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Agro-Industrial Waste: A Potential Feedstock for Pullulan Production

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Nowadays, the growing interest of using of biopolymer to replace petroleum based material asare increasing tremendously. Microbial biopolymers are usually water-soluble gum which have innovative and unique physical characteristics.Pullulan is a biodegradable and water soluble exopolysaccharide synthesized by the yeast-like fungus *Aureobasidium pullulans*. This polymorphic fungus is well known as producer of the polysaccharide, pullulan and other by-products such as oil, organic acids, pigment, and others. Pullulan has extensive applications in pharmaceutical, cosmetic, biomedical, and food industries because of its advantageous chemical and physical properties. Pullulan's structure is co-existence of á-(1, 4) and á-(1, 6) linkages which is nontoxic, tasteless and non-mutagenic. Some of its excellent properties are low viscosity, non-toxicity, slow digestibility, high plasticity, and excellent film-forming capabilities. Although pullulan shows great potential in several industries, its high production cost is a major drawback. Therefore, cheaper and accessible substrate which can minimize the production cost is needed. This review highlights the potential use of agro-industrial waste as an alternative source feedstock for pullulan production and its biosynthesis, chemical structure, production process and applications.

Keywords: *Aureobasidium pullulans*, Pullulan,Biopolymer, Agriculture waste, Bioprocess**.**

Since the beginning of the twentieth century, the function of microbe and microbial biotechnology in enhancing the quality of human life in each perspective has been perceived around the world. Biotechnology knowledge related to microbial bioprocessing for the production of bioproducts like biopolymers, enzymes, antibiotics and high cell mass have matured tremendously. Biopolymers are the most abundant molecules in living matter. Microorganism is proficient of producing a wide variety of biopolymers, including carbohydrates, polynucleotides, polyamides, polyesters, glycoproteins, polysaccharides and many others. Pullulan is an alternative type of valuable biopolymer as they can substitute the ordinary polysaccharides produced by plants.

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It has been reported that the retail price of pullulan is around $25 per kilogram (Ma *et al*., 2012). Pullulan has been chosen as the biotechnology product manufactured since 1976 by the Hayashibara Company Ltd 9 (Okayama, Japan), which remains the leader supplier (Singh *et al*., 2008). Pullulan produced by non-pathogenic polymorphic and oligotrophic yeast-like fungus, namely *Aureobasidium pullulans*. *A. pullulans* is a species of the class Ascomycetous yeast, which belongs to the family Dothideaceae of the order Dothideales. *A. pullulans* is a black yeast, which can be found mainly on leaves and other environment surface like concrete, limestone wood, soil, forest barks, fresh and sea water, plant and animal tissues (Shingel, 2004). It was first isolated and observed by Bernier (1958) and found to play as a valuable product in biotechnology business. Bender at the year 1959 discovered the unique glucan and proposed it "pullulan". At the year of 1960, the basic empirical structure of pullulan was established. Pullulan is broadly used as biomaterial applied in the food and medical sector because of its characteristics such as structure flexibility, low viscosity, nontoxicity, slow digestibility, high plasticity, and biofilm. Furthermore, pullulan is also edible and biodegradable in the environment. This review will explore the potential of feedstock from agro-industrial waste. Several low-cost feedstock that potentially can be used for supporting pullulan production includes potato starch waste, olive oil wastes, carob pod, corn steep liquor, coconut by-products, jaggery and rice hull hydrolysate. Bioprocess including basic medium optimization, cultivation in shake flask level up to batch bioreactor were discussed. The metabolic pathways of pullulan synthesis and applications of pullulan are discussed extensively.

Pullulan specifications:Chemical structure, molecular, and physical properties

The chemical formula of the natural biopolymer secreted from *A. pullulans* has been investigated by many authors and well established (Sugumaran and Ponnusami, 2017; Kumar *et al*., 2012; Singh *et al*., 2008; Rekha and Sharma, 2007; Shingel, 2004; Jakovljevic *et al*., 2001). Pullulan is a neutral polymer consist of repeating glucose units with á-1,6 and á-1,4 glyosidic bonds and no branching (Figure 1). The linear pullulan chemical structure may also contain maltotetrose subunits. Basic linkages in extracellular polymer and its enzymatic hydrolysis sites is shown in Figure 2. Pullulan contain both hydrophobic and hydrophilic features which are appropriate for its unique structure. The chemical formula derived from IR spectroscopic of pullulan is ($C_6H_{10}O_5$) with molecular weight reaching 45-600 kDa and optical rotation of +192 in a 1 g/dL solution (Shingel, 2004) (Table 1). The final purified of polysaccharide has a molecular weight of ca. 250 kDa. The structure consists of hydroxyl groups and the well-ordered alternation of á (1-4) and á (1-6) bonds on pullulan chains provides the polymer with characteristic physiological activity, structural flexibility and increasing solubility established (Sugumaran and Ponnusami, 2017; Ma *et al*., 2012; Kumar *et al*., 2012; Singh *et al*., 2008; Rekha nad Sharma, 2007; Shingel, 2004; Jakovljevic *et al*., 2001).

Pullulanase (EC 3.2.1.41, pullulan 6-glucanohydrolase), is well known as debranching enzyme is able to hydrolysis the á-1,6- glucosidic bond in pullulan structures and convert it to amylaceous polysaccharides. The pullulan also undergoes enzymatic hydrolysis by both á-1,6 and á-1,4 glycosidic bonds D pullulanases. The pullulanase enzyme, acting to cleave the (1-6) á-D-glucopyranoside linkages. From this actions, it can contribute perfect hydrolysis process of pullulan using (1-6)-á-D pullulanase yields maltotriose as an utmost outcome along with traces of maltotetraose. Furthermore, the (1-4)-á-D-pullulanases act on (1-4)-á-D-glucosidic linkages at their reducing ends adjacent to (1-6)- á-D linkages. Complete hydrolysis of pullulan with (1-4)-á-D-pullulanase present with isopanose as the primary product. Products of enzymatic pullulan degradation are usefulness in food and pharmaceutical industry (Oðuzhan and Yangýlar2013).

Pullulan is white colored, odorless and tasteless powder currently exploited in the food industry due to its variety of unique properties. Its natures are nontoxic, nonimmunogenic, non-mutagenic, non-hygroscopic in nature and non–carcinogenic. Pullulan is also highly soluble in water and insoluble in organic solvents. For the molecular weight, pullulan molar mass was stated in the range of 58-9000 kDa. Pullulan can be converted to other components or derivatives or chemically modified by using several steps

such as esterification, carboxymethylation and sulfation (Prasongsuk *et al*., 2018; Sugumaran and Ponnusami, 2017; Kumar *et al*., 2012; Ma *et al*., 2012; Singh *et al*., 2008; Rekha nad Sharma, 2007; Shingel, 2004; Jakovljevic *et al*., 2001). The great potential of pullulan ion vast variety of areas and applications ensures its bright future in microbial biotechnology. The aqueous solutions of pullulan are stable and its viscosity is relatively low compared to other polysaccharides. Pullulan can withstand and decompose at 250-280°C (Singh *et al*., 2008). The main quality parameters of pullulan are shown in Table 1.

Mechanism of pullulan biosynthesis

Exopolysaccharides produced serves as an outer protection for the producer containing high water content, to ensure greater resistance against desiccation and predation (Kumar *et al*., 2017). *A. pullulans*is known as the major producer of pullulan and aubasidan-like components (Sheng *et al*., 2015). This fungus disperses due to the production of yeast-like propagules and found globally but reported in the intense cold environment, as investigations on fungal diversity are limited to frozen Antarctic soils and Siberian permafrost where basidiomycetous yeasts were found (Gaur *et al*., 2010). It has unique metabolic features and the cellular morphologies characteristics of *A. pullulan* are more luxuriant.

Pullulan biosynthesis is accomplished through mediation of sugar-nucleotide-lipid carrier intermediates associated with the cell membrane fraction. It is synthesized extracellular at the cell of the membrane wall and secreted out to the cell surface to form amorphous solid which consists of maltotriose and maltotetraose with bond á-(136) and á-(134) linkages. For instance, the regular alternation of á-1,4 and á-1,6 bonds results in two distinctive properties, structural flexibility and enhanced solubility (Moubasher *et al*., 2014).

There are 3 main stages of the precursor of the pullulan molecule. The first stage is formation of Lph-Glu, through the intermediary uridine-diphosphate-glucose (UPDG) which is catalysed by ATP. Next stage is transfer an additional D-glucose produced by UPDG to form isomaltose molecule (Lph-Glu-(1-6)-Glu). Lastly, in the final stages, isomaltose will interact with the glycosyl lipid precursor from stage one to produce molecule of isopanosyl (Lph-Glu-(1-6)-Glu-(1-4)-Glu). The isopanosyl molecules will polymerised into a pullulan chain (Donot *et al*., 2012). The biosynthesis of pullulan is mainly performed by key enzymes such as uridine diphosphate glucose pyrophosphorylase (UDPG-pyrophosphorylase), á-phosphoglucose mutase and glucosyltransferase.

A. pullulans is able to consume various carbon sources such as mannose, sucrose, maltose, fructose, galactose, xylose and even the agro-industrial waste. The presence of isomerase and hexokinase are necessary for carbon source to be converted to UDPG which is an important precursor to synthesis pullulan (Sugumaran *et al*., 2017). UDPG is important in medium for pullulan production where *A. pullulans*incorporates 14C-labeled glucose into lipid-linked glucose, isomaltose, panose and isopanose that participate in reaction with lipid-linked glucose (Leathers *et al*., 2003). In addition, they proposed a reaction mechanism in which pullulan is formed by the polymerization of isopanosyl into the pullulan chain using glucosyl tranferase enzyme. However, there are limited studies about the exact mechanism of pullulan synthesis by *A. pullulans* which has not been understood due to the complex physiological andcytological characteristics of the microorganism (Cheng *et al*., 2011). The proposed pathway of pullulan synthesis is summarized in Figure 3.

Pullulan can be synthesized from sucrose with enzymes from *A. pullulans* when both ATP and UPDG participate in reaction mixture. ADPG cannot be replaced by the UDPG because the pullulan precursor is originated from UPDG. Unfortunately, the formation pathway still remains unclear. It is only known that, maltose containing media, the carbohydrate metabolites needed for the polymer formation, which are panose [á-Glc-(1fi6)-á-Glc-(1fi4)-á-Glc] and or isomaltose [á-Glc-(1fi6)-á-Glc] can be synthesized via a glucosyl transfer reaction in *A. pullulans*.

Cheng *et al*. (2011) explained that *A. pullulans* does not convert glucose directly into polysaccharide instead it involved in polymerization of carbohydrate precursors stored inside the cells. The cells will accumulate sugars and consume the carbohydrate for their last stage of life cycle in pullulan production. The hypothesis

was proved that an inverse correlation between the concentration of pullulan and the content of intracellular glycogen.

Besides *A. pullulans*, the newly isolated pullulan producing fungus *Eurotium chevalieri* has also been reported to produce non-melanin pullulan which demonstrate the higher yield of pullulan production (Gaur *et al*., 2010). Hydrolytic products of the polysaccharide produced by *E. chevalieri* using pullulanase specifically hydrolyses the á-1,6 linkage of the linear á-d-glucan, releasing maltotriose with the reducing end from pullulan to determine the purity of the polysaccharide produced. Forabosco *et al*. (2006) stated that *Cryphonectria parasitica* which is fungal virulence of chesnut cranker also produced pullulan where all cases pullulan was much richer in a-(1'!6) maltotetraose subunits than the pullulan(s). The maximum amount of a-(1'!6) maltotetraose subunits was believed to be 7%.

As studied by Olivia *et al*. (1986), they mentioned that pullulan is also produced by

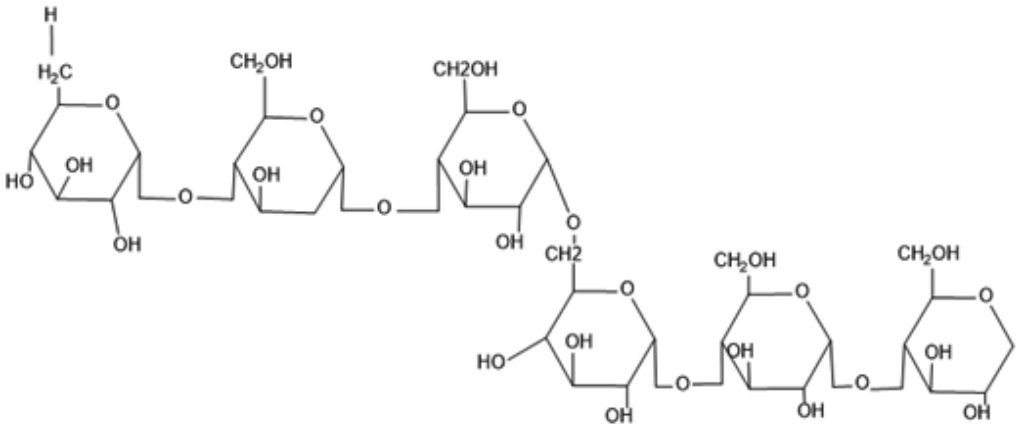

Fig. 1. Molecular structure of pullulan (CAS Number, 9057-02-7)

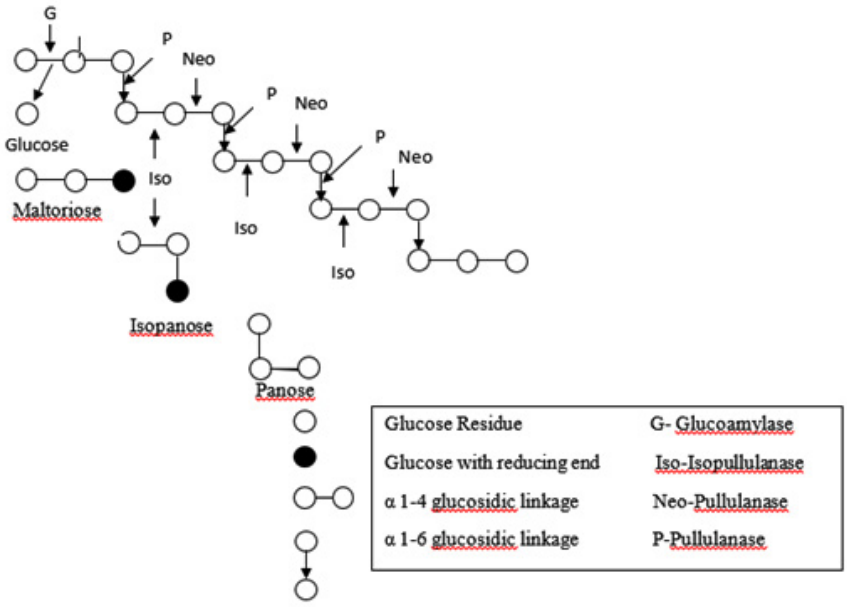

Fig. 2. Basic bond in pullulan and enzymatic hydrolysis site

Cyttaria darwinii which is a fungal species that form a tumour that infect the tree.The pullulan structure of *Cyttaria darwinii* was confirmed by the pululanase treatment. Chi and Zhao (2003) have found new pullulan-producing yeast strain *Rhodotorula baracum* that was collected from Chinese plant leaves from South China which produced large amount of pullulan and did not produce melanin pigment. Pullulanase hydrolyzes â-1,3-glucan was carried out. The result was confirmed that polysaccharide produced by *R. bacarum* is pullulan. Apart from *A. pullulans*, some other microbial strains are also reported as pullulan producer such as *Eurotium chevalieri* (Gaur *et al*., 2010), *Cryphonectria parasitica* (Forabosco *et al*., 2006) *Rhodotorula baracum* (Chi and Zhao, 2003) and *Cytaria darwinii* (Olivia *et al*., 1986).

Table 1. Pullulan molecular information

Name	IUPAC name	Pullulan
	Other names	E1204
Identifiers	CAS Number	9057-02-7
	EINECS Number	232-945-1
	ChemSpider	none
	E number	E1204
	ECHA InfoCard	100.029.938
	UNII	8ZQ0AYU1TT
Properties	Molecular formula	$(C_6H_{10}O_5)_n$

Production process

In production process, there are many factors that affect the production of pullulan. Some of the factors that contributed towards the efficiency of pullulan production in the industry are the medium components, processing parameters and other factors such as labour skills, bioreactor type and design used. Some of the factors that may influencing pullulan production are summarize in Figure 4.

Carbon source

Carbon sources is an important nutrient in living cells under the category as macronutrient as

Table 2. Quality parameters of pullulan (Ma *et al*., 2013; Singh *et al*., 2008)

Parameter	Specification
Appearance (external)	A white or yellowish-white powder
The degree of water of solubility (25C)	Soluble very well
Specific optical activity [á] D_2O (1% in water)	Min. +160
Polypeptidies (%)	Max. 0.5
pH (solution)	Within 5-7 scale
Mineral residue-ash (sulfated, %)	Max. 3
Moisture level (loss of drying, %)	Max. 6
Molecular weight (kDa)	Range between 100-250

Table 3. Characteristics of microbial Pullulan (Prasongsuk *et al*., 2018; Sugumaran and Ponnusami, 2017; Kumar *et al*., 2012; Shingel, 2004; Rekha and Sharma, 2007; Jakovljevic *et al*., 2001; Singh *et al*., 2008; Ma *et al*., 2013)

Characteristics of microbial pullulan	
Non-toxic	Non -carcinogenic
Non-mutagenic	Odourless
Tasteless	Edible
Biodegradable	Transparent/ Impermeable to oxygen
Low viscosity	Non-hygroscopic
Insoluble in organic solvents	Oil resistant
Water soluble	Non-reducing
High adhesion and film forming abilities	Film; thermostable, anti-static, elastic
Non-ionic polysaccharide	Blood compatible
Non-immunogenic	Dilute alkali
Insoluble in alcohol	Edible
Low viscosity	White to off-white Colour

they are needed mostly for energy. Quite numbers of previous studies reported that sucrose was the best carbon source supporting for high pullulan production. Jiang *et al*. (2018) reported that the production of pullulan obtained by *Aureobasidium melanogenum* TN1-2 strain isolated from natural honey was 97 g/L when sucrose was used as carbon source in the cultivation medium. Earlier, Özcanaa *et al*. (2014) reported that maximal pullulan production of 38.77 g/L achieved using 95.2 g/L of sucrose concentration. Another study conducted by Sheng *et al*. (2016) using various types of carbon

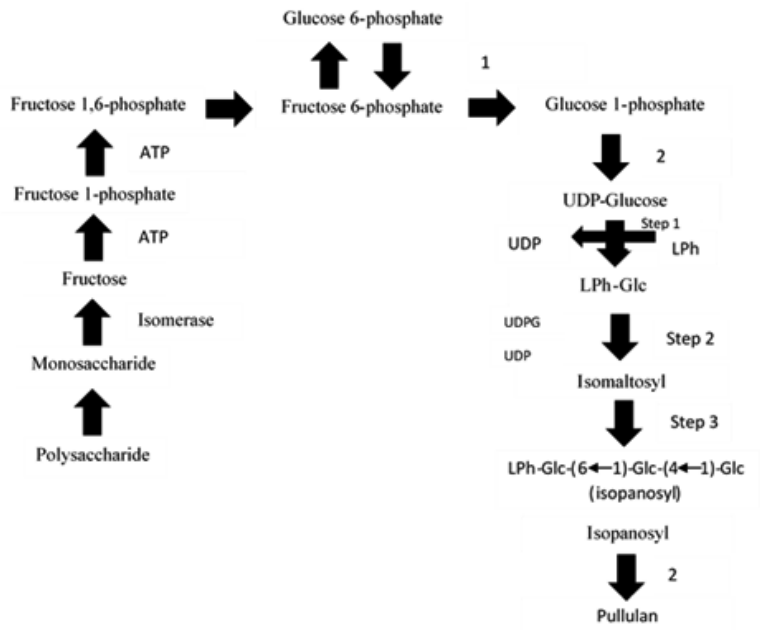

Fig. 3. Biosynthesis of pullulan (1,á-phosphoglucose mutase; 2,UDPG-pyrophosphorylase; 3,glucosyltransferase) Cheng *et al*. (2011)

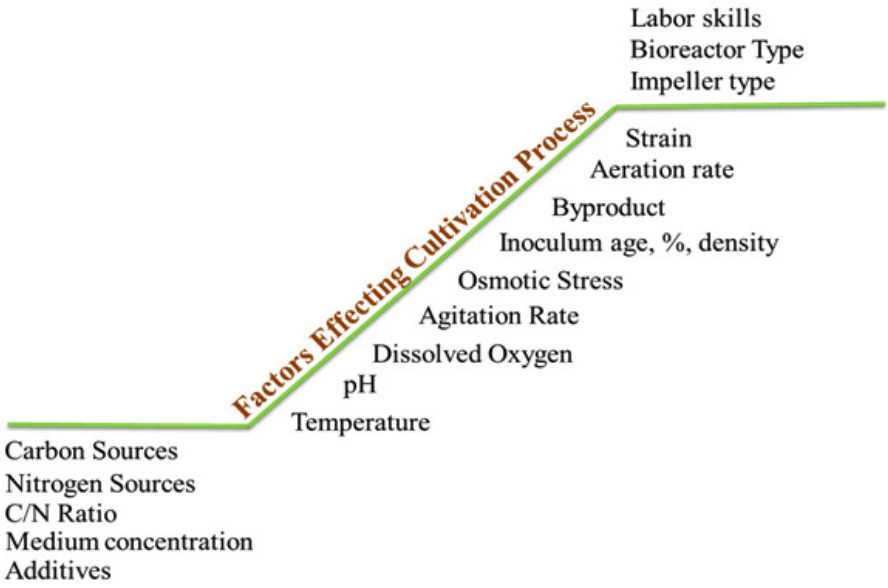

Fig. 4. Factors effecting pullulan production in industry

sources for pullulan production such as sucrose, maltose, glucose, fructose, mannose, galactose xylose and soluble starch. They found that highest pullulan yield obtained when cells were cultivated in medium containing sucrose. Ma *et al*., (2014) reported that maximal pullulan production of 65.3 g/L obtained when 120 g/L of sucrose was used in the cultivation medium**.** Nevertheless, there are also other studies reported that other carbon sources besides sucrose is best when used for pullulan production. Chen *et al*., (2017) reported that 19.8 g/L of pullulan obtained and highest compared to other carbon sources tested such as sucrose, fructose and maltose using mutant *A. pullulans*. By using fructose as carbon source, 50.1 g/L of pullulan obtained for *A. pullulans* NCPS2016 and was the highest among other carbon sources tested that were glucose, sucrose, maltose, xylose and soluble starch. (Yang *et al*., 2018). This is probably due to the capability of strain used for pullulan production were different as well as the cultivation conditions.

pH of cultivation

The pH of cultivation medium is highly influencing not only to the pullulan production but also to the morphology and cultivation time of *A. pullulans*. It was reported that the pullulan production increasing as the pH increase from 2.5 to 5.5 and decreased after that (Ponnusami *et al*., 2014). This result was similar with the result obtained by Singh *et al*. (2012) in which they found that pH 5.5 was optimal for pullulan production. Chen *et al*., (2017) reported that the optimal pH for pullulan production was pH 4 using mutant *A. pullulans*. Study conducted by Sheoran*et al*. (2012) showed that optimal pH is pH 5.9 and that pH ranging from 5.3 to 6.2 did not gave significant effect on pullulan production. Singh *et al*. (2018) reported that pH 5 was best in producing pullulan. Optimal pH values for pullulan production are varied. This is probably due to the variety of strains and cultivation condition used.

Temperature

Cultivation temperature is one of the most crucial factors influencing the production of pullulan. It was reported by Singh *et al*. (2018) that the optimal temperature for high pullulan production was 37 °C. Another interesting study conducted by Singh *et al*. (2012) where they have isolated a pullulan producer strain that is thermotolerant and non-melanin producer that can be grown and produced pullulan at temperature up to 42 °C. Nevertheless, it was reported that the pullulan production was favoured in lower temperature and as low as 25 °C (Hilares *et al*., 2019) and 26 °C (Xia *et al*., 2011). This variation could be mostly due to the capability and origin of the strain being isolated.

Fermentation time

Pullulan production is directly related to the fermentation time. It was reported by previous researches that the fermentation time takes to produce the maximum yield of pullulan is different to one another. This is probably due to different cultivation conditions used for each experiment reported for pullulan production. Ponnusami and Sugumaran (2014) reported that the maximum yield of pullulan obtained was maximum on day 4. Another study by Göksungur*et al*. (2014) reported that the pullulan production was maximum when cultivated for 5.36 days in an air lift bioreactor. Lin and Thibault (2013) reported that highest pullulan concentration of 23.3 g/L produced at 78 hours of cultivation time. In other study, it was reported that the maximal production of pullulan can be achieved within 48 hours of cultivation time (Singh *et al*., 2012). Therefore, in order to get high pullulan production, the fermentation time can be in the range of 48 to 120 hours depending muchly on the strains and cultivation conditions.

Agro-industrial waste as feedstock

In a recent review, it is estimated the cost of the raw materials for pullulan production is three times higher than other polysaccharides and 30% of the total production cost comes from the raw material (Mishra *et al*., 2017). There are many approaches have been taken to reduce the cost for pullulan production which include using genetically modified strains, engineering innovations but the best solution so far is by identifying cheaper and effective carbon source. Agro-industrial waste which is nutritionally rich enough to support the growth of the microorganisms as well as the production of pullulan can be used as an alternative approach in reducing the production cost as they are abundant available to be used.

Pullulan can be produced using different type of substrates incorporated into either the defined (synthetic) or non-synthetic media. Using agro-industrial waste as substrate, it can be sound

advantages for both ecological and economical. This is because it can lower down the negative costs when synthetic chemicals are being used as the sole substrate. Since the usage of agro-industrial waste as feedstock can reduce the environmental pollution, it is desired to find the suitable material that can be used as substrate. The pollution problem which is associated with the accumulation of agro-waste and by-products increased the usage of bioconversion of the plant biomass to value-added compounds economically.

Starch waste

Potato is a cheap and easily available agriculture product. Potato mainly constitute of starch and little amount of sugar. Normally the wastes of potato starch from the manufactures of the potato crisp or other potato processing industries are in the form of homogenous substrate which normally free from extraneous materials. Starch from potato has been used as an alternative carbon source for various industrial fermentations. Certain *A. pullulans* strains possess the starch degrading enzymes but this activity was greater against linear á-1,4-glucans, but very little if against any polysaccharides with á-1,6-linkages. Therefore, in order for the starch waste to be considered as a good substrate for the production of pullulan, it must be hydrolysed partially. Normally, the starch should be hydrolysed to become sugar right before the fermentation process starts. But, there is no need of adding the expensive â-amylase when the potato is being used as a carbon source. This is mainly because potato contains considerable amount of highly active â-amylase. Besides that, the production of pullulan is largely dependent on the degree of the hydrolysis of the starch or dextrose equivalent. This indicates that the total amount of reducing sugar as a percentage of glucose. Some of the unhydrolyzed starch has the dextrose equivalent of 0 and glucose has 100 of dextrose equivalent. By using the hydrolysed starch waste as the substrate, the pullulan content of the agglutinating substances increased during the course of the fermentation process and reached more than 90% (w/w) on day six.

Olive oil waste

Olive oil fruit contains large amount of bioactive compounds and substances which is highly interest. This olive oil is known for its health properties which help to contribute to form a protective effect towards human body. During the olive oil processing, most of them remain as olive oil wastes. Although the olive oil processing generated by two phase extraction process, it represents the major disposal and potentially can be the best solution for most of the pollution problem. The waste from the olive oil is basically the effluent caused by the mills that produce by the olive oil. It is considered to be one of the major pollutants and can cause huge problems in olive tree cultivation areas mainly in Mediterranean countries. The fresh waste from this olive oil can be phytotoxic due to the presence of phenolic compounds and for the time being there is no ecological or economic

Table 4. List of agro-industrial by-products for potential used as feedstock in pullulan production

Agro-industry by-products	Strain	Pullulan yield (g/L)	References
Sugarcane bagasse			
Starch waste	A. pullulans LB83		
A. pullulans P56	25.19		
79.40	Hilares et al., 2019		
Barnett et al., 1998			
Olive oil wastes	A. pullulans RBF 4A3	73.98	Israillides et al., 1999
Carob pod	A. pullulans SU No. M18	68.20	Mishra et al., 2018
Corn Steep Liquor	A. pullulans RBF 4A3	77.92	Mishra et al., 2018
Coconut byproduct	A. pullulans MTCC2195	38.30 (Coconut water); 58.00 (Coconut Milk)	Thirumavalavan et al., 2009
Jaggery	A. pullulans CFR-77	50.00	Vijayendra et al., 2001
Rice Hull Hydrolysate	A. pullulans CCTCC M2012259	22.20	Wang et al., 2014

Table 5. List of applications of pullulan in food and pharmaceutical industries

Industry	Pullulans	Application	Reference
Food industry	Chitosan-PU	Preservation of fresh-cut pineapple	(Treviño-Garza *et al*., 2017)
	CS-BOPP	Food packaging	(Cozzolino *et al*., 2016)
	Pullulan-CMC-TP	Food preservation	(Shao *et al*., 2018)
	Pullulan Coating	Delaying deterioration and controlling microbial growth on blueberry	(Kraœniewska *et al*., 2017)
	Pullulan coating containing oregano essential oil	Preservation of Brussels Sprouts	(Kraœniewska *et al*., 2016)
	Pu-OSA	Edible coating on fruits	(Shah *et al*., 2016)
	Laminaria japonica-incorporatedpullulan coatings	Preservation of cherry tomatoes	(Wu, Lu and Wang, 2016)
	WPI-pullulan	Better oxidativestability of tuna oilinside microcapsules	(Bakry *et al*., 2016)
	WPI/PUL/NS nanocomposite	Increasing the shelf life of food products	(Hassannia-Kolaee *et al*., 2016)
Pharmaceutical	CAPL/PBAE/PLGA nanoparticles	Excellent hepatoma-targeting capability and increased synergistic effects of PTX and CA4 on tumor growth and tumor angiogenesis	(Zhang *et al*., 2016)
	CHAP	Drug carrier for tumor treatment	(Tao *et al*., 2016)
	CMC-pullulan hydrogel	Injectable in situ anti-adhesive agent	(Bang *et al*., 2017)
	Crosslinked carboxymethylated pullulan/chondroitin sulfate hydrogel	Facilitate chondrogenesis	(Chen *et al*., 2016)
	Crosslinked pullulan/ cellulose acetate fibrous scaffolds	Bone tissue engineering	(Atila, Keskin and Tezcaner, 2016)
	CS-ADH/oxPL hydrogel	Cell delivery carrier scaffold in cartilage tissue engineering	(Li *et al*., 2018)
	Curcumin pullulan acetate	Effective hepato-protective agent against diethyl nitrosamine induced liver damage	(Ganeshkumar *et al*., 2016)
	FA-Pull-LAPTX CLNPs	Anti-tumor Liver Drug Delivery	(Huang, Tu and Sun, 2017)
	FA-CP/SeNPs	Anticancer drug template in drug delivery systems	(Chen *et al*., 2018)
	FPA NPs	Drug carriers for targeted therapy of the folate-receptor overexpressed cancers	(Chen *et al*., 2016)

FPDP	Co-delivery of DOX and shBeclin1 for cancer therapy	(Nonsuwan *et al.*, 2018)
HA-g-Pu	Wound healing materials	(Li *et al.*, 2018)
OGG3P	Geneticphotodynamic therapy	(Zhou *et al.*, 2018)
PABA-QP	Humancancer treatment	(Laksee *et al.*, 2018)
PAMAM-pullulan	Delivering gene into liver cells	(Askarian *et al.*, 2017)
PDP	Co-delivery of drug and gene for potential cancer therapy	(Chen *et al.*, 2017)
PPSS	As gene delivery vector and efflux inhibitor	(S. and R., 2016)
PSCFO	Therapeutic applications in targeting tumors	(Eslaminejad,
	Nematollahi-Mahani and Ansari, 2016)	
Pullulan	Pharmaceutical oral films	(Vuddanda *et al.*, 2017)
Pullulan-CMCS	Wound dressing	(Wang *et al.*, 2016)
Pull-LA-CLNPs	Delivery of paclitaxel into ASGPR over-expressed cancer cells	(Huang *et al.*, 2017)
PuPGEA)	Codeliver lncRNA and pDNA to treat Hepatocellular carcinoma	(Ren *et al.*, 2016)
Pullulan-g-poly	Controlled delivery of indomethacin	(Constantin *et al.*, 2017)
Pullulan-poly(vinyl alcohol)	As controlled release drug delivery system	(Soni and Ghosh, 2017)
ssPBAE-oxPL-DOX	Exhibited in vitro hepatoma-targeting property and condensing genes including plasmid DNA and fluorescein-labeled oligoDNA	(Wang *et al.*, 2016)

solution to overcome this problem. Although there are many microorganisms which cannot grown with the presence of the olive oil waste which is mainly due to the presence of toxic phenol compounds, *A. pullulans*has the ability to grow and produce pullulan. According to the experiment conducted by Israilides *et al.,* 1993, the *A. pullulans* grown well regardless of the presence of the olive oil waste but most importantly it reduced the phenolic compound content up to 41% during the sixth day of observation. The concentration of the pullulan that agglutinate ethanol substance increased when the phenols content was completely removed which indicates the absence of the phenols plays pivotal inhibitory role during the pullulan production (Israillides *et al.,* 1999).

Carob pod

The carob pod is a type of fruit from the carob tree *(Ceratonia siliqua)*. This tree mainly can be found at the Mediterranean regions and at some semiarid regions of North America. The carob pods contain special polyphenolics compounds, carbohydrates and also contain low level of insoluble dietary fibres, minerals and lipids and proteins. It consists of high amount of soluble sugars which is around 40% to 60% that enables it to be used as good substrates. When it comes to the ripen seeded carob cod, it contains high level of tannins which makes it to be used partially for the production of health confections. It is also mainly being used for animal feeding besides the fact that the presence of the tannin decreases the nutritional value of the pod. Somehow due to the difficulties and the high cost for the harvesting purposes, most of the carob pods are left unutilised. Due to the compositions of the carob pods, it is highly used as an animal feed (Mishra *et al.,* 2018). According to Rouks and Billaderis (1994), the yield of pullulan increased up to 89% when carob cod was used as the substrate during the fermentation process.

Corn Steep Liquor

Corn steep liquor (CSL) is the by-product from the corn wet milling process and also from other commercial corn processes. This waste which comes from the corn milling processing plant considered being non-synthetic and it has been used as non-synthetic input for most of the liquid fertilizer formulations for any organic crop production. It normally comprises of 10 g fiber/kg dry matter, 130-220 g carbohydrate/kg dry matter,

205 g crude protein /kg dry matter, 525 g/kg dry matter, 88 g ash/kg dry matter and a small amount of sulfurous acid (<0.01 g/kg DM) (Chiani *et al*., 2010). CSL also contains 42% of protein (Mishra *et al.,* 2018).

According to the experiment done by Sharma *et al*. (2013), when five different types of agricultural wastes which are rice bran oil cake, soya bean oil, cotton seed oil cake, mustard seed oil cake and corn steep liquor used as substrate for pullulan production,the corn steep liquor gave the highest pullulan production up to 77.92 g/L. This fermentation procedure also validated in 7-L fermenter where the economics of the process was analysed and it was found that, CSL can reduce the cost of raw material up to three times compared to the conventional process. This finding can be used for the development of cost-effective pullulan production.

Coconut by-product

Coconut water contains naturally occurring lipid which can be found inside the coconut. The coconut milk which tastes sweet can be derived from the meat of the mature coconut. The coconut water particularly considered as a waste product especially from the factories that produce copra desiccated coconut and other meat coconut product. Moreover, the coconut water can be an active pollutant due to its high biological oxygen demand. Increasing pollution problem also increased the interest on coconut water and motivated for its utilization for industrially important biopolymer production. Coconut water contains easily digestible carbohydrate which normally can form simple sugars and electrolytes. Due to the high demand for biological oxygen demand of the by-product from coconut, this agro waste has been used as a substrate for efficient pullulan production (Mishra *et al.,* 2018). Several researches has been done on utilizing this by-product as a substrate in attempt to reduce its waste in the environment. One of it is, according to the study done by Thirumavalavan *et al.,* (2009), both the coconut water and coconut milk can be used as a good substrate for the production of pullulan. However, the coconut milk tends to be more efficient when it comes to pullulan production comparing with coconut water. According to Thirumavalavan *et al.,*(2009), this mainly due to the higher amount of carbon and nitrogen ratio in coconut milk than in coconut water.

According to the results obtained by Thirumavalavan *et al.,* (2009), coconut water contains around 40 g/L of reducing sugar while the tender coconut water contains 22 g/L of reducing sugar. Coconut milk contains around 48 g/L of reducing sugar. The highest production of pullulan which is around 54 g/L was obtained from coconut milk during the fermentation period of 144 hours. This is mainly because the coconut milk and coconut water are rich with mineral source and amino acids. Besides that, both of the by-product does not require any additional pre-treatment methods like other substrates to enhance the pullulan production.

Jaggery

Jaggery can be defined as natural and traditional sweetener which can be made from concentrated sugarcane juice and it is known in different local names according to the country all over the world. It is the traditional unrefined and non-centrifugal sugar that being consumed in regions likes Asia, Latin America and Africa. It contains all the basic minerals and vitamins which also present in sugarcane juice which make it to be one of the healthiest sugar in the world (Singh *et al.,* 2018). Jaggery is largely produced in India which is around 70% of total. Basically, jaggery is prepared by concentrating the sugarcane juice and there are three types of jaggery which are solid jaggery (cube shape), liquid jaggery and granular or powder. Moreover, the sap that being collected from some of the palm trees like coconut palm, wild date palm and sago palm is being used for the preparation of jaggery (Nath *et al.,* 2015).

Since jaggery contains different sugar and minerals like sucrose, glucose, sucrose which is about 75% to 85%, potassium and calcium, it can be used as important components for the growth media for *A. pullulans.* According to the experiment done by Ganduri *et al*., 2016, it was revealed that jaggery can be a good carbon source due to high composition of sucrose which can be utilized by *A. pullulans* for pullulan production. This can be a good strategy to deliver cost effective pullulan production. Likewise, it was reported that the pullulan yield was highly dependent on the concentration of the jaggery used (Vijayendra *et al.*,

2001). Therefore, the pullulan yield was examined by adding different concentration of jaggery which were at 50%, 75% and 100% respectively and 50% concentration showed the highest pullulan production which is up to 6 g/L.

Rice hull hydrolysate

Rice hull is one of the most widely used and available agricultural by product in many rice producing countries like Thailand and China. 1000 kg of paddy grain can produce about 200 kg (20%) of rice hulls (Hossain *et al*., 2018). Only some small part of the rice hull used for variety of purposes like building materials, fertilizers, fuel, insulation material and more importantly most of them are being thrown away as wastes (Ma *et al.,* 2011). Owing the presence of lignocellulosic material as the main component, the rice hull is highly used for the fermentable sugar production in recent time where the rice hull also been categorised as desirable candidate to be used as carbon source for production of bioethanol and other bio-based materials. Although the rice hull hydrolysate contains little amount of silicon and ashes, it consists significant amount of glycans that can be decomposed into fermentable sugars by acids (Rahman *et al.,* 2011).

For the best conversion of rice hull into the fermentable sugars, the important part is the physiochemical pre-treatment of the biomass. Normally, the diluted sulphuric acid hydrolysis method was used to recover the sugars from the lignocellulosic material under high efficiency. But during this process, most of the inhibitory compounds for example furfurals and acetic acid will be generated along with the released of fermentable sugars (Hickert *et al.,* 2013). According to research done by Wang *et al*. (2014), the presence of acetic acid during hydrolysis process exert some negative effect on pullulan production. This indicates that acetic acid might have function as an inhibitor for the pullulan production. This is somehow lowers down the yield of pullulan due to the high level of acetic acid in the rice hull hydrolysate. To overcome the negative effects of the acetic acid, two of these methods which is detoxification of the hydrolysate or adaptive evolution of the microorganisms can be applied. Table 4 shows the list of agro-industrial by-products for potential used as feedstock in pullulan production.

Pullulan applications

Polysaccharides are ubiquitous in nature. Apart from cellulose, which is the plentiful biomass material on earth, there are other natural polysaccharides such as pullulan. The biologically important natural polysaccharides can be used for developing functional bio-based polymer (Danjo *et al*., 2017). Currently, pullulan is used in film and food industry extensively (Rekha and Sharma, 2007).It is a molecule which is tasteless and digested slowly in human. Hence, resulted in rising of blood glucose level slowly(Wolf *et al*., 2003). Nevertheless, recently pullulan is being focused in pharmaceutical applications such as targeted drug or gene delivery, nanoparticles for drug or gene delivery, cancer therapy, medical imaging, molecular chaperone plasma expander and tissue engineering (Rekha and Sharma, 2007).

Food industry

Over the past decades, pullulan films and coatings have received gigantic attention in the food industry (Farris *et al*., 2014). Extending shelf life, minimizing foodborne illness, improving postharvest quality is very pivotal in industry (Trinetta and Cutter, 2016). Hence, the development and utilization of pullulan film and coating are used to enhance the quality and strengthen the shelf life of agricultural products (Shao *et al*., 2018).

Edible films or coatings can be defined as the thin layers that applied and isolate the food products. Thus, the fruits or vegetable can be protected from chemical, physical and microbiological activity (Falguera *et al*., 2011).Wu *et al*. (2016) stated that Laminaria japonica-derived oligosaccharides (LJOs) incorporated pullulan coatings were found to diminish respiratory intensity, weight and vitamin C loss. In another study, an edible coating based on chitosan and pullulan were found to increase the quality and strengthen the shelf lifetime of fresh pineapple (Treviño-Garza *et al*., 2017). A similar finding was found in Kraœniewska *et al* (2017), pullulan was found to delay deterioration and prevent the drying and wilting of the fruits especially in high temperature conditions. In addition, it was found to inhibit microbes. Pullulan coating containing oregano essential oil were found to inhibit the yeast and mold and populations of *Aspergillus niger* (Kraœniewska *et al*., 2016).

Pullulan has been applied inprotection systems for omega 3 oils and development of inulin-based encapsulation technology.In order to prolong the oxidative stability and shelf life of the microencapsulated fish oils, whey protein isolate and pullulan were used as emulsifier and stabilizers respectively to prepare tuna oil microcapsules (Bakry *et al*., 2016). Besides, pullulan was a thickener that can use to form semipermeable films. Pullulan coating based incorporated with antibacterial agents which consists of 1% pullulan, 0.8% glutathione + 1% chitooligosaccharides, and 0.8% glutathione + 1% chitooligosaccharides + 1% pullulan on apple was used to examine during cold storage. It was found to be effective to prolong the shelf life of apple as delaying the browning, inhibiting the microbial growth and maintaining the firmness (Wu and Chen, 2013). It can be utilized in various ways as thickeners in beverage or sauces. It also stabilizes emulsions. This property created smooth and viscous texture. Moreover, the consistency of pullulan to high salt and pH are utilized to impart viscosity to foods such as barbecue and soy sauces (Chaen, 2009).

In addition, pullulan can be used as low calories food ingredient as it only slightly depolymerized by digestive enzymes. It has been demonstrated that replacing flour with pullulan to make biscuit or doughnut in the baking industry (Tsujisaka and Mitsuhashi, 1993). Furthermore, pullulan acts as a humectant and binder by retaining moisture. It has been applied by adding of pullulan to have a fluffy sponge cake. Additionally, it can used as a binding agent to bind food pastes or glazing agents due to its strong adhesive property (Chaen, 2009).

Pharmaceutical industry

Over the past decades, intense research has been carried out in order to understand bioactive polysaccharides utilize its medical properties in naturally produced pharmaceuticals (Giavasis, 2014). Pullulan is non-toxic, water soluble, non-mutagenic, odorless, low oxygen, and moisture permeability (Aguilar-Vázquez *et al*., 2018). These mechanical properties resulting pullulan can be used as another alternative for gelatin in the production of the capsule coating for dietary supplements and medical products (Park and Khan, 2009). In addition, it can be used as a denture adhesive. Adhesive can be prepared by dissolving pullulan ester in a mixture of water and acetone. Sugar-coated pharmaceutical compositions contain pullulan in sugar layer of tablet can prolong the shelf life (Singh, Saini and Kennedy, 2008).Pullulan films can be applied in oral care product and have been commercialized (Leathers, 2003).

Tissues engineering

Bone is a dynamic tissue that capable of altering its structure and mass throughout the lifetime (Weatherholt, Fuchs and Warden, 2012). Osteogenesis with appropriate scaffolds for bone regeneration can be enhanced by applying tissue engineering technique to bone defects (Moreau *et al*., 2007). Cholesteryl group- and acryloyl group-bearing pullulan (CHPOA) nanogels is used to deliver two distinct growth factors FGF18 and Bone Morphogenetic Protein 2 (BMP-2) to a critical size skull bone defect for bone repair using CHPOA/hydrogel systems. Studied indicate that synergistic effect between FGF18 and BMP-2 increase the thickness of the bone. This hydrogel is having potential as a drug delivery systems containing multiple growth factors to regulate and induce osteogenesis. Thus, aided in developing of an efficient delivery system of osteogenic factors that contribute a very stable bone regeneration (Fujioka-Kobayashi *et al*., 2012). Pullulan provides good solubility and its hydrogels demonstrate great mechanical stability with high water retention capacity (Li *et al*., 2011). Hence, it is used as a composite based of photocrosslinkable polysaccharide hydrogel for human co-culture model of human osteoblast and endothelial cells. In this study, pullulan-amylose hydrogel composites are demonstrated to have great potential as carrier systems, especially concerning endothelial enhancement by addition of SDF-1. After incubation of hydrogels with the growth factor BMP-2 and SDF-1 respectively, the cell growth occurred and this highlighted the retained function of growth factors after entrapment and release from the hydrogel matrix (Ritz *et al*., 2016).Li *et al*. (2016)reported HLC/pullulan hydrogel may enhance the fibroblasts attachment and inhibit the cell death.

Besides, the great mechanical strength with reduced inflammation delayed hydrogel degradation may posse advantages in vivo applications. A scaffold composed of pullulan

and dextran with hydroxyapatite particles (nHA) was developed to examine bone healing process. This study revealed that the composite based-polysaccharide scaffold (Matrix + nHA) retained subcutaneously local growth factors like BMP-2, induced the formation of dense mineralized tissue in mice. After that, implanted this scaffold in different size of animal models. High mineralized tissue was observed in all the animal models which including rat and goat. Therefore, proposing this composite matrix able to stimulate bone cell differentiation and bone formation(Fricain *et al*., 2013). In another similar study, pullulan has been supplemented with nHA in a rat model. The result showed an increasing of newly formed tissue and osteoid tissue around the scaffold. This study suggests pullulan based scaffold favored bone mineralization and formation. Besides, it also enhances vessel ingrowth into the defect site. Therefore, this suggests the scaffold possible meet the clinical trial as it capable of repairing small size defect(Schlaubitz *et al*., 2014). An enzymatically crosslinked biocompatible hydrogels were established using pullulan and silk fibroin under condition presence of horseradish peroxidase (HRP) and hydrogen peroxide (H_2O_2) as an oxidant. Besides, the rabbit bone marrow-derived mesenchymal stem cell was encapsulated in silk fibroin/ pullulan hydrogels for 7 days. The result showed that about 90% live cell was present in this hydrogel. This indicates silk fibroin/ pullulan hydrogel had good cytocompatibility and this can be proposed as a cell carrier candidate to have application in musculoskeletal tissue engineering (Li *et al*., 2018).

Film industry

Pullulan coating prolonged the shelf life of kiwifruits and strawberries. Accumulation of ethylene in pullulan coated fruits prevent ethylene translocation from the internal to external fruit atmosphere and maintain the firmness during the storage (Diab *et al*., 2001). Pullulan based films are clear, low toxicity, highly oxygen-impermeable with excellent mechanical properties and good biodegradability (Farris *et al*., 2012). As a result, it is known as "edible" packing. Normally, they function as protecting food from lengthening the shelf life of food products against moisture and gases (Rinaudo, 2008). In this study, glutaraldehyde and glycerol were used to enhance physical properties and water resistance of pullulan films. The result possess that film will have stronger tensile strength when 2% (w/w) of glutaraldehyde added. Furthermore, glycerol act as plasticizer assists to ameliorate flexibility of films though with reduced water resistance (Chen *et al*., 2016). In addition, pullulan can be used as wound healing film. In this study,hyaluronic acid grafted pullulan (HA-g-Pu) polymers with hyaluronic acid were synthesized to examine the rate of wound healing process. Results showed applying of HA-g-Pu film will speed up the healing process compared to the natural wound healing process. Due to the HA composition with porous microstructure, high swelling ratio, prevent accumulation of exudates and fast hemostasis ability, hence, HA-g-Pu film is used as wound healing materials(Li *et al*., 2018). Oral thin films (OTF) is a thin film that composed of the drug molecule and other excipients. It can be produced through extrusion method or solvent casting that capable dissolves rapidly on patient's tongue (Chowdary *et al*., 2012). Pullulan as hydrophilic polymers is used as film formers for OTF.In this study, pullulan based oral thin film (OTF) of zolmitriptan was made with PEG 400 as a plasticizer and sucralose as a sweetener in lab scale. Result showed PEG400 and sucralose are compatible and have a good quality overall. In addition, PEG 400 showed having no negative effect on drug release rate and having excellent stability in aluminium sachet stored at 40 ÚC(Prajapati *et al*., 2017). In another similar study, protein loaded orodispersible films (ODFs) were prepared based on blends of trehalose/ pullulan by air- and freeze-drying. Based on the excellent protein stabilizing capacity of trehalose and film-forming ability of pullulan, these two carbohydrates were selected. Trehalose has a very weak performance on film former, therefore, pullulan is being selected as the main material for ODFs. Combination of these 2 materials are believed can have strong protein stability and exhibit great film-forming properties (Tian *et al*., 2018). Another pullulan-based nanocomposite films composed of lysozyme nanofiber (LNFs) for functional food packaging were developed. LNFs able to maintain with good mechanical properties and several functionalities such as withstand temperature up to 225 ÚC, antioxidant activity and especially antibacterial activity

towards *Staphylococcus aureus* food pathogenic bacteria increase with the content of nanofibers. These properties not only showed pullulan based nanocomposite is an edible film for food packaging and serve as multifunctional purposes to protect and prolonged the shelf life (Silva *et al*., 2018).

Nanoparticles drug delivery

Emerging in biotechnology have led to research and development of the protein-based drug. However, the bioavailability of protein drugs is low as it always being filtered out from the body via proteolysis and renal filtration. Thus, developed a delivery of therapeutic proteins is attractive in the biopharmaceutical industry and biotechnology research community (Nuttall and Walsh, 2008). In this study, Hybrid hyaluronan (HA) hydrogel modified with 2-aminoethyl methacrylate with the presence of cholesteryl group-bearing pullulan (CHP) nanogel. Just immersing hybrid hydrogels into the drug solutions will allow therapeutic proteins to be trapped in the CHP nanogel in the HA gel. In vitro and in vivo, CHP/protein complex nanogel will be released from the hybrid hydrogel in a sustained manner. Hence, this hybrid hydrogel system possess biocompatible sustained for releasing the protein without denaturation of protein(Hirakura *et al*., 2010). Finding appropriate strategies to deliver proteins has become a crucial issue and transmucosal administration is the first-line option for their systemic delivery. Recently, nanoparticles have been suggested as protein carriers due to its structural flexibility and biodegradability and biocompatibility (Antosova *et al*., 2009). In this study, pullulan based nanoparticles were produced with sulfated and aminated derivatives of the polymer. These derivatives were then complexed with carrageenan and chitosan to synthesize nanocarrier. In this work, pullulan based nanoparticles capable to release 30% of protein up to 24 hours. In addition, there is evidence indicating an absence of toxicity of the pullulan based nano-delivery systems on a respiratory cell line(Dionísio *et al*., 2013). Generally, infectious pathogens invade their hosts through mucosal surfaces of respiratory and gastrointestinal tracts. Nasal and oral vaccines are developed to target various infectious disease (Bahamondez-Canas and Cui, 2018). In this study, cationic pullulan nanogel is being proposed as a mucosal drug delivery system. The nanogel composed of cholesteryl-group-bearing pullulan (CHP) which make protein easier incorporated within the internal space of CHP nanogel. These unique properties make it function as a molecular chaperone. Cationic cholesteryl-group-bearing pullulan recognized as safety and its efficacy in generating antigen-specific protective immunity (Nakahashi-Ouchida, Yuki and Kiyono, 2018). Nanosystem has been focused for drug delivery system to the tumor cell. In this study, pullulan serves as a multifunctional function such as vehicle and design which further assisted by folic acid and disulfide crosslinking, a PTX-loaded redox-responsive nanoplatform was designed for dual targeted liver cancer treatment. In vivo, therapeutic efficacy studies indicated enhancing of antitumor effect and reducing systemic toxicity compared taxol were achieved using FA-Pull-LAPTX CLNPs. In addition,a reversibly disulfide-crosslinked pullulan nanoparticle with folic acid (FA-Pull-LA CLNPs) was reported could target ASGPR and FR-positive human hepatic tumor xenografts. In conclusion, combining dual-targeting and reversible crosslinking can serve as drug delivery systems for the transport of lipophilic drugs (Huang *et al*., 2017).

Medical imaging

Nanotechnology has been applied for earlier detection of cancerous cell grow in the body. Quantum dots are a nano-size semiconductor which gaining a lot of interest in biological field. The main purpose of quantum dots is used for cell tracking as fluorescent probes.Cholesterol pullulan and amino group modified cholesterol pullulan nanogel is developed for the delivery of quantum dots into cells in comparison to a conventional cationic liposome which having the difficulty forming aggregates ones gets into the cells. They compared the intensity of fluorescence per cell with conventional cationic liposome. They concluded that cellular uptake of cholesterol pullulan was improved by introducing cationic groups and simultaneously the quantum dot better than the conventional cationic liposomes and these nanoparticles could be a promising fluorescent probe for medical imaging(Prajapati, Jani and Khanda, 2013).

Molecular chaperons

A molecule having chaperon-like activity are capable to catch or release proteins. It will bind

to the denatured protein to prevent irreversible aggregation. Then the chaperons release the protein in its refolded form. Water-soluble polymers such as polyethylene oxide (PEO) will try to increase the recovery yield of native protein during refolding (Cleland *et al*., 1992). This polymer will block the exposed hydrophobic surface to prohibit aggregation of proteins. Nomural *et al*. (2003) developed hydrophobized pullulan nanogel possessing properties of molecular chaperons. In the presence of cyclodextrins, complexed proteins will release rapidly from nanogels in their refolded forms. They concluded that this denatured protein and cyclodextrin will be trapped by these amphiphilic nanogels and it acts as an effector to control the binding ability of chaperon molecule to proteins.

Plasma expander

Pullulan explored as a potential blood plasma substitute. Polymer which is highly water-soluble can be used as plasma expanders. Due to its unique structure, pullulan is water soluble in nature. It has been reported that pullulan should have molecular weight about 60 kDa then only can be used as plasma expanders (Rekha and Sharma, 2007). It was stated that pullulan with high molecular weight will raise the venous pressure whereas pullulans with low molecular weight will exclude from the organism leaving the stage of secondary hemorrhagic shock. Therefore, pullulan should be in the therapeutic range of molecular weight in order to be used as a plasma expander. An anionically modified pullulan is being developed through gamma irradiation which was used as a base for blood plasma substitute (Shingel and Petrov, 2002). Table 5 summarize the potential applications of pullulan mainly in the food and pharmaceutical industries.

CONCLUSION

The advancement and needs to design for an efficient bioprocess is the most important step in any biotechnology industry be it food, pharmaceutical or any other. There are needs to have an economical and robust process that can be reproducible effortlessly. Agro-industrial wastes are produced in huge amounts every year and they are rich in nutrients comprising variability of sugars and minerals. This nutrient offers good supports for cell growth and pullulan production. In addition to that, it can help to contribute in minimizing waste and creates environmental eco-friendly.

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