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Purification of Pullulan from Microorganisms for Food and Biomedical Applications.

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ABSTRACT

The emergence of modern microbial technology and product biotechnology had significantly made over the way, scientists and researchers view differently the microbes and the product they biosynthesize. Polymers create a significant role inside the natural environment and also play a vital action in the fashionable industrial economics. Some of the natural biopolymers like proteins and nucleic acids pass the biological information both in the intracellular and extracellular environment. Other polymers such as sugar based polysaccharides provide metabolic energy for the cellular activity and play important role with their potential applications in the different sectors. For instance, those can be used as lubricants, absorbents, adhesives, cosmetics, drug delivery, structural materials, lithography and for the manufacturing of computational switching devices). The main constraints and major obstacles for the full commercialization are due to their production cost, which is related to cost of the substrates and downstream processing. In this research study purification and invitroanalysis of pullulan will be discussed.

Keywords: microorganism, pullulan, polysaccharide.

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INTRODUCTION

The polysaccharides synthesized by the microbes have been used commercially with wide range of applications. Presently, the polysaccharides produced from algae (examples: alginate, agar), crustacean (example: chitin), plants (examples: guar gum, gum arabic) still demand the need and dominate the market along with polysaccharides obtained from the microbes like xanthan gum, gellan, levan, dextran, curdlan etc [1,19,20]. The microbial polysaccharide contributes very small fraction of the biopolymers market. Recent times the exopolysaccharides synthesized from the microbe are used in a wide range of the applications as foods, cosmetics and pharmaceuticals etc. The plant-derived polysaccharides like Guar gum, Arabic gum, Chitin, Agar, Alginate etc., still dominate the market with the microbial polysaccharides like gellan, xanthan, bacterial alginate etc.

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PULLULAN; AN EMERGING MICROBIAL POLYSACCHARIDE

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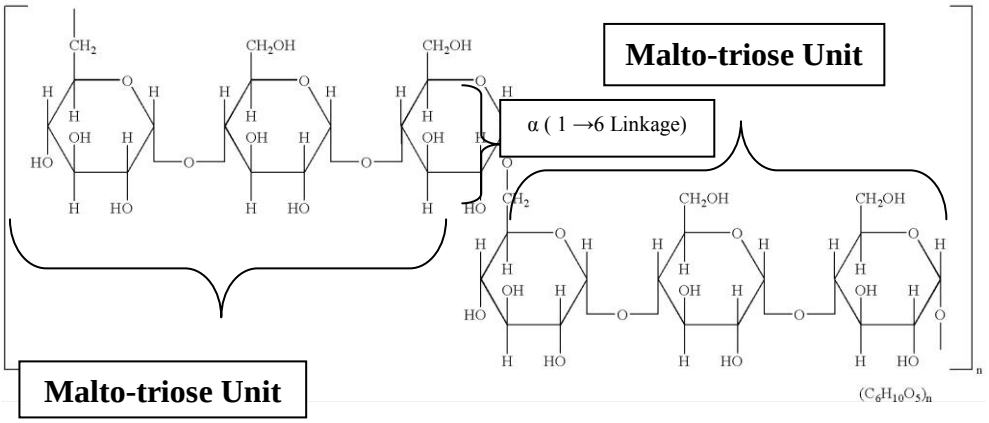

Figure 1: General structure of the pullulan

Table-1 Properties of Pullulan

Parameter	Specifications
pH of solution	5–7
Water solubility	Easily soluble
Appearance	White or yellowish-white powder
Specific optical activity $[\alpha]_{\lambda}^{T}$ in 1% water	Minimum +160°
Polypeptides	Maximum 0.5 %
Molecular weight	100–250 kDa
Loss of moisture on drying	Maximum 6 %

Figure 2: Different types of decorated food items prepared by pullulan

The microbial pullulan can able to make a thin film which is oil resistant, transparent, odourless, colourless, tenacious, and impermeable to In many cases, pullulan can be used in place of the starch for the preparation of low calorific foods [2,7,8,13,14,17].

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Recently, many researchers have investigated that the surface modified pullulan with the substitution of different chemical groups have been used in various bio-medical, pharmaceutical and nutraceutical application [3-6].

MATERIAL AND METHODS

Screening and Isolation of a New Potential Microbial Strain for the Pullulan Production

Sample Collection

The various leaf samples from totally different culinary plant species have been collected from different places like Amrithi forest (12.91651°N, 79.132498°E), Yelagiri (12.5781°N, 78.6407°E), Iruvaram (13.2000°N, 77.1167°E) and Chittoor (13.2000°N, 79.1167°E). All these samples were collected aseptically by using sterile gloves and stored in the sterilised polypropylene bag and brought to the laboratory for further processing [3,5,9,10].

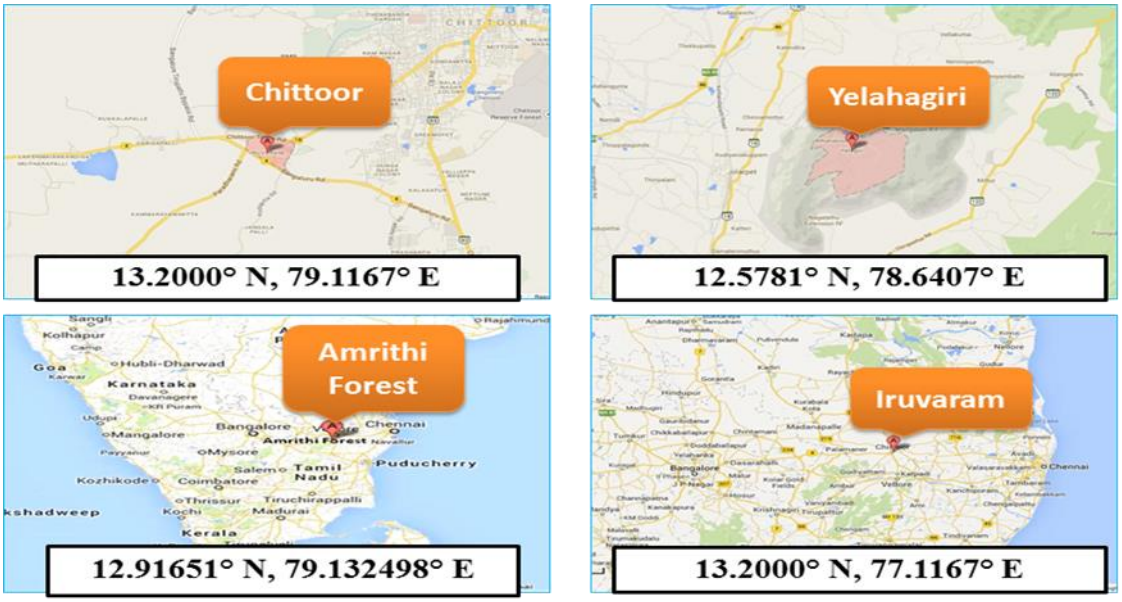

Figure 3: Different geographical locations from Tamil Nadu and Andhra Pradesh where, leaf samples have been collected

Exopolysaccharide Production and Purification

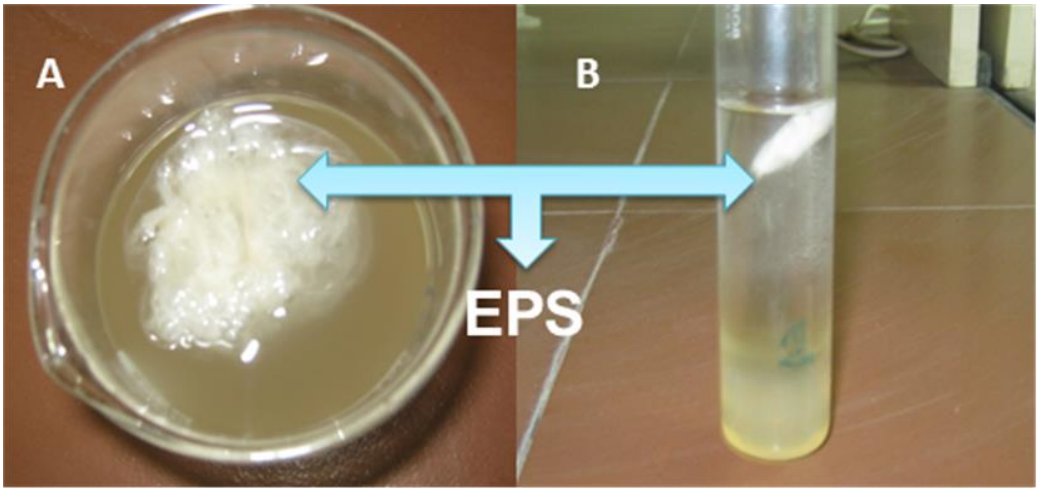

Figure 4: Precipitation of pullulan followed by the addition of organic solvent (A: top view; B: side view)

The assay for the exopolysaccharide (pullulan) production was carried out with the addition of isopropyl alcohol to the centrifuged culture filtrate of the fermentation broth and precipitated pullulan was obtained on the upper layer of the broth. This layer was recovered by separating and was kept for drying Further it was converted in to powdered form The quantity of the crude pullulan was calculated as the grams of dry weight made per 100 mL of fermentation broth, and it was found to be 3.9±0.02 % (Calculated as the means ± s.e.m. of three independent experiments) on the 6th day of the fermentation. The rate of production of pullulan decreased drastically after the 6th day of the fermentation. This might be due to the hydrolysis of pullulan by the action of endogenous enzymes produced by this strain. Additionally, during the later stages of the fermentation, the carbon sources in the medium depleted. So the pullulan produced by the organism was utilized as the carbon source for its survival and growth. However, the biomass was increased during the whole experimental period.

Downstream Processing for the Production of Pullulan from *Aspergillus japonicus-VIT-SB1* and Purification

Activated charcoal is generally used for the separation of melanin pigments from the production medium. In this study different concentration of activated charcoal was used and its effect on pullulan recovery along with the pigment separation was evaluated. It was found that the melanin content was decreased when the concentration of the activated charcoal was increased. But the decrease in the melanin content was found up to 1% (w/v) of the activated charcoal. After that, melanin content was increased. This may be due to the saturation of the activated charcoal with the melanin pigment. Moreover, it was found that the recovery of pullulan also decreased gradually with increase in the concentration of melanin pigment. This was occurred due to the adsorption of pullulan along with melanin by activated charcoal.

Adsorption of Pigments with Solvents Blends

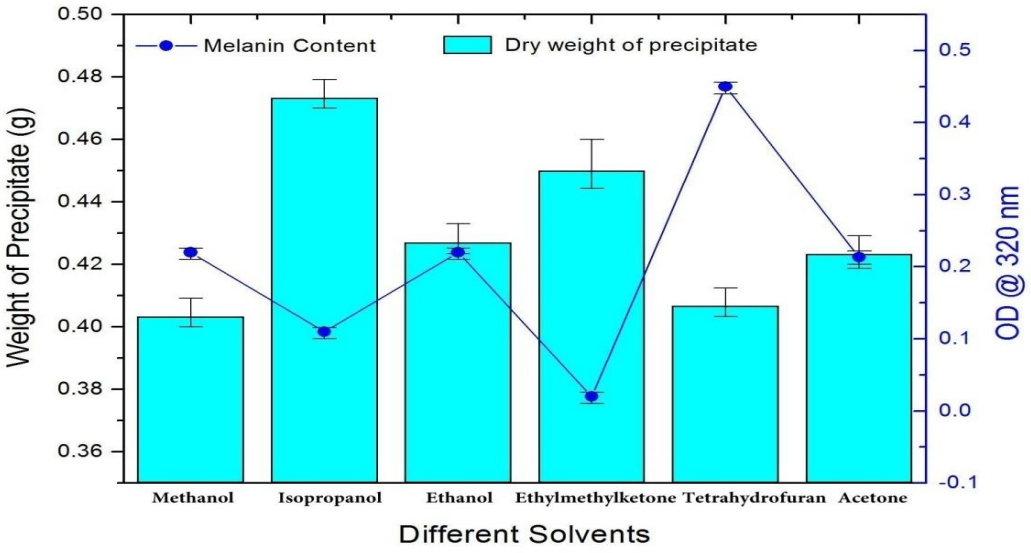

Figure 5: Effect of different solvents for adsorption of melanin along with the dry weight of the precipitated EPS (Values were representative of three separate experiments)

Different types of organic solvents like a. methanol, b. Isopropanol, c. ethanol, d. ethylmethylketone, e. tetrahydrofuran, f. acetone were used to study their efficiency for the adsorption of the melanin pigments. it was found that most of the melanin pigments were adsorbed when ethylmethylketone was used as the organic solvent. At the same time maximum amount of the dry precipitate were formed with isopropanol . Additionally, all the other solvents included in this study except ethylmethylketone gave the precipitate of yellow to brown colour and these were further darkened during storage. In this study, the basic objective was to make a solvent combination, which can absorb more amount of the pigments and precipitate maximum amount of the polymer.

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Extraction of the Pigment Using Combination of Solvents and Salts

Table 2: Different combination of organic solvents and salt (KCl) to find its effect on pullulan recovery and pigments separation

Combination of Solvents and Salt (KCl)	Pullulan Recovery (% w/w)	Melanin Content (OD @ 320 nm)
Ethanol	73	0.18
Ethanol+0.5% KCl	70	0.16
Ethanol+1.0% KCl	71	0.19
Ethanol+1.5% KCl	68	0.19
Ethanol+2.0% KCl	69	0.24
Isopropanol	76	0.23
Isopropanol+0.5% KCl	72	0.21
Isopropanol+1.0% KCl	**73**	**0.25**
Isopropanol+1.5% KCl	70	0.27
Isopropanol+2.0% KCl	70	0.27
Ethyl methyl ketone	71	0.14
Ethyl methyl ketone+0.5% KCl	**68**	**0.14**
Ethyl methyl ketone+1.0% KCl	67	0.16
Ethyl methyl ketone+1.5% KCl	65	0.17
Ethyl methyl ketone+2.0% KCl	63	0.17

It was found that, ethanol, isopropanol and ethylmethylketone gave more precipitates than other organioc solvents. Hence, different combination of these organic solvents (ethanol, isopropanol, ethylmethylketone) and salt (KCl) were taken into consideration for this study. It was found that 73% of pullulan was recovered with less amount of melanin pigment at the combination of isopropanol with 1.0% KCl. At the same time 68% of pullulan was recovered at ethylmethylketone + 0.5% KCl , solvent salt combination with least amount of melanin content. Hence ethylmethylketone + 0.5% KCl was the optimum condition for better production of pullulan with more amount of melanin adsorption. This method was not satisfactory, as the recovery of pullulan was very less .

Hydrogen Peroxide Treatment for the Removal of Melanin

Although different methodologies like activated charcoal method, solvent blends and different combination of solvent and salts were followed in order to separate the melanin from the fermentation broth , the recovery of pullulan was not satisfactory in all these methods. Hence hydrogen peroxide treatment was followed in order to separate the melanin pigments. Generally hydrogen peroxide is used as a decolorizing agent and it has the ability to oxidize the pigment . In this study, the effect of the concentration of hydrogen peroxide on melanin separation was investigated. It was found that as the concentration of hydrogen peroxide increased, the melanin content decreased up to 4% of hydrogen peroxide used. When the amount of hydrogen peroxide was increased more than 4% , no further decrease in OD value at 320nm was observed. This indicated that at 4% of hydrogen peroxide, all the pigments were oxidized and removed. Hence 4% of hydrogen peroxide was considered to be the most optimal amount for the removal of melanin from the fermentation broth and involved in the purification of pullulan.

Structural Analysis by ^{1}H-NMR Spectrometry

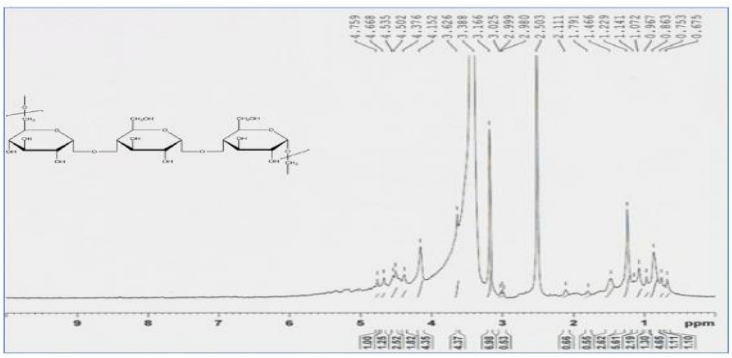

Figure 6

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