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Pullulan, a biopolymer with potential applications in pharmaceutical and cosmeceutical: A review

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Pullulan is an important polysaccharide with unique characteristics. This natural biopolymer is water-soluble, non-mutagenic, non-immunogenic, and non-toxic. It produced naturally as extracellular polysaccharide by the yeast-like fungi *Aureobasidium pullulans*. Due to the chemical, biological, and physical characteristics, pullulan has become a desired compound for many applications in pharmaceutical industry including drug and gene delivery, tissue engineering, medical imaging, plasma expander and also in the cosmeceutical industries. This article presents a review of the state-of-the-art applications of pullulan by *Aureobasidium* sp. in the pharmaceutical and cosmeceutical field. Such important knowledge was organized and updated on the basis of latest research directions in pharmaceutical and cosmeceutical area. The presented information emphasizes an actual outlook and the essential steps to improve the utmost exploitation of the scientific advancement documented in the pullulan area up to date.

Keywords: Pullulan, biopolymer, polysaccharide production, pharmaceutical applications, cosmeceutical applications.

INTRODUCTION

Polysaccharides, at first acquired from plant or animal sources, turned out to be effectively accessible for a wide scope of uses, particularly when they were commercially produced by microbial cells using fermentation techniques (El Enshasy et al., 2011; El Enshasy et al., 2012; Esawy et al. 2013; Dailin et al., 2016 and Elsayed et al., 2016). Microorganisms have the capacity to generate a wide variety of polysaccharides of many desired biological, physical and chemical properties (Then et al., 2012; Maftoun et al., 2013 and Dailin et al., 2019). In general, exo-polysaccharides can be homo-polysaccharides or hetero-polysaccharides and the biological functional characteristics are highly dependent on composition, molecular weight, branching and molecular configuration (Soltani et al., 2013; Elsayed et al., 2017; Masri et al., 2017 and Esawy et al., 2019). Pullulan is water soluble, biodegradable and biocompatible microbial biopolymer produced by yeast-like fungus *A. pullulans.* This biopolymer has received gigantic attention from worldwide researchers (Su et al., 2019). It was devoted in optimal cultivation while maintaining a high yield, low cost, short cultivation

time and high purity of the final product yield (Singh et al., 2015). It is proposed for industrial and medical purposes as alternation of α-1,4 and α-1,6 bonds results in two distinctive properties. This special linkage pattern is responsible for the solubility and flexibility of pullulan (Leathers, 2003).

Pullulan is a linear macromolecule linked by α-(1,6) glycosidic linkages of maltotriose repeating units (Yang et al., 2018). The chemical formula of pullulan is $(C_6H_{10}O_5)_n$, stable at a wide range of pH, can remain stable at temperature up to 280°C and the level of solubility can be managed with reactive groups by using chemical derivatization (Shingel, 2004 and Singh et al., 2015). The molecular weight of pullulan is ranging between 45-600 kDa, optical rotation of 192°C in $1gdL^{-1}$ solution and used as ingredients to develop blood plasma substitutes with weight distribution (Mw/Mn) of 1.2. Pullulan with molecular weight less than 15 kDa can initiate damage to kidneys and higher than 150kDa can increase venous pressure (Shingel, 2004 and Singh et al., 2015). The average molecular weight and molecular weight disseminations are significant for its bioactivities, for instance, chemical releasing capacity and immunomodulatory development (Cheng et al., 2011).

Pullulan has many potential applications in the industries. They found to have outstanding medical applications since the mid of 20th century exhibiting antitumor, anti-cancer effects, and medical devices (Bataille et al., 2011; Mishra et al. 2011; Mishra and Vuppu, 2012 and Moscovici, 2015). One of the examples of successful commercialized product is a film-based oral containing high value added pullulan marketed in many countries under the brand name Listerine and in capsule types (NPcaps® from Capsugel) targeting consumers those who are vegetarians, diabetics and obesity patients (Bataille et al., 2011). Pullulan with such properties of non-toxic, non-immunogenic, non-mutagenic, and non-carcinogenic make it suitable for various pharmaceutical uses including granulation and coating tablets, biodegradable targeted drug and gene delivery, tissue engineering, non-animal capsules, oral and wound care products (Mishra et al., 2012; Singh and Saini, 2012 and Oğuzhan and Yangılar, 2013). This review present current findings for applications of pullulan in the pharmaceutical and cosmeceutical industries. The presented information highlight a real outlook for maximal exploitation of the scientific progress recorded for pullulan applications up to date.

Biosynthesis of pullulan

Pullulan is a slimy layer that synthesized in the cell wall membrane and being released to the cell surface. There are several possible pathways for synthesis of pullulan (Cheng et al., 2011). To date, only α-phospho glucose mutase, uridine diphospho glucose pyrophosphorylase glucosyltransferase and pullulansynthase are confirmed to involve in biosynthesis of pullulan (Chen et al., 2018). Not only glucose or sucrose, *A. pullulans* capable to utilize mannose, fructose, galactose or even other carbon sources. The pathways with these media for biosynthesis of pullulan are still undefined. However, it is only known that for the maltose-containing medium, carbohydrate metabolites are the precursor for the polymer formation which is panose or isomaltose can be utilized and synthesized via glucosyl-transfer reaction in *A. pullulans* (Shingel, 2004). UDPG, the pullulan precursor which plays a crucial role in the biosynthesis of pullulan. D-glucose residue is attached to the lipid molecules (LPh) triggered by UDPG with a phosphoester bridge. Then, D-glucose residue is further transfer from UDPG gives lipid-linked isomaltose. Afterwards, isopanosyl residue is generated by participated of isomaltosyl in the reaction with lipid- linked glucose. Eventually, isopanosyl residue as a precursor is polymerized into pullulan chain (Cheng et al., 2011). The proposed pathway of pullulan synthesis is summarized in Figure 1.

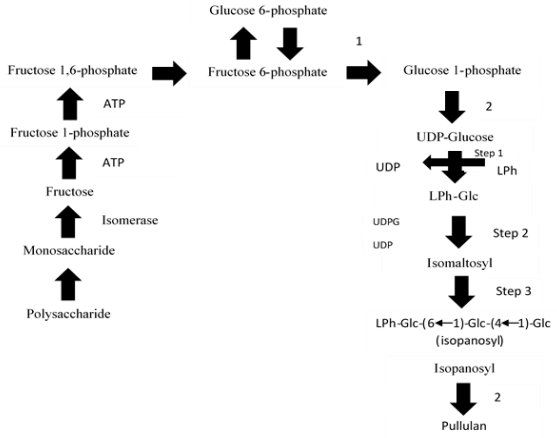

Figure (1): Biosynthesis of pullulan (1,α-phospho glucose mutase; 2,UDPG-pyrophosphorylase; 3,glucosyltransferase) (Adapted from Cheng et al. 2011).

Pullulan is being synthesized from sucrose by cell-free enzymes of *A. pullulans* when both ATP

and UDPG is available in the reaction mixture (Cheng et al., 2011). UDPG cannot be replaced by ADPG which specifying that from UDPG is the initiator of pullulan precursors. In addition, it was proved that the processes of transglycosylation of ADP- glucose are not involved in biosynthesis of pullulan (Shingel, 2004). Singh and Saini (2012) reported that the polymerization of the carbohydrate precursors stored inside the cell apart from the direct conversion of glucose residues into EPS. It is being assumed that cells will mount up sugars and later will be utilized for pullulan production in later stages of cultures life cycle.

Factors affecting pullulan production

Carbon and nitrogen source

Černáková et al., (1980) reported that pullulan can be produced using wide range of carbon sources such as rhamnose, sucrose, maltose, lactose, xylose, galactose, inulin, glycerol and soluble starch. In general, most reports showed that sucrose was the most suitable substrate for pullulan production compared to glucose (Ravella et al., 2010; Ma et al., 2014 and Sheng et al., 2016). This is because the activity of β-Fructo furanosidase, an enzyme that convert sucrose to glucose and fructose when the sucrose concentration is low, was the highest when sucrose used as the main carbon source (Sheng et al. 2016). However, some studies reported glucose (Wang et al., 2013 and Tu et al., 2015) and fructose (Yang et al., 2018) can produce higher amount of pullulan. Fermentation medium containing xylose resulted in low cell biomass and pullulan production (Duan et al., 2008). Nevertheless, other studies found out that the biomass obtained was the highest when using xylose as carbon source, but lower pullulan production (Sheng et al., 2016 and Yang et al., 2018). These disagreement on the optimum C sources used for pullulan and biomass production might due to different strains of *A. pullulans* used and culture conditions. When galactose was used as C source, almost no or very small amount of pullulan can be produced (Wang et al., 2014 and Sheng et al., 2016). Agro-industrial wastes, which are rich in nutrients were also used as cheap substrates for pullulan production, such as De-oiled rice ban (Singh and Kaur, 2019), beet molasses (Lazaridou et al., 2002; Goksungur et al., 2004 and Srikanth et al., 2014), cassava bagasse (Sugumaran and Ponnusami, 2017), sugarcane bagasse (Hilares et al., 2019), potato starch water (An et al., 2017), and rice hull (Wang et al., 2014).

Pullulan production was strongly affected by the nitrogen source, where decreasing in the amount of nitrogen source often indicating the production of pullulan in the media (Gibbs and Seviour, 1996; Bulmer et al. 1987). Alternate nitrogen source from agro-industrial wastes were also used for pullulan production, such as corn steep liquor (Sharma et al., 2013), jatropha seedcake (Choudhury et al., 2012) and soybean pomace (Seo et al., 2004 and Sheoran et al., 2012). Jiang et al., (2011) studied the fermentation time, pullulan production, biomass, and UDPG-pyrophosphorylase activity affected by different nitrogen sources. It showed that $NaNO_2$ and $(NH_4)_2SO_4$ supported both cell growth and pullulan production. Their study also showed that nitrogen source influenced the optimum pullulan production time and the UDPG-pyrophosphorylase activity.

pH and temperature

Generally, optimum pH for biomass production is different from the optimum pH for pullulan production, lower pH increases the cell growth but causes reduction in pullulan production (Israilides et al., 1998). Several studies suggested that pH in the range of 5.5 to 7.5 was found to be optimal for pullulan production (Lee et al., 2001 and Li et al., 2009). At lower pH, pullulan production was halted but it stimulates the synthesis of insoluble glucan (Madi et al., 1997). Wang et al., (2013) stated that the production of pullulan decreased due to the acid stress if the pH is below 2.5. Ji-Hyun et al., (2002) reported that letting the pH of the medium to drop naturally was found to enhance the pullulan production compared to the constant pH.

To optimize the pH for both biomass and pullulan production, two-stage pH profile was first proposed by Lacroix et al., (1985). The pH was first adjusted to 2.0 to increase the biomass production, which was later changed to pH 5.0 for pullulan production. A similar study by Wu et al., (2010) which employed a two-stage temperature fermentation process for pullulan production by optimizing the temperature. This is because lower temperature is optimum for pullulan production while higher temperature for biomass production. Wu et al., (2010) found out that pullulan production achieved 27.4 gL^{-1} at optimum temperature of 26ºC, and cell growth achieved 10.0 gL^{-1} at 32ºC. Typically, temperature ranging from 25ºC to 30ºC is optimum for pullulan production. A two-stage controlled pH and

temperature for pullulan and biomass production was also studied by Xia et al., (2011).

Mineral salts and surfactants

West and Reed-Hamer (1992) first reported that metal ions like Fe^{3+}, Mn^{2+} and Zn^{2+} increased the production of *A. pullulans* ATCC 42023. Similar studies by Gao et al., (2010) showed increase in cell growth and pullulan production by optimizing the mineral salts in the medium formulation. Besides, Wang et al., (2016) studied the effect of five different mineral salts ($FeSO_4$, $CuSO_4$, $ZnSO_4$, $MnSO_4$ and $CaCl_2$) on pullulan production, it showed that adding $CuSO_4$ at the concentration of 0.2 mgL^{-1} to the media increased the production of pullulan from glucose by 36.2% when compared to control. Another recent study by Wang et al., (2018) showed that the pullulan titer was increased by 26.7% when 3 gL^{-1} of NaCl was added, but the molecular weight of the pullulan was reduced to 46.8%.

Tween 80, a non-ionic surfactant was found to improve pullulan production. Sheng et al., (2013) studied how different concentration of Tween 80 (0.1%, 0.5%, 1.0%) affected the production of pullulan and cell biomass. The result showed that the pullulan production was increased with the addition of Tween 80, where the best concentration was 0.5%. However, there was no increase in biomass, suggesting that Tween 80 was not degraded to be used as C source by the strain. Presence of Tween 80 enhances the releasing of pullulan from the cell due to the interaction of the surfactant with the cell membrane's permeability. Further study by Sheng et al. (2015) showed that with the addition of 0.5% Tween 80, there was 41% improvement of pullulan production compared to the control, with a maximum yield of 53.04 gL^{-1}. Another study by Tu et al. (2014) showed that polymalic acid and pullulan yield was increased to 75.08% and 27.21% respectively compared to the control when 0.05% of Tween 80 was added to the media.

Light intensity

Ruly et al. (2017) studied the effect of different wavelengths' light-emitting diodes in the pullulan production by a wild type strain *A. pullulans* LB8, focusing on pullulan with low-melanin content in different carbon sources. The study found out that when using both white and blue LED lights (450-470 nm), the pullulan produced (approximately 20 gL^{-1}) was lower in melanin content in sugarcane bagasse (SCB) hydrolysate medium, compared to using only white light in glucose-based medium, which resulted in lower yield and higher melanin pullulan.

Applications of pullulan in pharmaceutical field

Tissue engineering

Tissue engineering is a favorable substitute to allografts for the rejuvenation of huge bone defects. The significance of tissue engineering shows restrictions in tissue grafting is increasingly clear for a huge variety of diseases including osteoarticular pathologies (Fricain et al., 2013). Tissue engineering aim to regenerate the injured tissue and reinstate a biologically valid articular surface (Fisher et al., 2017). The surface characteristics of bio materials applied for medical implants have been shown directly impacts on the active interaction that happen at tissue implant boundary. These characteristics and changes may take place over time in-vivo and need to be recognized for designing biomaterial principally pullulan for particular applications (Mishra et al., 2011).

Iswariya et al., (2016) developed a good absorbent collagen-pullulan hydrogel with enhanced mechanical firmness and well-defined biocompatibility for skin tissue engineering. The scaffolds were constructed using pullulan which is mixed with sodium trimetaphosphate and collagen to create polymeric linkages. Chen et al., (2016) well developed an enzymatically cross-linked injectable and biodegradable hydrogel system using a micture of carboxymethyl pullulan-tyramine and chondroitin sulfate-tyramine conjugates for cartilage tissue engineering. Fricain et al., (2013) reported scaffolds composed of pullulan and dextran which is in combination or not with nanocrystalline hydroxyapatite particles (nHA). This composite matrix is used for encouraging bone cell differentiation of host mesenchymal stem cells.

Drug delivery

Current progresses in the field of drug delivery are targeted at discovering sufficient strategies for the administration of different drugs. One of the most central characteristics of drug to be deliver is their stability. It is essential to provide an appropriate protection of the drugs against stresses both during manufacturing and storage. Studies have shown that pullulan is well-matched when injected in blood vessel (U.S Congress Publications, 1993). In targeted drug delivery

system such as for tumor drug delivery, pullulan has been used for surface modification of the drug carrier to reduce the hematological toxicity to the neighboring cells (Wang et al., 2013 and 2014). Pullulan was used to coat magnetic nanoparticles for better compatibility when used in various biomedical applications such as for hyperthermia therapy for heating cancer cell (Saranya et al., 2015). Henry et al., (2017) propose the design of a new injectable biphasic system, based on the association of pullulan microbeads into a cellulose-based hydrogel used for the TGF-β1 and GDF-5 growth factors. Their loading and discharge capacities were able to show a persistent release of both growth factors for up to 28 days. Chassot et al., (2016) reported the preparation of poly (ε-caprolactone) nano-capsules using pullulan as a stabilizer for drug delivery. The formulations develop showed physicochemical characteristics steady with nanocarriers for drug delivery.

Gene delivery

Non-viral vectors are preferred to deliver nucleic acid materials, to improve the transport and avoid degradation by lysosomal enzymes (Moscovici, 2015). Pullulan due to its specificity for liver has been widely used for biomedical applications. Successful delivery of genes was reported in the presence of carrier made of cationized pullulan. The polycations cause DNA condensation, enhance the DNA half-life and prevent it from being degraded by plasma nucleases (Askarian et al., 2017). Yang et al., (2014) reported that a tailor-made bio cleavable pullulan-based gene vector with good hemo compatibility was successfully proposed via atom transfer radical polymerization for efficient liver cell-targeting gene delivery. Polyethyleneimne (PEI) is a very efficient transfecting agent but is toxic due to high charge density. Ambattu et al., (2015) reported that PEI conjugated with pullulan is hemocompatible and nontoxic while ensuring remarkable transfection efficiency.

Antimicrobial activity

Pullulan itself does not show antimicrobial activity. However, due to their promising outcome to be used as coating in biomedical applications there are several studies that had been conducted showing the functionalization of pullulan for anti-microbial activities. Fernandes et al., (2014) reported that functionalized pullulan powder chemically modified with 3-aminopropyltrimethoxysilane showed antimicrobial activity toward *S. aureus* and *E. coli* attributed to the presence of aminopropyl groups in the pullulan chains. Another study reported by Synowiec et al., (2014) shows the antimicrobial activities of pullulan films containing sweet basil extract (SBE). Pullulan coating with SBE showed low antibacterial activity on mesophilic bacteria and good antifungal protection against *Rhizopus arrhizus*.

Medical Imaging

Medical imaging is a technique used for visual representation of body interior by labeling the inner body cells with fluorescent probes. Quantum dots (QDs) can be used as fluorescent probes for live cell imaging to track whole cells or intracellular biomolecules. These QDs are semiconductor nanocrystals with excellent properties such as broad excitation, bright fluorescence, high photo-stability and narrow emission spectra. Prajapati et al., (2013) reported that pullulan-coated iron oxide nano-particles were used for medical imaging such as lymph node, receptor, perfusion, vascular compartment imaging, and target specific imaging. Jo et al., (2010) reported that pullulan coated iron oxide nanoparticles were used as magnetic resonance contrast agent for labelling mesenchymal stem cells.

Plasma expander

Pullulan is also used as plasma expander to treat volume deficiency of the vascular system. Treatment with blood is not a compulsory when blood loss does not exceed 30% of the total blood volume. Alternatively, plasma expander has almost infinite supply, has longer shelf life, independence on blood type and free of pathogens, hence could alternatively be used to replace blood for certain cases. Plasma expander acts as blood plasma substitute to maintain blood circulation and the osmotic pressure of blood vessels (Singh et al., 2017). Derivatized pullulan colloid has great therapeutic potential to be used as blood plasma substitute (Kulicke and Heinze, 2006). Pullulan needs to be modified to enhance biocompatibility as well as to prevent it from amylase attack. Such biocompatibility properties are lower molecular weight and lower viscosity (Shingel and Petrov, 2002).

Film forming agent

Pullulan has also been used as film forming agent for orally disintegrating drug delivery system. The hydrophilic characteristic allows it to

disintegrate quickly in the mouth, thus releasing incorporated active pharmaceutical ingredient within seconds. Furthermore, pullulan possesses high mechanical strength property and thermal stability, thus easy to handle (Irfan et al., 2016). When tested for film forming properties, pullulan or pullulan in combination with other organic polymers formed an excellent film with high tensile strength and fast disintegration and dissolution times (Kulkarni et al, 2010; Murata et al, 2010; Choudhary et al., 2011 and Saini et al., 2011). Evaluation of pullulan as a film agent for various active pharmaceutical ingredients resulted in excellent performance for high and fast drug release. These are summarized in Table 1.

Table (1): **Applications of pullulan as film forming agent for various active pharmaceutical ingredients.**

Film agent formulation	Active pharmaceutical ingredient	References
HPMC, pullulan, polyvinyl pyrrolidone (PVP)	Nebivolol HCl	Parejiya et al. (2012)
Pullulan and HPMC	Granisetron hydrochloride	Chaudhary et al. (2013)
Pullulan	Cetirizine	Mishra & Amin (2011)
Modified pea starch (Lycoat RS 720) and pullulan	Tramadol Hydrochloride	Kathpalia et al. (2013)
Pullulan	Ropinirole hydrochloride	Panchal et al. (2012)

Vaccination

Pullulan has also been used as antigen delivery system for vaccines via nasal administration. Mucosal vaccines, especially nasal, when compared to major subcutaneous and intramuscular route are more effective in preventing infection via the respiratory tract and it induces immunity in both the systemic and mucosal parts (Nakahashi-Ouchida et al., 2018). Cholesteryl pullulan (CHP) is amphiphilic copolymers that form hydrophobic internal core space and hydrophilic external surface. The internal space could entrap various molecules by hydrophobic forces and the hydrophilic surfaces stabilizes interface between the hydrophobic core and the external aqueous environment. The CHP serves as a great carrier for active compounds as it protects the encapsulated molecule from attacks from the surrounding environment and it allows slow release of the entrapped materials (Ohta, 2016). CHP was used as a carrier for NY-ESO-1 protein cancer vaccine and the safety and immunogenicity has been confirmed by testing with patients with advanced/metastatic esophageal cancer (Kageyama et al., 2013). The CHP was also proven safe and effective in delivering PspA nasal vaccine with specific target for pneumococcal respiratory infection (Kong et al. 2013) and tumor necrosis factor-α (TNF-α) (Ohta, 2016).

Molecular chaperons

Amphiphilic polymer can also be used to mimic protein chaperone for regulating protein folding. This system could enhance the protein thermal stability, assist protein folding and prevent aggregation. Nomura et al., (2003) has developed amphiphilic polysaccharide self-assembled nanogels using CHP. The hydrophobic cholesteryl groups form hydrophobic binding sites in the core structure to bind the denatured proteins through hydrophobic interactions when heated. The release of protein was induced by addition of β-cyclodextrin which would bind to the cholesteryl groups as the temperature was cooling down. Dynamic cholesteryl-group-bearing pullulan (CHP)-CD supramolecular polysaccharide nanogel was also developed as artificial chaperone to enhance the thermal stability of protein (Takeda et al., 2013). Recently, the polysaccharide self-assembled nano-gel has been tested for protein or protein-based drug carrier for therapeutic purposes, hence serves as a promising protein carrier for effective protein drug delivery system (Hashimoto et al., 2018).

Applications of pullulan in cosmeceutical field

Pullulan is one of the bioactive exopolysaccharides which involved in many mechanisms from attachment of intra- and interspecific communication and competition. It can be produced either by bacteria, fungi and other microalgae by fermentation process. However, bacteria are more preferable due to its large production of these exopolysaccharides. Examples of several microorganisms that are able to produce these exopolysacchrides include *Agrobacterium* sp., *Alcaligenes faecalis,*

Xanthomonas campestris, *Bacillus* sp., *Zymonas mobilis* and *A. pullulans* (Corinaldesi et al., 2017). The special characteristic and physical properties imparted by pullulan makes the biopolymer a good feature to be applied in the cosmeceuticals industries. The quality and effect of cosmeceutical products can be upgraded and improved when fused with pullulan (Nakashio et al., 1976). One of the excellent applications is in oral care products. Pullulan-based oral care products have been widely commercialized all over the world (Singh and Saini, 2012). The biopolymer has been characterized to have several advantages such as having the ability to form an excellent transparent film, significant moisture absorptivity, tackiness, tolerable water solubility, edibility, good dispersibility and non-toxicity. The different linkages of α(1-4) and α(1-6) in the pullulan structure has made pullulan nonimmunogenic, non-mutagenic and having non-cancerous traits (Dubey & Kashyap, 2018). Additionally, pullulan is non-irritant to the human skin while being tasteless and odorless as well. The relatively-low viscosity exerted by pullulan has made it a good binder in various kind of fields including cosmeceuticals.

Conventionally, the cosmeceutical industries have been utilizing high polymers which have water-soluble polysaccharides characteristic such as starch, methyl cellulose, carboxy-methyl cellulose, hydroxyethyl cellulose and sodium alginate. Nevertheless, pullulan has a different molecular structure and thereof better properties such as easily soluble even in cold water, and the aqueous solution containing pullulan is stable over a long period of time without any gelation formation or 'aging' phenomenon occur. These kind of excellent properties can overtake the starch with the additional advantage being low viscosity in aqueous solution even when in the same solute concentration or molecular weight. Due to these excellent features of pullulan, it may be applied as one of the cosmetic ingredients in body lotion, compact and loose powders, hair shampoos and tooth care products. The potentiality of pullulan to be used as facial pack has long time been patented by Japan inventors 20 years ago (Ozaki et al., 1995). The facial pack was prepared by mixing homogeneously 0.5 parts by weight of linolenic acid with a mixture consisting 1.5 parts by weight of squalane, 0.5 parts by weight of polyoxyethylene hydrogenated castor oil, 5.5 parts by weight of L-sodium lactate, 4.0 parts by weight of glycerine, 50.0 parts by weight of 40% pullulan mixture, 10.0 parts by weight of ethyl alcohol, and 33.0 parts by weight of refined water. The product developed is claimed to be suitable for skin-whitening agent and also very useful to prevent and treat local and systemic hyperpigmentation in the skin such as freckle, sunburn and chloasma. This high values of pullulan have increase its interest to be widely used for the development of cosmeceutical and skin regeneration products. Table 2 summarizes the general features of pullulan and main applications in cosmeceutical industries.

Table (2): The features of pullulan and its application in the cosmeceutical field.

Features of pullulan	Application in cosmeceutical industries
Odorlessness, tastelessness, non-toxicity, high water solubility and tackiness. Covering power, adsorptivity and tackiness. Ingredient for rouges (liquid rouges and paste rouges)	Ingredient for rouges (liquid rouges and paste rouges)
Non-toxicity, non-irritability, and film- forming ability	Ingredient for cosmetics around the eyes (eye liners and eye shadows)
Film-forming ability, adsorptivity, water retainability, continuity of film at peeling time, and ability of giving tension to the skin due to shrinkage of film at drying time	Ingredient for facial packs
Foaming-promoting effect and builder effect	Ingredient in hair shampoos
Tackiness, ability of forming tough film, hair setting ability, and high water solubility necessary for removal after use	Ingredient in body lotions and hair lacquers
Excellent coherence (protective colloid-forming ability), foamability, high viscosity, non-toxicity and storage stability	Ingredient in tooth paste
Excellent water-soluble film forming with transparent in color	Face mask and hair styling

As pullulan is safe and non-toxic biopolymer, it can be used freely without any limitation in terms of the amount and composition when preparing the cosmetics. It may be combined and mixed with other polymers such as low molecular weight compounds, inorganic compounds, fragrance and preservatives during the time of preparation for the cosmetics. The pullulan obtained from microbial strain may varies in the physical properties depending on the type of strain from which it is produced. However, this does not greatly impact the efficiency to be used in the cosmeceutical products. Pullulan, being a carbon neutral, sustainable, biodegradable and palatable substrate in nature has provide a good alternative to the conventional polysaccharides substrates (Dubey and Kashyap, 2018). Alternatively, pullulan represent sustainable, low-cost with fast-production compared to other natural molecules which can be used in photo-protection, skin-whitening and anti-aging products for total body care (face, body and hair) (Corinaldesi et al., 2017). The photo-protective characteristic of pullulan has increased the demand for skin-care and hair-care products with the surplus of consumers' demand for natural products instead of chemical-based cosmetics. The mixture of pullulan with other ingredients obtained from fermentation products has enhance the synthesis of collagen I which contribute towards the amelioration of structural properties on human skin (Martins et al., 2014). As nowadays, people are looking into natural products for their everyday usage, pullulan has represented the future for cosmeceutical industries. The biological properties of pullulan have received great attention. The natural and biodegradable pullulan extracted from selected microorganism may reduce the use of synthetic compound in the cosmetics.

Nevertheless, despite the high benefits being imparted by pullulan, its high production cost greatly limits its wide application. Thus, optimization of the fermentation condition for pullulan production is highly needed for an effective production with reduced production cost (Yang et al., 2018). For these reasons, Yang et al., (2018) have manipulated several parameters such as varying the carbon source, hydrolysate content, and medium composition and the results obtained has shed some lights for further pilot-scale production.

CONCLUSION

Research studies in the ground of polysaccharides have shown that pullulan is a distinctive polysaccharide with a vast variety of potential in pharmaceutical and cosmeceutical applications. The unique and promising characteristics of pullulan including thermal stability and non-poisonous allows them to be utilized in many different ways. More research need to be done to explore in depth the technology of applying pullulan in the field of pharmaceutical and cosmeceutical.

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

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AUTHOR CONTRIBUTIONS

DJD designed, wrote and reviewed the manuscript. LZMIL, RAM, NIWA, NHAM and HCK wrote the review paper. DS and HEE reviewed the review paper. All authors read and approved the final version.

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