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International Journal of Current Research
Vol. 9, Issue, 07, pp.53735-53737, July, 2017

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

ISSN: 0975-833X

RESEARCH ARTICLE

ISOLATION OF AUREOBASIDIUM PULLULAN FROM SOIL AND THE PRODUCTION OF PULLULAN

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ARTICLE INFO

Article History:
Received 18th April, 2017
Received in revised form
20th May, 2017
Accepted 08th June, 2017
Published online 22nd July, 2017

Key words:

Aureobasidium pullulan,
Pullulan, Production,
Soyabean oil.

ABSTRACT

Polysaccharides produced by microorganisms are utilized for a variety of purposes, including the use in cosmetics and as food additives. More recently, polysaccharides have been exploited by the medical and pharmaceutical industries. The production and synthesis of these compounds is costly and time consuming. In the present work isolation of *Aureobasidium pullulan* was done from soil sample. The production of pullulan was done by using complex nitrogen medium with different concentration of soyabean oil. Pullulan yield was determined and optimum soyabean oil concentration in the growth medium for pullulan production was determined

Citation: Monal V. Ingawale, Mukesh Tiwary and Dr. Kale, S.C. 2017. "Isolation of Aureobasidium pullulan from soil and the production of pullulan", *International Journal of Current Research*, 9, (07), 53735-53737.

INTRODUCTION

Exopolysaccharides (EPSs) produced by a number of microorganisms are chemically well defined and have attracted worldwide attention due to their novel and unique physical properties. These are rapidly emerging as new and industrially important source of polymeric materials, which are gradually becoming economically competitive. Microorganisms that produce a large amount of slime have the greatest potential for commercialization, since EPSs can be recovered from the fermentation broth easily. During recent years, microbial EPSs have become available for use in many applications that are not only compatible with human lifestyle but also are friendly to the environment (Steinbüchel 2001). Polysaccharides are long carbohydrate molecules of repeated monomer units joined together by glycosidic bonds. They range in structure from linear to highly branched. Polysaccharides are often quite heterogeneous, containing slight modifications of the repeating unit. Depending on the structure, these macromolecules can have distinct properties from their monosaccharide building blocks. They may be amorphous or even insoluble in water. (Pepler and Priman 2nd edition) Pullulan is a polysaccharide polymer consisting of maltotriose units, also known as α-1,4-;α-1,6-glucan'. Three glucose units in maltotriose are connected by an α-1, 4 glycosidic bond, whereas consecutive maltotriose units are connected to each other by an α-1,6 glycosidic bond. Pullulan is produced from starch by the fungus *Aureobasidium pullulans* Pullulan has a wide range of commercial and industrial applications in many fields, including the food and cosmetic industries, environmental treatment, pharmacy and healthcare, agricultural, and chemical industries and even in lithography (Chi and Zhao, 2003; Singh *et al*., 2008a). Despite these applications, the price of pullulan is still three times higher than that of other polysaccharides such as xanthan and dextran, which limits its large-scale utilizations. The high price of pullulan is attributed to several factors, including low product yield. However, with the limitations of low pullulan yield and raw material utilization ratio, it is important to enhance the yield of pullulan during fermentation which will make the process economically compatible. During fermentation process, product concentration and yield depends on several factors and one of them being interaction among the media components

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Aims and Objectives

- To isolate *Aureobasidium spp* from the soil sample
- To characterise the isolate by studying morphological and cultural properties and identify it.
- To carry out pullulan production from the isolated strain of *Aureobasidium spp*.

- To optimize the pullulan production medium with respect to soyabean oil concentration.

MATERIALS AND METHODS

Collection of soil sample

Soil sample was collected from the campus of Krishna Institute of medical sciences deemed university, Karad in polythene bag and immediately brought to laboratory and kept in refrigerator at 4°C till further use.

Isolation of *Aureobasidium spp:* Isolation was done on Sabourauds agar plate and these plates were incubated at 28°C for 4-5 days.

Identification and characterisation of isolate: Isolates were subjected to cultural and morphological and microscopic examination. Microscopic examination was done by wet mount using Lactophenol cotton blue and observing the slide.

Recovery of pullulan: Fermented broth was centrifuged at 3500 rpm for 15 minutes to separate fungal biomass. Supernatant was collected and pullulan was precipitated from the supernatant using one volume of propanol per volume of supernatant. The precipitate was separated by centrifugation at 3000 rpm for 10 mins and dried at 40°C in hot air oven.

Confirmation of the product as Pullulan: Product was subjectedto various below mentioned tests.

Determination of viscosity: Viscosity was determined by viscometer.

Determination of sugar content: Estimation of sugar content of pullulan was done by phenol- sulphuric acid method.

Enhancement of pullulan production by using different concentration of soyabean oil in sucrose solution

Soyabean oil (SBO) was added in different concentration such as 1%.2%, 3%,4%,and 5% in fermentation medium and one

Table 1. The result of colony characteristics are shown below

Isolate	Size	shape	colour	Margin	Elevation	Spore colour	Mycelium	opacity	consistency
Ab1	4mm	circular	Black	Irregular	Convex	Black	Arial	Opaque	Dry

Sr. No	Soyabean oil concentration	O.D at 450nm
1	0%	0.40
2	1%	0.49
3	2%	0.53
4	3%	0.58
5	4%	0.62
6	5%	0.67

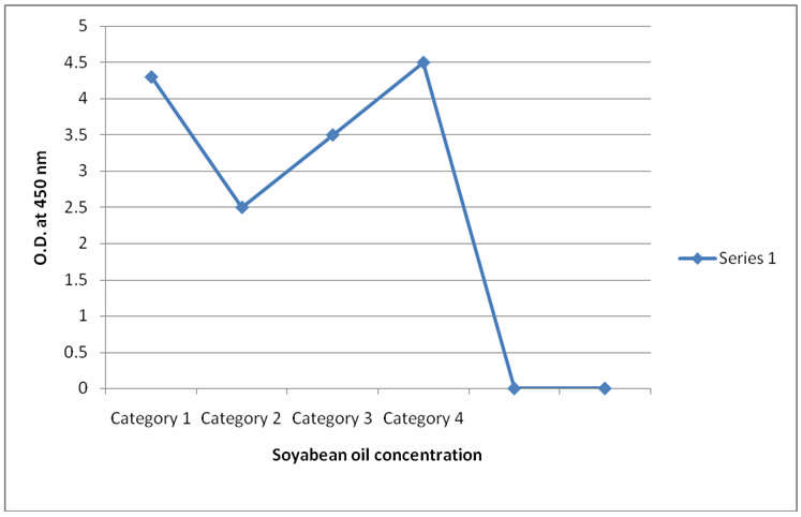

Figure 1. A graph showing result of carbohydrate content of pullulan supplemented by soyabean oil (SBO) using phenol sulphuric acid method

Production of Pullulan

Preparation of inoculum: A loopful of suspension of isolate Ab1 was inoculated in 50ml sterile seed medium. And the flask was incubated at 28°C for 4 days on a rotarory shaker.

Fermentation: 100ml of sterile complex nitrogen broth containing 5% sucrose was inoculated with 5% v/v inoculum from seed culture. These flask was incubated at 28°C and 250 rpm on a rotatory shaker for 3-4 days.

flask without soybean was used for fermentation to evaluate its effectiveness.

RESULTS AND DISCUSSION

Isolation of the isolate Ab1

After spreading the soil dilution on Sabouraud's agar plate black mycelial growth was observed on 4th day. After

incubation typical black coloured colonies of 4-5 mm were observed.

Identification of isolate: The isolate Ab1 was subjected to cultural, morphological characterization. The isolate was mounted on slide using lactophenol cotton blue and was observed under 45X objective lens.

Production of pullulan: The isolate Ab1 based upon it's growth response was used to produce pullulan using complex nitrogen medium and sucrose.

Determination of carbohydrate content of pullulan by phenol sulphuric acid method: Table no.4.2: Results of carbohydrate content of pullulan supplemented by soyabean oil (SBO) using phenol sulphuric acid method

Determination of Viscosity: Determination of viscosity was done by Searle's viscometer. The viscosity of pullulan was found to be 1.85cp.

Determination of sugar content

Table 4. The result of the pullulan production at varying concentration of the soyabean oil

Sr. No	Soyabean oil concentration	Pullulan production
1	Control	57%
2	1%	70%
3	2%	76%
4	3%	83%
5	4%	88%
6	5%	96%

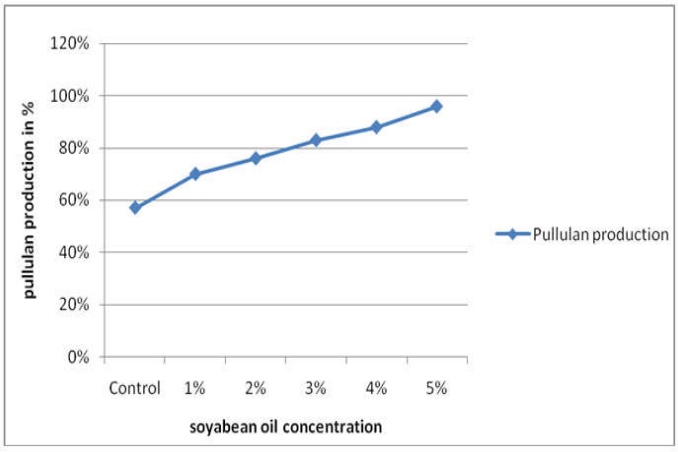

Figure 4. The result of the pullulan production at varying concentration of the soyabean oil

Conclusion

Aureobasidium puluulan was used for the production of pullulan. Maximum production of Pullulan i.e 96% was obtained when grown in 5% concentration of soyabean oil.

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