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Curdlan Production by Agrobacterium Radiobacter and Wide Array of its Applications in Medicine

KEYWORDS: Curdlan, EtBr, FT-IR, UV-Vis-Spectrophotometry

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ABSTRACT *In this study, we carried out curdlan production from Agrobacterium radiobacter [NCIM 2443] bacterial strain. This strain produces two types of colonies viz... white & blue colonies on Aniline blue media plates. In which white colonies are succinoglucan producers & are heteropolysaccharides which are water soluble & Blue colonies are β-[1→3] glucan producers & are homopolysaccharides which are water insoluble. Blue colonies were the curdlan producers by which curdlan production carried out. Further, chemical mutagenesis with Ethidium bromide [EtBr] was also carried out for high production of curdlan. FTIR and UV-Vis-Spectrophometry also carried out to analyze curdlan.*

Introduction

Curdlan is an extracellular unbranched homo β-(1→3] glucan, which is water insoluble at a neutral pH [1, 2]. Curdlan forms a firm gel when heated at or above 54^{0}C in water bath [3]. Since it can be used as gelling agent in chemical and food industries. It acts as a structural macromolecule in the cell wall of yeast, mushrooms and other higher plants or excreted as exopolysaccharides by bacteria[3]. Curdlan is a neutral gel forming β–(1→3)D- glucan[5]. It was first detected in A. biovar [6]. It is a secondary metabolite synthesized by *A. radiobacter* under nitrogen-limiting conditions [7, 8].It is Gram negative,motile & bacilli or rod shaped bacteria.It is used for curdlan production because it produces distinct blue & white colonies on AB media plates. A minimal salt medium (MSM) containing mineral salts and a phosphate buffer to maintain a suitable pH during culture source in the medium is considered as critical factor for changing intracellular metabolism, because isoprenoid lipids that play a vital role in carrying cellular oligosaccharides would be more available for curdlan synthesis instead of cellular lipopolysaccharides synthesis under nitrogen limiting conditions [9]. Curdlan drawn considerable interest because of its unique thermal rheological and gelling properties. Curdlan shows wide range of applications in medicine, food,cosmetic and pharmaceutical industries. It is extensively used as an ingredient in animal feed since it acts as immune stimulator [10,11]. curdlan suplhate is developed as an antiviral agent against human immunodeficiency virus factors [12,13]. It shows various immunomodulating activities like phagocytic activity [14], cytotoxic activity on macrophages and other biological activity [15,16,17]. It shows antitumorigenicity, antiinfective activates against bacterial, fungal, viral and protozoal agents, anti inflammatory activity, wound repair, protection against Radiation & anticoagulant actively [15,18,19] shows probiotic activities and supports/stimulate growth of probiotics Also shows antioxidant properties [20]. Considering its wide applications in medicine and pharmaceutical area. In this study we carried out its production. Further we also carried out chemical mutagenesis of strain for high production of curdlan.

Material and Method

Selection of Microorganism:

A pure culture of *Agrobacterium radiobacter* (NCIM 2443) was purchased from NCIM, Pune. This strain was used for curdlan production. As aniline blue reacts specifically with curdlan, curdlan producing colonies were selected by using Aniline Blue medium (AB medium) containing (per liter) : glucose 10gm, yeast extract 5gm, aniline blue 0.05gm, agar 20gm, pH 7.2 [21]. The *Agrobacterium radiobacter* was spread on AB medium and incubated for 24 hrs at 37^{0}C. After incubatetion blue colour producing colonies on medium are selected for production of curdlan.

Production of Curdlan:

The seed culture was prepared by transferring selected microorganism colony to 50ml of growth media in 250ml Erlenmeyer flask containing (per liter): glucose 10gm, yeast extract 10gm, $(NH_4)_2SO_4$ 1gm, KH_2PO_4 0.25gm[23] and incubated for 72hrs at 30^0 C at 180 rpm. After incubation seed culture was transferred to 150 ml Minimal salt medium (MSM) containing (per liter):KH_2PO_4 1.74gm, K_2HPO_4 0.49gm, $Na_2SO_4.10H_2O$ 3gm, $MgCl_2.6H_2O$ 0.25gm, $FeCl_3.6H_2O$ 0.024gm, $CaCl2.2H_2O$ 0.015gm, $MnCl_2.4H_2O$ 0.01gm, Citrate 0.21gm, NH_4Cl 1.5gm, sucrose 20gm [22] aseptically in 500ml Erlenmeyer flask and incubated for 5 days at 30^{0}C & 180rpm.

Recovery:

After incubation curdlan was recovered by adding equal volume of 0.6N NaOH with 30 min stirring to production medium. The bacterial cells were removed from production medium by centrifugation at 10,000 rpm for 10 min. The supernatant was neutralized to pH 7 by adding 4N acetic acid. After neutralization it is heated for 100^{0}C for 10 min. After heating again centrifuged at 10,000 rpm for 10 min. Then pellet was then stored in ethanol at 4^{0}C [7].

Analytical methods: To confirm curdlan confirmatory tests were carried out on the basis of its property. The curdlan was checked for its solubility in 0.25N NaOH. Gel forming property was carried out by heating 2% of aqueous suspension in boiling water bath for 10mins. & cooling. The test for polysaccharide was performed by Anthrone method. Further the FTIR and UV Vis spectrophotometric analysis of curdlan was done.

Strain Improvement:

The culture was inoculated in growth medium and incu-

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bated at 30^{0}C for 72hrs after incubation its was centrifuged at 10,000 rpm for 10 min. The pellet was recovered and then treated with 0.2% Ethidium bromide (EtBr) [7]. These treated cultures were again used for curdlan production as above steps and recovered.

Results:

Strain Producing Curdlan

Agrobacterium radiobacter after incubation on AB medium produced mainly two types of colonies, water soluble white colonies & water insoluble blue colonies as shown in Fig.1 A&B. These results confirm that water insoluble polysaccharide (curdlan) forms a complex with aniline blue as previously reported. [22].

Analysis of curdlan:

The curdlan was checked for its solubility in 0.25N NaOH shows its solubility whereas it is insoluble in water. Gel forming property was carried out by heating 2% of aqueous suspension in boiling water bath for 10mins. & cooling shows the gelling nature of curdlan. The test for polysaccharide was performed by Anthrone method shows blues green complex indicates presence of polysaccharide. From this preliminary analysis its shows curdlan property.

In FT-IR, IR spectra of curdlan obtained showed absorption band near 1020.17 cm-1 & 1110.83 cm^{-1} indicative of ester (C-O) bonds. The spectrum of curdlan showed a strong band at 1643.27 cm-1, which is because of C-C. The band at 3434.68 cm-1 was indicative of O-H group. These groups indicate curdlan presence Fig. 2.

The curdlan produced is the subjected to UV-Vis spectrophotometry analysis the peak of absorption was observed at 487 nm as shown in Fig. 3.

Strain Improvement:

In strain improvement the blue colonies treated with 0.2 % EtBr. It gives more intensive blue colonies on AB media plates Fig. 1 C. These strains are then selected and used for curdlan production shows increased yield of curdlan.

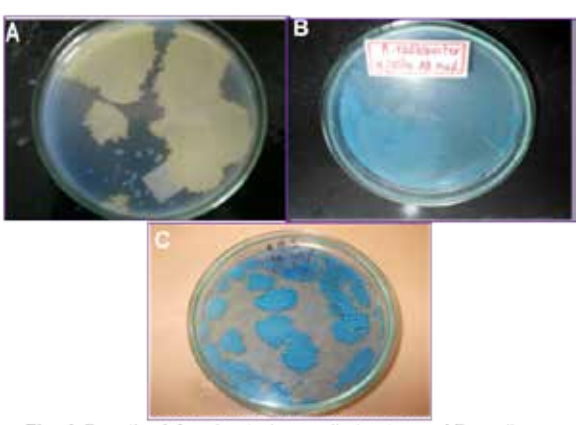
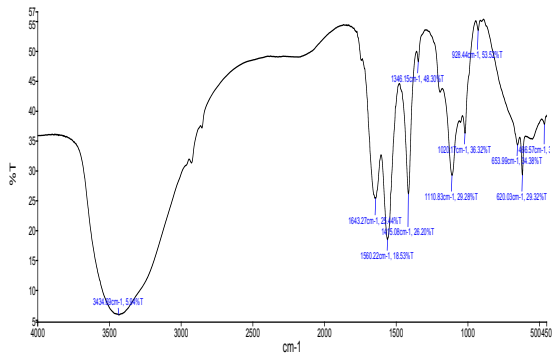
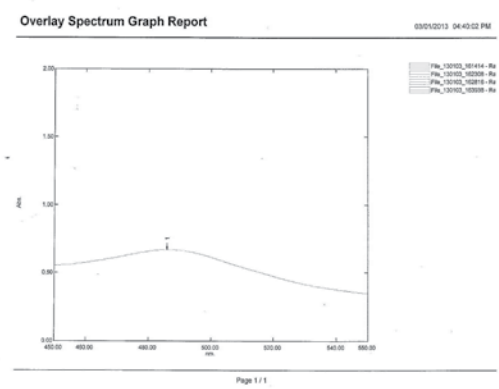


Fig. 1 Growth of *Agrobacterium radiobacter* on AB medium
A] Shows white colonies producing succinoglycn
B] Shows blue colonies producing Curdlan
C] EtBr treated colonies



FTIR Sectrum Band Of Curdlan

Fig.3 UV- VIS Spectrophotometry analysis of curdlan



From results of FT-IR and UV-Vis spectrophotometry and checking the properties of curdlan such as solubility, test for polysaccharide and gelling nature it is concluded that curdlan was produced from *Agrobacterium radiobacter*. The treatment with EtBr with strain of *Agrobacterium radiobacter* produced high curdlan polysaccharide in MSM medium, so this method can be used to produce high yield of curdlan to provide demand of curdlan.

Today, polysaccharides recovered from plants, algae & animal sources are still major contributors to the all over market. This is mainly because of higher prices of bacterial polysaccharides due use of high value of carbon sources & of associated downstream costs. Nevertheless, the research interest in bacterial production of polysaccharides is continuously growing & is focused on using low cost substrates & improving downstream processing that reduces cost. β-(1→3)-glucans could have significative development in next few years, notably in medicine & pharmaceutical industries as there compounds have been described as immunomodulators, antitumorogenic, antiviral (AIDS), agents for treatment of hypercholesterolemia & agents for stabilization of glycaemia. Ultimately the cosmetics & pharmaceutical industry may provide new markets for glucans modified by chemistry.[2] considering these future aspects we still continue our study by using sugar cane molasses as carbon source & corn steep liquor as nitrogen source. So as to reduce its cost which ultimately lead to its high production & use.

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