ISSN 1996-3351

Asian Journal of Biological Sciences

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Asian Journal of Biological Sciences 6 (8): 376-381, 2013
ISSN 1996-3351 / DOI: 10.3923/ajbs.2013.376.381

Towards Safer Alginate Production from a Strain of *Pseudomonades*

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ABSTRACT

Pseudomonas aeruginosa produces a unique type of soft alginate. But, being an opportunistic pathogen, its alginate production is one of the missed biotechnological products. Few *Pseudomonades*, other than *P. aeruginosa*, are able to produce alginate. The safest ones those isolated from plant source. In this study, *Pseudomonades* like strain that was isolated from plant source, showed the ability to produce alginate. Its alginate was produced under static cultivation condition. Alginate produced from *P. aeruginosa* ATCC 9027 was used as a control and underwent the same conditions. The net amount of the produced alginate from the new *Pseudomonades* like isolate was 3 g L^{-1}, which is less by 33.3% than that produced by *P. aeruginosa*. FT-IR and DSC were used to evaluate the yielded alginates. The FT-IR and DSC results showed that the alginate extracted from the new isolate is almost compared to that produced by *P. aeruginosa*. This study showed the ability to produce alginate using safe strain of *Psedomonades* bacteria.

Key words: *Psedomonades*, alginate, safe production

INTRODUCTION

Pseudomonas aeruginosa is one of the opportunistic bacteria (Satsuta *et al*., 1984). It causes nearly no problem for healthy individuals; however, it can cause severe diseases for immunocompromised patients (Stephenson *et al*., 1985; Boukadida *et al*., 1991; El Baze *et al*., 1991; Fergie *et al*., 1991, 1994; Furukawa *et al*., 1993; Qu *et al*., 2002; Lang *et al*., 2004; Kalai *et al*., 2005).

Because *P. aeruginosa* is able to show pathogenic properties, its has few biotechnological applications. Despite of that, the advances in the fermentation processes control enable using *P. aeruginosa* as a package for producing some biotechnological products, however, the risk is still existing (Buning-Pfaue and Rehm, 1972; Chayabutra and Ju, 2001; El-Bessoumy *et al*., 2004; Benkert *et al*., 2008). Therefore, it is worth to search for more friendly *Pseudomonades* like. *Pseudomonades* alginate is flexible while that produced from *Azotobacter vinelandii* is rigid upon react with Ca^{2+} (Sherbrock-Cox *et al*., 1984).

Many authors reported that *Pseudomonades* like which isolated from plant source are able to produce alginate. Those which are harmless for human can be used for commercial alginate production.

So, the main aim of this study is to investigate the efficiency of an isolate of *Pseudomonades* like strain got from plant to produce alginate compound close to that produced by *P. aeruginosa*. The successful of new plant isolated strain in producing alginate in an acceptable quantity will result in a safe and economic production of alginate from *Pseudomonades*.

MATERIALS AND METHODS

Strains: *P. aeruginosa* ATCC 9027 was used in this study. It was routinely cultivated on LB medium or Muller-Hinton agar at 37°C (Sambrook *et al*., 1989; Atlas, 1995).

The new *Pseudomonades* like strain was got from vegetables waste collected from local market. These vegetables waste was screened for *Pseudomonades* using M-Cetrimide medium (Atlas, 1995). The new isolate was cultivated routinely on LB medium or Muller-Hinton agar at 37°C.

Biochemical test: The new isolate was subjected to basic biochemical tests to prove that it is a *Pseudomonades* like isolate and that it is not *P. aeruginosa*. Those tests include Gram-stain, its ability to grow in Citramid-medium, production of fluorescent on Muller Hinton agar, production of alginate on LB medium, hemolytic on Blood agar, motility and oxidase test.

Alginate production: Alginate was produced from the tow invastigated strains using 100 mL flasks contain, each, 25 mL of LB medium. The flasks were incubated at static incubator at 37°C for 72 h.

Alginate isolation and purification: Alginates from the used *Pseudomonades* were isolated and purified according to the following method. After 72 h static cultivation using LB medium at 37°C the cells free supernatant was collected (for each strain) by decantation. Two point five volumes of 50 mM EDTA was added to each cells pellet. The pellets then re-suspended and vortex aggressively for 5 min followed by centrifugation at 1500 rpm. Each supernatant was collected by decantation and two volumes of cold ethanol were added to each followed by incubating the mixtures at -80°C for 30 min. The cold supernatant was centrifuged at 13000 rpm for 15 min. The resultant pellets were collected for each preparation and dried. For further purification, the pellets were dissolved in double distilled water and dialyzed for overnight. The alginate for each preparation was collected using cold ethanol precipitation method that mentioned above. The final amount for each type of alginate was dried using airflow drying process in suitable container and the net recovered alginate was weighted. The dry alginate was preserved in a clean and sterile glass container for further use.

Fourier transform infrared spectroscopy: The Fourier Transform Infrared Spectroscopy (FT-IR) spectra of the produced alginates were recorded by FT-IR spectrophotometer (FT-IR-8400S, Shimadzu, Kyoto, Japan). The investigated sample of alginate was mixed with potassium bromide (KBr) and converted the mixture into a disk by compressing the mixture using hydraulic press. The resultant disk was scanned from 4000-600 cm^{-1} according to Sherbrock-Cox *et al*. (1984).

Differential scanning calorimetry: Differential Scanning Calorimetry (DSC) DSC-60 (Shimadzu, Japan) was used to investigate the produced alginates. Three to five milligram of the investigated alginates were loaded in an aluminum pans and covered by a lid using a crimper. The thermal behavior of each sample of alginate was studied by raising the temperature form 25-300°C in a rate of 10°C min^{-1} under nitrogen. Thermograph was got using the TA-60WS thermal analysis software.

RESULTS

Biochemical identification for the new isolate: The new isolate was a gram-negative motile road. This isolate showed the ability to grow on Citramid agar and produced fluorescent on Muller Hinton agar. This new isolate showed the ability to produce alginate when cultivated in LB medium, which are the main feature of *Pseudomonades*. But, it is oxidase negative and non-hemolytic when cultivated on Blood agar.

Alginate production and purification: The net gain from the alginate produced by the new isolate under the described condition was equal to 3 g L^{-1}, whereas, for *P. aeruginosa* ATTC 9027 under the same cultivation and purification conditions was 4.5 g L^{-1}.

FT-IR analysis: The results from the analysis of the alginate produced and purified from both of the new isolated strain and *P. aeruginosa* were shown as in Fig. 1 and 2. Both figures (Fig. 1 and 2) represent typical alginate plateaus.

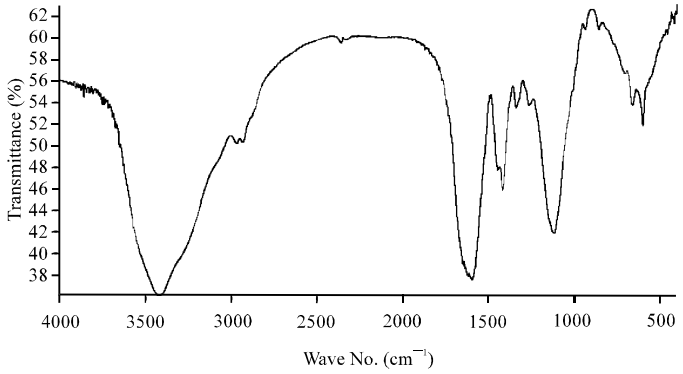

Fig. 1: FT-IR alginate analysis of the new *Pseudomonades* like isolate

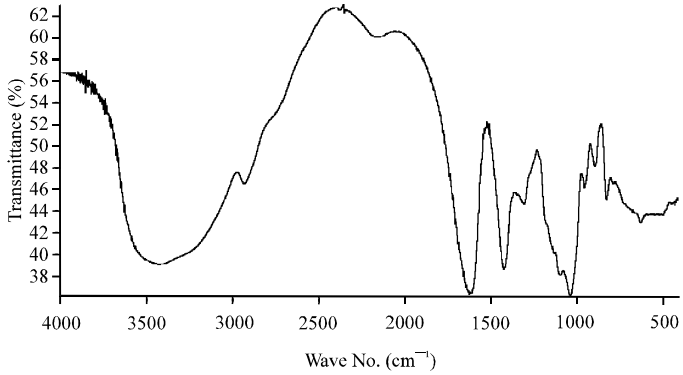

Fig. 2: FT-IR alginate analysis of the *Pseudomonas aeruginosa* ATCC 9027

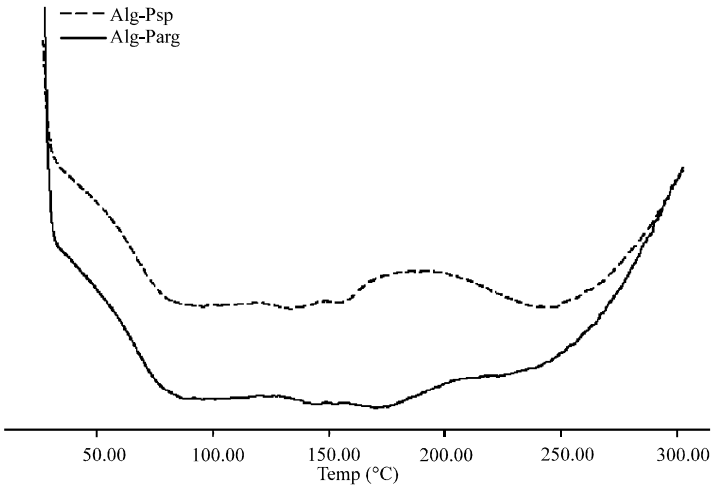

Fig. 3: DSC trace of alginate got from *Pseudomonas aeruginosa* ATCC 9027 (Alg-Parg) and the new *Pseudomonades* like isolate (Alg-Psp)

DSC analysis: Despite the absence of a characteristic and define transition beak for alginate, the thermogarms of the investigated samples (the alginates got from new isolate and *Pseudomonas aeruginosa*) showed almost the same behavior (Fig. 3).

DISCUSSION

Alginate is a biopolymer produced by algae and few bacterial species. Alginates from different sources and found its way in many industrial, pharmaceutical and medicinal applications. Based on its type and monomeric composition, the application is variable. *P. aeruginosa* has a unique type of soft alginate. Being an opportunistic pathogen, *P. aeruginosa*'s did not commercialize yet. *P. aeruginosa* other products were cloned and expressed out of host using the molecular biology and genetic engineering tools.

Unfortunately, its alginate did not produce. This is because of that, alginate production in *P. aeruginosa* is a complicated and multi-step process. It does not follow the role of "from gene to protein". Its production governs by a complicated process where genes/proteins, transport system, excretion system, signaling system are involved. Alginate production is host dependent products; however, future might show a success for producing it in an independent host.

Alternatively, scientists have investigated different ways to produce the same type of alginate using species except *P. aeruginosa*. Their concept based on using *Pseudomonades* like isolated from non-human source such as plant. *Pseudomonades* isolated from plant proved historically that it is none or less harmful for human. But, some risk is still existed. The system of *Pseudomonades* associated with plant is adapted for manipulating plant material, which reduce many important virulence factors for human.

The current study is designed to explore *Pseudomonades* got from plant wastes collected from local market using the Citramid agar as enrichment medium. The biochemical analysis of the new strain revel that this strain related to the *Pseudomonades* and it is not *P. aeruginosa*. For comparisons purposes, *P. aeruginosa* strain ATCC 9027 was used. Both strains were cultivated on LB medium, which might not be the optimum alginate production condition for both strains and particularly for the new *Pseudomonades*. Meanwhile, LB media insure

alginate production under static condition. Static cultivation induce algT(U) mutations which occur in vitro when organisms are grown under static conditions in ambient air as reported by Hassett (1996).

After 72 h incubation under static condition at 37°C the produced alginate was recovered and purified using simple method as described in this study. The method ensures maximum recovery and high purity. Both alginate were characterized using FT-IR spectrophotometer and DSC and proved to be nearly identical.

This study simply summarizes a process for isolation of *Pseudomonades* like strain from plant source other than *P. aeruginosa*. This study showed the possibility to produce and purify the alginate produced by both strains (using static cultivation). The production condition did not maintain optimum alginate production. However, static cultivation ensures producing alginate whenever their related genes are existed. Simple isolation and purification process can be used for better comparison between the different types of alginates.

This study recommended using plant *Pseudomonades* for alginate large scale production. Further study about to medium optimization and molecular identification of the new isolate should be done in future study.

CONCLUSION

In this study, a pseudomonade from plant source was isolated. The strain shows ability to produce alginate. The produced alginate is similar to that produced from *P. aeruginosa* 9027. When *Pseudomonades* from plant source could be a safe force for alginate, this study open the way for a safe soft alginate production.

ACKNOWLEDGMENT

The authors extend their appreciation to the Deanship of Scientific Research at King Saud University for funding the work through the research group project No. RGPRGP-VPP-038.

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