenzoic acid, pyridoxin, riboflavin, biotin, folic acid and nicotinic acid) were affected on cell growth and BC production by both tested strains but *Komgataeibacter saccharivorans* PE 5 was more responsible for vitamins addition than *Acetobacter xylinum* ATCC 10245. Where *Komgataeibacter saccharivorans* PE 5 gave the highest BC yield (40.93%) in modified GAM medium supplement by nicotinic acid followed by P-aminobenzoic acid (38.43%) and riboflavine (37.47%). The medium supplement with calcium pantothenate or pyridoxin recorded approximately the same value of BC yield and its dry weight comparing to control (without vitamin). Slight increase in BC was recorded by biotin whereas the drastic effect occurred after folic acid addition. The latter vitamin recorded the maximum BC yield by *Acetobacter xylinum*ATCC 10245 (36.15%) which increased about 1.24 fold compared to control. Addition of calcium pantothenate or pyridoxin was not affected on BC production by *Acetobacterxylinum*ATCC 10245 whereas P-aminobezoic acid, riboflavin and nicotinic acid gave a drastic effect on BC yieldwhich decreased about 13%, 20% and 9%respectively. Moreover, this strain recorded the bacterial growth and cellulose production lower than *Komgataeibacter saccharivorans* PE 5 at different vitamins treatments. Also, datainFig. (6) presentthe response of studied bacteria to different concentrations of the most effective vitamin for each one ranged from 0.00001to 0.00008%. Itclearly showthat the maximum bacterial growth and BC production by *Acetobacter xylinum* ATCC 10245 and *Komgataeibacter saccharivorans* PE 5were attained in media supplemented by 0.0004 gl^{-1}folic acid and 0.0002 gl^{-1} nicotinic acid,respectively. Increasing the concentration of these vitamins in GAM medium led to decrease the cell growth and BC production by both strains. In similar study **Son *et al*. (2003)** found that the maximum bacterial cellulose production by *Acetobacter sp.* V6 being 4.16$gl^{-1}$was attained at0.00005% nicotinamide.

Fig(5): Effect of different vitamins on the growth and bacterial cellulose production by *Acetobacter xylinum* ATCC 10245 and *Komagataeibacter saccharivorans* PE 5 after 7 daysat 30ºC using static culture.

Columns in the same parameters followed bythesame letterdon't significantly differ from each other, according to Duncan's at 5% level.

Fig(6): Effect of different folic acid and nicotinic acid concentration on the growth and bacterial cellulose production by *Acetobacter xylinum* ATCC 10245 and *Komagataeibacter saccharivorans* PE 5, respectively after 7 days at 30ºC using static culture.

Columns in the same parameters followed bythesame letterdon't significantly differ from each other, according to Duncan's at 5% level.

5. Different mineral salts

All mineral salts treatments recorded increasing in the value of BC yield by *Acetobacter xylinum*ATCC 10245 and *Komgataeibacter saccharivorans* PE 5 except $FeSO_4.7H_2O$ on the latter strain as shown in Table (4). The highest figures of cell growth (O.D) and BC concentration were obtained in modified GAM medium supplemented with 0.01% NaCl (0.80 &6.39 $gl^{-1)}$ and0.01% $CaCl_2.2H_2O$ (0.85 &7.05 gl^{-1}) by*Acetobacter xylinum* ATCC 10245 and *Komgataeibacter saccharivorans* PE 5, respectively. Generally, it could be concluded that the BC concentration (gl^{-1}dry weight) produced by *Acetobacter xylinum* ATCC 10245 and *Komgataeibacter saccharivorans* PE 5, increased up to 1.39 fold and 4.86 fold in modified GAM medium, respectively as compared with thatobserved in table (2). Therefore, it could berecommended to use modified GAM mediumwhich containing glucose, 15;yeast extract, 3; peptone, 3; ethanol, 5 mll^{-1};acetic acid, 3 $mll^{-1;}$ folic acid, 0.0004 and NaCl, 0.1 $gl^{-1}$for growth of*Acetobacterxylinum*ATCC 10245 and BC production and using modified GAM medium which containing mannitol, 15;tryptone, 6; ethanol, 7 mll^{-1};acetic acid, 2 $mll^{-1;}$ nicotinic acid, 0.0002 and $CaCl_2.2H_2O$, 0.1 gl^{-1}forthe growth of *Komgataeibacter saccharivorans* PE 5 and BC production. So, modified GAM medium were used to study the effect of some environmental factors on the growth and BC production by tested strain.

Table (4): Effect of different mineral salts on bacterial cellulose production by *Acetobacter xylinum*ATCC 10245 and *Komagataeibacter saccharivorans* PE 5 strain grown on GAM medium after 7 days at 30 ºC using static culture**.**

Salt (g/l)	***Acetobacterxylinum*ATCC 10245**			***Komagataeibacter saccharivorans* PE5**		
	O.D/620nm**	**Dry wt. (gl^{-1})***	**Yield(%)**	**O.D/620nm**	**Dry wt.(gl^{-1})**	**Yield(%)**
Without salts (Control)	0.61± 0.06	5.53±0.12	36.88	0.66±0.03	6.05±0.2	40.35
$MgSO_4.7H_2O$ (0.1)	0.61±0.07	5.61±0.11	37.41	0.68±0.04	6.41±0.3	42.75
$CaCl_2.2H_2O$ (0.1)	0.66 ±0.06	5.83±0.14	38.88	0.85±0.02	7.05±0.5	47.04
NaCl (0.1)	0.80±0.09	6.39±0.2	42.62	0.68±0.04	6.33±0.16	42.22
$FeSO_4.7H_2O$ (0.005)	0.67±0.04	5.69±0.13	38.00	0.65±0.03	6.09±0.4	40.62
H_3BO_3 (0.003)	0.61±0.05	5.62±0.12	37.48	0.67±0.05	6.23±0.21	41.55

Results are expressed as the means± standard error of three replicates. *.Dry wt. :dry weight **. O.D/ 620nm: optical density at 620nm

B. Effect of environmental factors

The main environmental factors of interest arepH, incubated temperature and dissolved oxygen(as shaking culture). Data illustrated in Fig (7) show the effect of different values of initial pH, different degrees of incubated temperature and shaking culture on the bacterial growth and cellulose production by *AcetobacterxylinumATCC* 10245 and *Komgataeibactersaccharivorans* PE 5 after 7days fermentation period. Generally it could be noticed that the maximum bacterial growth and cellulose production of both strains were observed on modified GAM medium adjusted to pH 3.5 after 7 days at 30 °C incubation temperature using static flask as a batch culture technique. Increasing or decreasing the value of pH and temperature led to decrease of the cell growth and BC production. Moreover, using shaking flask led to decrease the cell growth and BC production about 45.3% and 53.5% for *Acetobacterxylinum*ATCC 10245 and about 74.7% and 64.01% for*Komgataeibactersaccharivorans* PE 5. The previous results are in line with those obtained by**Hanmougjai *et al*. (2007) and Castro *et al* (2012).** They observed that good growth in the presents of 0.35% acetic acid at pH 3.5, whereas the optimum pH for BC production was ranged from 4 to 7 wasreported by **Jahan *et al*. (2012)**. Also, maximum BC was produced at30 °C bysome investigator **as Aydin and Askoy (2009) and Castro *et al*. (2012). Yoshinaga *et al*. (1997)** stated that when *A. xylinum* grown in agitated conditions the results often give a poor yield.

C-Use of sugar cane molasses for cellulose production

Data presented in Table (5) revealed that all the treatments varied in their effect on growth of the tested strains. Drastic cut was observed by *Acetobacter xylinum* ATCC 10245 followed by *Komgataeibacter saccharivorans* PE 5 at the treatment using molasses (T4) only or molasses supplemented with nitrogen source (T3) as whole medium. Also, at these treatments, both tested strains loss 21.26 to 49.6% of their cellules production (g/l) comparing to control treatment.On the other hand, the treatment of molasses supplemented with both ethanol and acetic acid in the presence of nitrogen source (T1) gave the higher cellulose concentration than in the absence of nitrogen source (T2). The first treatment (T1) enhanced the bacterial cellulose yield

which increased about 28.5% for *Acetobacter xylinum*ATCC 10245 and about 76.99%for *Komgataeibacter saccharivorans* PE 5 comparing to control treatment (modified GAM medium) after 7days incubation period. This result was agreement with **Keshk *et al* (2006)** they found that using of beet molasses increased cellulose production by *Gluconacebacter xylinus* 10245 about 1.31 fold comparing to HS medium.

Fig(7): Effect of some environmental factors on the growth and bacterial cellulose production *by Acetobacter xylinum* ATCC 10245 and *Komagataeibacter saccharivorans*PE 5after 7 days.
Columns in the same parameters followed by the same letter don't significantly differ from each other, according to Duncan's at 5% level

Table(5): Production of BC from *Acetobacter xylinum* ATCC 10245 & *Komagataeibacter saccharivorans* PE 5 using molasses as a sole carbon source on GAM medium after 7 days at 30 ºC using static culture.

Treatment	Code	*Acetobacter xylinum*ATCC 10245			*Komagataeibacter saccharivorans* PE 5		
		O.D/620nm **	Dry wt. (g/l)*	Yield(%)	O.D/620nm	Dry wt. (g/l)	Yield(%)
Molasse+N source+ ethanol+acetic acid	**TI**	0.832a	**8.16 a**	54.42	0.999 ab	12.43a	82.88
Molasse+ ethanol+ acetic acid	**T2**	0.769c	6.03 b	40.25	0.972 a	10.12b	67.50
Molasse+N source	**T3**	0.407d	5.00 c	33.36	0.799 ab	5.28d	35.22
Molasse	**T4**	0.199e	3.46 d	23.07	0.511b	3.54e	23.59
Modified GAM medium	**control**	0.804b	6.35 b	42.38	0.842 ab	7.02c	46.84

Values in the same parameter followed by the same latter do not significantly differ from each other, according to Duncan's at 5% level..
*.Dry.wt :dry weight **. O.D/ 620nm: optical density at 620nm

Conclusion:

The study concluded that the optimum conditions for bacterial growth and BC production were varied from one tested strain to another. The *Komagataeibactersaccharivorans* PE 5 strain was isolated from rotten peach and selected as high efficient cellulose producing bacteria. On the other hand, the highest BC production was obtained by the reference strain *Acetobacterxylinum* ATCC 10245 and *Komagataeibactersaccharivorans* PE5 in modified GAMmedium at pH 3.5 after 7 days fermentation period at 30°C, using static culture. While the BC production by both strains increased when molasses used as a sole carbon source in the modified media about 1.29 fold and 1.77 fold for *Acetobacterxylinum* ATCC 10245 and *Komagataeibactersaccharivorans* PE 5, respectively, comparing to control treatment (modified GAM medium).

References

1. Altschul, S.F.;T.L. Madden; A.A.Schaffer;J. Zhang; Z.Zhang;W. Miller and D.J. Lipman.(1997). Gapped BLAST and PSIBLAST: a new generation of protein database search programs.Nucleic Acids Res.25:3389-3402.
2. Ausubell, F.M.; R.Brent; R.E. Kingsto; D.D.Moore; J.G Seidman; J.A. Smithandand K. Struhl.(1987). Current Protocols in Molecular Biology, Greene Publishing Associates,WileyInterscience, New York.
3. Aydin,Y.A. and N.D.Aksoy(2009).Isolation of cellulose producing bacteriafrom wastes of vinegar fermentation. ISBN: 978-988.
4. Brown, A.J.(1886). On an acetic ferment which forms cellulose, *J. Chem. Soc. Trans. 49:*432–439.
5. Castro, C.; R.Zuluagaa; C.Alvareza; J. Putauxb; G.Caroa;Orland; J. Rojasc; d.I. Mondragone and Piedad.(2012). Bacterial cellulose produced by a new acid-resistant strain of *Gluconacetobacter* Genus. Carbohydrate Polymers89:1033– 1037.
6. Castro, C.;R. Zuluaga.; J.L. Putaux; G. Caro; I. Mondragon and P. Gañán. (2011).Structural characterization of bacterial cellulose produced by *Gluconacetobacterswingsii*from Colombian agroindustrial wastes.Carbohydr. Polym. 84: 96-102.
7. Chawla. P.; B. Ishwar; B. Shrikant; A. Survase and R. Singhal.(2009).Microbial cellulose: Fermentative production and applications. FoodTechnol. Biotechnol. 47 (2): 107–124
8. De ley J;M.Gills and J. Swings.(1984).FamilyVI.Acetobacteracae.Bergy's Manual of systematic Bacteriology, Voll, 19th edn.williams and willkens, MD, USA,PP.267-274.
9. Foster, Jeremy j.(2001). Data Analysis UsingSPSS for Windows Versions 8-10 Sage Publication Ltd., London.
10. Gamal,RawiaF.;FatmaR.Nasser; Hemmat,M.Abdelhady and M. EL-Sawy.(1991).Glycerol production by osmotolerant yeast strain using fermentor as fed batch and continous culture techniques.Annals,Agric, Ain Shams Univ. Cairo.36(2):319-321.
11. Hanmougjai,W.; E.Chakeatiirote; W.Pathom-aree;Y. Yamada and S.Lumyoung. (2007). Identification of acid tolerant Acetic Acid bacteria isolated from Thailand sources. Research Journal of Microbiology 2 (2):194-197.
12. Hestrin, S. and M. Schramm. (1954).Synthesis of cellulose by *Acetobacterxylinum*.Preparation of freeze-dried cells capable of polymerizing glucose to cellulose.Biochem. J.,58: 345-352
13. Jahan, F.;V.Kumar; G.Rawat and R. K. Saxena (2012). Production of microbial cellulose by a bacterium isolated.Appl, Biochem. Biotechnol. 167:1157–1171.
14. Kamide,K.; Y. Matsuda; H. Iijima and K. Okajima (1990). Effect of culture conditions of acetic acid bacteria on cellulose biosynthesis. British Polymer Journal 22: 167–171.
15. Keshk, S. M.; T. Razek and k. Sameshima, (2006). Bacterial cellulose production from beet molasses. African Journal of Biotechnology 7 (15):1519-1523.
16. Kim S.Y.; J.N. Kim; Y.J. Wee; D.H. Park and H.W. Ryu.(2006). Production of bacterial cellulose by *Gluconacetobacter* sp. RKY5 isolated from persimmon vinegar Appl. Biochem.Biotechnol.131:705–715.
17. Lane, D.J. (1991). 16S/23S rRNA sequencing. In: Stackebrandt E. and M. Goodfellow, (eds.), Nucleic Acid Techniques in Bacterial Systematics. John Wiley & Sons, Inc New York, pp. 115-148.
18. Lin, D.; P. Lopez-Sanchez; L. Rui and L. Zhixi (2014).Production of bacterial cellulose by *Gluconacetobacterhansenii* CGMCC 3917 using only waste beer yeast as nutrient source. Bioresource technology 151: 113-119.
19. Masaoka, S.; T. Ohe and N. Sakota (1993). Production of cellulose from glucose by *Acetobacterxylinum*, J.Ferment. Bioeng. 75:18–22.
20. Pourramezen, G. Z.; A. M. Roayaeiand Q. R.Qezelbash. (2009).Optimization of culture conditions for bacterial cellulose production by *Acetobacter* sp. 4B-2. Biotechnology 8: 150–154.

21. Shoda, M. and Y. Sugano.(2005). Recent advances in bacterial cellulose production. Biotechnol. BioprocessEng. 10:1–8.
22. Son, H.J.; H.G. Kim; K.K. Kim; H.S. Kim; Y.G. Kim and S.J. Lee.(2003).Increased production of bacterial cellulose by *Acetobacter* sp. V6 in synthetic media under shaking culture condition.Bioresour. Technol. 86: 215–219.
23. Sudsakda,S.;W. Srichareon and W. Pathom-aree.(2007).Comparison of three enrichment broths for the isolation of thermotolerant acetic acid bacteria from flowers and fruits. Research Journal of Microbiology2 (10):792-795.
24. Suwanposri, A.; P. Yukphan; Y.Yamada and D.Ochaikul.(2014). Statistical optimization of culture conditions for biocelluloseproduction by *Komagataeibacter*sp. PAP1 using soya bean whey. Maejo Int. J. Sci. Technol. 8(01): 1-14.
25. Timke, M.; D. Wolking; N. Q. Wang-Lieu;K. Altendorf, and A. Lipski. (2005). MicrobialComposition of biofilms in a brewery investigated by fatty acid analysis, fluorescence *in Situ* hybridization and isolation techniques.*Applied Microbiology and Biotechnology 66: 100-107.*
26. Yoshinaga, F.; N. Tonouchi and K.Watanabe.(1997). Research progress in production of bacterial cellulose by aeration and agitation culture and its application as a new industrial material. Biosci. Biotechnol. Biochem. 61(2): 219–224.
27. Zeng, X.; P.Small and W. Wan.(2011). Statistical optimization of culture conditions for bacterial cellulose production by *Acetobacterxylinum* BPR 2001 from maple syrup. Carbohydrate Polymers 85:506–513.

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