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Research article

Production, optimization, and characterization of cellulose produced from *Pseudomonas* spp

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ABSTRACT

Bacterial cellulose is a type of biopolymer produced by many Gram-negative bacteria such as *Acetobacter*, *Azotobacter*, *Rhizobium*, *Pseudomonas*, *Salmonella*, *Alcaligenes*, and Gram positive bacteria such as *Sarcina ventriculi*. Bacterial cellulose characterized by high purity containing no hemicellulose or lignin, high water holding capacity and hydrophilicity, good mechanical strength, elasticity and high crystallinity. This article aimed to study the optimum conditions for the production and extraction of cellulose produced from different species of *Pseudomonas* isolated from different samples of food. The results showed that the best production was obtained in cellulose production medium (HS- medium) containing 2% dates molasses, 1% yeast extract, pH 5, inoculated with 10% of bacterial culture and incubated at 30 ^{0}C for one week in shaker incubator. Fourier transform infrared spectroscopy (FTIR) analysis for *Pseudomonas* $_{f3}$ cellulose showed peaks at 3365.55 $^{cm-1}$, 2968.24 $^{cm-1}$, 2852.52 $^{cm-1}$, 1118.64 $^{cm-1}$ and 1436.87 $^{cm-1}$. These peaks indicating the presence of stretching O-H, C-H, H-C-H, C-O-C, and C-C, which identifying functional groups present in *Pseudomonas* $_{f3}$ cellulose.

Keywords: Cellulose, FTIR, *Pseudomonas* spp,

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INTRODUCTION

Cellulose is an organic compound with the formula $(C_6H_{10}O_5)n$, a polysaccharide consisting of a linear chain of several hundred to many thousands of β(1→4) linked D-glucose units [1]. While, cellulose is a basic structural material of most plant substances, it is also produced by Gram-negative bacteria such as *Acetobacter*, *Azotobacter*, *Rhizobium*, *Pseudomonas*, *Salmonella*, *Alcaligenes*, and Gram positive bacteria such as *Sarcina ventriculi* [2]. Plant cellulose is makes up the cell walls of most plants, is a tough, mesh-like bulkwork in which cellulose fibrils are the primary architectural elements. Bacterial cellulose has significantly different macromolecular properties and characteristics. In general, bacterial cellulose is more

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chemically pure, containing no hemicellulose or lignin, higher water holding capacity and hydrophilicity, greater tensile strength resulting from a larger amount of polymerization, ultrafine network architecture [3]. bacterial cellulose has a more crystalline structure compared to plant cellulose and forms characteristic ribbon-like microfibrils [4]. A hallmark of microbial cellulose, these thin microfibrils are significantly smaller than those in plant cellulose, making bacterial cellulose much more porous [5]. Bacterial cellulose could be an interesting alternative for the plant-derived material, especially since the bacterial cellulose is produced in a pure (free from other polymers) it does not have hemicellulose or lignin that need to be removed and can be grown to virtually any shape, crystalline form which makes its recovery relative simple, and it has exceptional physicochemical properties [6]. In the synthesis of bacterial cellulose, *Acetobacter xylinum* used the natural glucose present in the ineapple waste as carbon source. In the normal condition, *A. xylinum* produced thicker layer of bacterial cellulose by synthesizing more glucose resulting to higher yield of bacterial cellulose. For other case, the data indicated that as the concentration outside the optimum concentration range 75%-85%, resulted decrease in bacterial cellulose production due more gluconic acetic or lactic acid formed when more glucose is synthesized during fermentation process. This side product will accumulate into the medium and decreases the pH level, thus decrease the bacterial cellulose production and the optimal growth temperature for bacterial cellulose production is 25°C-30°C, although most researchers observed that 30^{o}C is the best temperature for cellulose yield. The highest value of bacterial cellulose yield is at temperature of 29.50°C -30.50°C and the optimum temperature is 30°C. During bacterial cultivation, gluconic acid and 5-keto-gluconic acid are responsible for the decrease of the pH value thus affect bacterial cellulose productivity [2]. Several studies were carried out in the same institution on Pseudomenase spp such as the ability of these bacteria to resistant several antibiotics and ability of these bacteria to bioremediation of oil contaminated the soil [7-9]. The present work was carried out to optimize the nutritional and environmental parameters for improving cellulose production by *Pseudomonas* spp.

MATERIALS AND METHODS

Sample collection

Fifty-five of different food samples (rice, meat, cream, cheese, eggs, bread, chicken, tomato, apple, peach and grape) were collected from different local markets in Baghdad governorate in sterile container and transported to the laboratory until using.

Pseudomonas Isolation and identification

One-gram of each food sample was added to 9 ml of sterilized peptone water, mixed thoroughly and serial dilutions for each sample were done separately, then 100 µl aliquots from the appropriate dilution were taken and spread on MacConkey agar and incubated at 37°C for 24 h. Fluorescing colonies was streaked on MacConkey agar plate and these steps were repeated until pure culture was obtained. Morphological, cultural characteristic and biochemical tests such as oxidase, catalase, nitrate reduction to nitrite, triple sugar iron (TSI), Voges Proskauer, indole test, pigment production on Macconkey agar and nutrient agar, hemolytic reaction on blood agar in addition to culture on selective agar containing inhibitors such as cetrimide agar were used for presumptive identification of *Pseudomonas* were performed for full identification of bacterial isolates.

Detection and extraction of cellulose produced from bacterial isolates

To screen bacterial isolates for their ability to produce cellulose, the standard method of Son *et al.* (2002) was used [10].

Cellulose analysis by FTIR

The highest dry weight of cellulose obtained was analysis by FTIR spectroscopy as follows: Sample of dried weight was mixed with potassium bromide (KBr) crystals at ratio 1:10 (w/w) using motor and pestle, placed in cap and compressed to form a thin pellet, the spectrum of the pellet was obtained by ashimadzu FTIR spectrophotometer [11].

Determination of optimum conditions for cellulose production from Pseudomonas spp

Effect of different carbon sources

Fifty ml of sterilized Hestrin-Schram medium (HS-medium) containing 2% (w/v) of different kinds of sugars instead of glucose (dates molasses, ethanol, maltose and fructose) were inoculated with 10% of activated bacterial culture broth (optical density, 0.1), and incubated at 30 °C for one week. Cellulose was extracted and dry weight was measured.

Effect of nitrogen source

Fifty ml of sterilized HS- media with optimum carbon source were prepared with different nitrogen sources (peptone, yeast extract, tryptone, malt extract, and sodium nitrate) each of these nitrogen sources were added to the production medium (HS medium) at a concentration of 1% (w/v) and inoculated with10% of activated bacterial culture (optical density, 0.1), and incubated at 30 °C for one week. Cellulose was extracted and dry weight was measured.

Effect of pH

Fifty ml of sterilized HS- media with optimum carbon and optimum nitrogen source were prepared at different pH values (5, 5.5, 6, 6.5, 7 and 7.5). The medium was inoculated with 10% of activated bacterial culture and

incubated at 30 °C for one week. Cellulose was extracted and dry weight was measured.

Effect of temperature

Cellulose production medium (HS- medium) was inoculated with 10% of activated bacterial culture and incubated at different temperatures (20, 25, 30, 37, 40 and 45°C) for one week. Cellulose was extracted and dry weight was measured.

RESULTS AND DISCUSSIONS

Pseudomonas Isolation and identification

Fifty-five of different food samples were collected from different local markets in Baghdad governorate. Thirty–five of bacterial isolates were belonged to *Pseudomonas* spp. depending on morphological and microscopic examination [12,13]. *Pseudomonas* isolated on MacConkey agar showed that the strains of this organism produced fluorescence on MacConkey agar. Fluorescing colonies were checked for oxidase activity and then subcultured to fresh MacConkey agar plates. Selective agar containing inhibitors such as cetrimide also used for isolation and presumptive identification of *Pseudomonas* spp. colonies of *Pseudomonas* spp. appeared as colourless, but white, off-white, cream, and yellow colony pigmentation when grew on cetrimide agar medium. Microscopic examination showed Gram-negative, rod-shaped and polar-flagellated bacteria. Non spore former bacteria.

Biochemical tests

The results showed that 35 isolates were identified as *Pseudomonas* spp., they were oxidase positive, catalase positive, nitrate reduction to nitrite was positive, triple sugar iron (TSI) alkaline (k)/alkaline (k) (slant/butt), negative Voges Proskauer, indole negative, pigment production on Macconkey agar and nutrient agar, a hemolytic reaction can be observed on blood agar [14].

Detection and extraction of cellulose produced from Pseudomonas isolates

The ability of *Pseudomonas* isolates on cellulose production was assayed after culturing in HS broth medium and incubating at 30 °C for one week, then dry weight of crude cellulose produced by isolates were determined. The results showed in **table 1** indicated that seven isolates of *Pseudomonas* isolates were cellulose producers with different ranged between (4.6-2.8 g/L) according to the formation of white pellicle on the surface of HS broth medium. *Pseudomonas* $_{f3}$ isolate from tomato gave the maximum cellulose production (4.6 g/L), and this may be attributed to physiological and genetic properties of this isolate.

Analysis of cellulose production from Pseudomonas f3 isolate by using FTIR

The results showed the presence of O-H stretching group in 3365.55^{cm-1}, bending groups C-H and H-C-H in 2968.24 and 2852.52 $^{cm-1}$, The C-O-C groups stretching vibration appears at 1118.64 $^{cm-1}$, C-C groups presence at 1436.87 (**fig. 1**).

Table1. The ability of *Pseudomonas* spp. on cellulose production in HS broth medium after incubation at 30°C for one week.

Isolate	Source of isolation	Cellulose weight (g/L)
Pseudomonas $_{f1}$	Cream	3.2
Pseudomonas $_{f2}$	cheese	2.8
Pseudomonas $_{f3}$	Tomato	4.6
Pseudomonas $_{f4}$	chicken	3.8
Pseudomonas $_{f5}$	Bread	2.9
Pseudomonas $_{f6}$.	rotting peach	3.7
Pseudomonas $_{f7}$	rotting apple	4.3

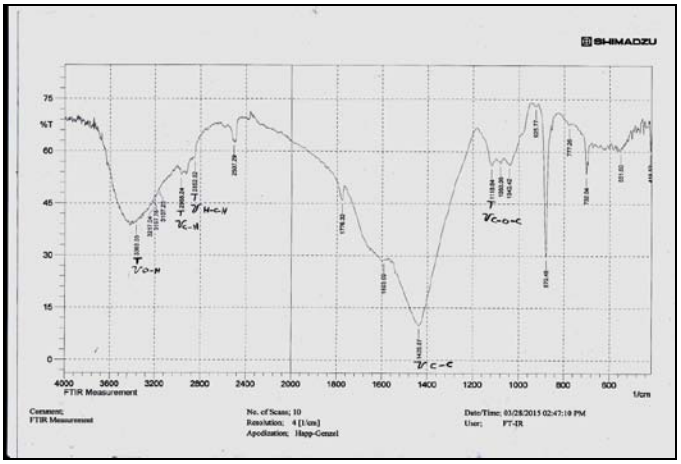

Fig. 1 FTIR analysis of cellulose produced from *Pseudomonas*$_{f3}$.

Effect of different culture conditions on cellulose produced from *Pseudomonas*$_{f3}$

Effect of different carbon sources

The results showed that the production medium (HS-medium) containing dates molasses was the best medium for cellulose production and the productivity of cellulose was less when other carbon sources were used to supplement production medium. The dry weights of cellulose were (dates molasses 6.3 g/L, glucose 5.6 g/L , fructose 3.4 g/L ,maltose 2.3 g/L, ethanol 0.8 g/L) (**fig. 2**). dates molasses promote cell growth and increase cellulose production beacouse it is a good nutrients [15]. Cell growth and cellulose yield were increased in the date syrup medium more than in the glucose medium [15].

Effect of nitrogen sources

The results showed that the highest cellulose production was obtained from HS- medium containing yeast extract followed by peptone, malt extract and tryptone, while the lowest production was observed in HS- medium containing sodium nitrate, the dry weights were 7.2 g/L , 6.8 g/L, 4.8 g/L, 2.3 g/L, 0.8 g/L, respectively (**fig. 3**).

Yeast extract serves to be a better nitrogen sources for the production of cellulose, yeast extract enhances and

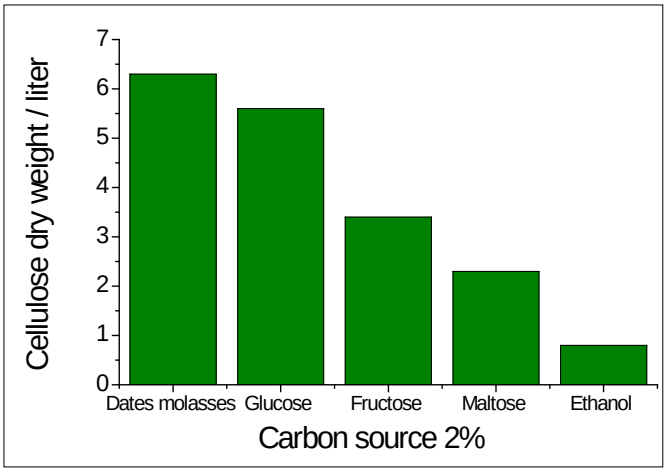

Fig. 2 Production of cellulose from *Pseudomonas* ($_{f3}$) in HS- medium containing different carbon sources, pH 5, inoculums size10%, incubated at 30 ^{0}C for one week.

increased bacterial growth, biosynthesis of an important molecules such as (nucleic acid, protein, and other important components), providing nitrogen, amino acid, carbon and vitamins, especially vitamin B complex, that gives the requirements for microorganism for growth and cellulose production [16].

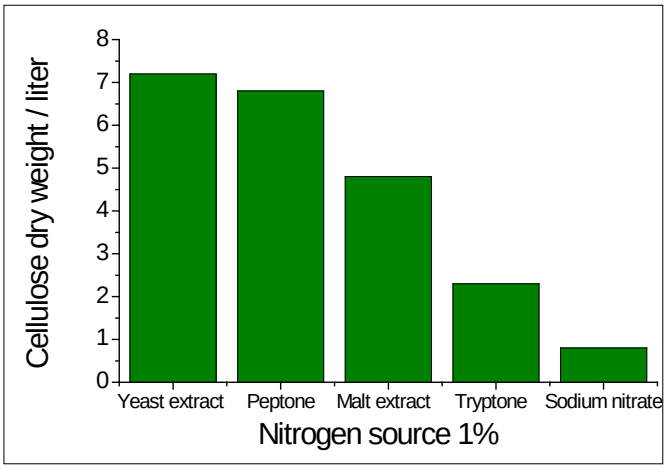

Fig. 3 Production of cellulose from *Pseudomonas* ($_{f3}$) in HS- medium containing 2% dates molasses and different nitrogen sources, pH 5, inoculums size10%, incubated at 30 ^{0}C for one week.

Effect of pH on cellulose production

The results showed that good productions were obtained at all pH values, and maximum productions were observed at pH (5), the dry weights ranged from (6.9-4.3 g/L) (fig.4).The optimum pH for bacterial cellulose production was range from (5.25-5.75) which resulted decrease in gluconic acid. Gluconic acid responsible for dropping pH value of the production medium then inhibited cell growth and cellulose productivity [5].

Effect of temperature on cellulose production

The results showed that the best temperature for cellulose production was 30°C (dry weight was 6.3 g/L), followed by 25°C (dry weight was 5.8 g/L). The production was decrease at temperature above than 30^{0}C (**fig. 5**).

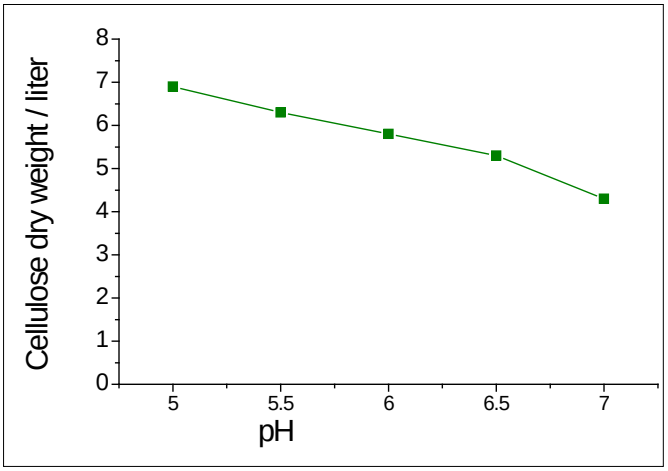

Fig. 4 The effect of pH on cellulose production from *Pseudomonas* ($_{f3}$) in HS- medium containing 2% dates molasses and 1% yeast extract, inoculums size 10 %, incubated at 30 ^{0}C for one week.

The optimum growth temperature for bacterial cellulose production was 30°C although most researchers observed that 30°C is the best temperature for cellulose yield and the conversion of glucose to cellulose is regulated by a multi steps carbon metabolism pathway involving a large number of both individual enzymes and complexes of catalytic and regulatory proteins [17].

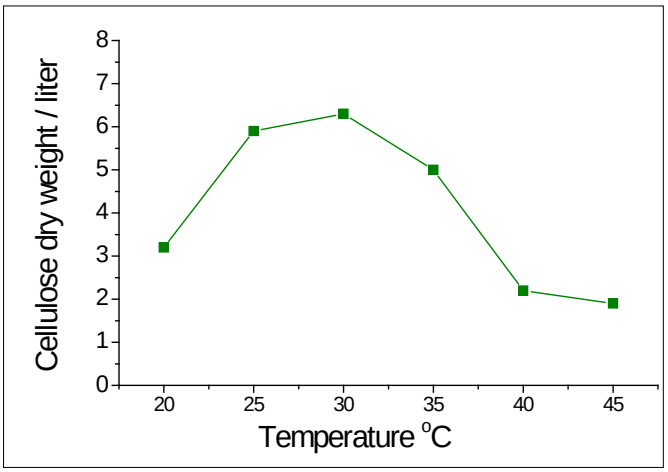

Fig.5. The effect of temperature on cellulose production from *Pseudomonas* ($_{f3}$) in HS- medium containing 2% dates molasses and 1% yeast extract, pH 5, inoculum size was 10%, incubated at different temperature for one week

For the temperature range 30.50°C to 31°C, the bacterial cellulose yield is decreased because of the enzyme cannot perform well when the temperature is over than optimum temperature and temperature between 28^{0}C-30^{0}C is suitable for enzyme to work optimum performance [17]. It can conclusion from present study that thirty five of bacterial isolates were belonged to *Pseudomonas* spp. depending on morphological, microscopic examination, and biochemical tests. Seven isolates from Thirty–five isolates of *Pseudomonas* spp., isolates were cellulose

producers with different ranged between (4.6-2.8 g/L) according to the formation of white pellicle on the surface of HS broth medium. *Pseudomonas* f3 isolate that isolated from tomato gave the maximum cellulose production (4.6 g/L), and this may be attributed to physiological and genetic properties of this isolate. FTIR analysis of cellulose produce from *Pseudomonas* f3 isolate showed the presence of functional groups O-H, C-H, H-C-H, C-O-C, and C-C groups. Date molasses and yeast extract were optimal carbon and nitrogen sources respectively for cellulose production in concentration of 2% and 1% respectively, and maximum productions were observed at pH (5), and incubation at 30°C for one week.

Conflict of interest

The author declares that she has no conflict of interests.

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