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Bioscience Research

Print ISSN: 1811-9506 Online ISSN: 2218-3973

Journal by Innovative Scientific Information & Services Network

RESEARCH ARTICLE BIOSCIENCE RESEARCH, 2023, 20(2):605-613. **OPEN ACCESS**

Applications of probiotic yeast *Kluyveromyces lactis* in industrial process

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Yeasts are considered as one of the oldest knows microbial biofactories with diverse applications in many ancient cultures. Since centuries, they have been used in many daily foods such as in bread preparation, beer and wine manufacturing. Nowadays, the use of yeasts in various industrial, biotechnological and food applications is increasingly growing. Among the emerging yeasts the species *Kluyveromyces lactis*. The latter is endowed with probiotic characteristics, which allow it to be used in the food industry and in biotherapy. This species is also widely used as a cell factory for the production of metabolites and proteins. this can be explained by its physiological and genetic characteristics and its adaptation to industrial processes. This review gives an overview on the physiological and probiotic characteristics of *K. lactis* and on the different processes of fermentation and development of probiotic product

Keywords: *Kluyveromyces lactis,* yeasts, Probiotic, industrial Process, Fermentation.

INTRODUCTION

It is currently widely known the key role that the gut microbiota plays critical role in human health and disease prevention (de Vos et al. 2022). It has been clearly proven that maintaining of gastrointestinal microbiota equilibrium can be improved by the efficiency of probiotic strains (Hashim et al. 2021; Dahiya and Nigam, 2022). Probiotics, as defined by Food and Agriculture Association of the United Nations (FAO) and World Health Organization (WHO), are living microorganisms which when consumed in sufficient amount can provide therapeutic effect to the host (Cook et al.2012; Selvamani et al. 2022; Yoha et al. 2022). The sufficient amount for probiotic intake that will give the therapeutic effect to the host is (10^6–10^7 cfu/g) which result in prevention of gastrointestinal infections and also improve the immune system of the host (Arslan et al. 2015).In heterogenous microbial system, bacteria composed about 10^{14} whereas yeast just consist around <0.1% from the gastrointestinal microbiota. Even though, yeast can be considered as minority from the microbiota, their cell size is usually larger than bacteria and this result in represent a significant stearic hindrance of bacteria. Usually, yeast can be found in the stomach and colon. They can resist high stress environment by the variation of pH they can withstand which is around 4.5-6.5 (Czerucka et al. 2007; Schulze and Sonnenborn, 2009).Among the yeasts most used in the food industry and in biotechnology, scientists describe the two genera *Saccharomyces* and *Kluyveromyces*. The species *Kluyveromyces lactis* is widely used in fermented foods and in the production of metabolites and proteins in biotechnology (Varela et al. 2019). The objective of this review is to provide a comprehensive updated information about physiological features, bioprocessing and the different potential applications of this species in industry.

2. Basic features of *Kluyveromyces lactis*

The genus *Kluyveromyces* (named in honour of microbiologist Albert Jan Kluyver) was proposed by van der Walt in 1956. The best-known species of the genus are *K. lactis* and *Kluyveromyces marxianus*. They have the ability to use lactose, xylose, arabinose, xylitol and cellobiose (Lachance 2007; Spohner et al. 2016).

Kluyveromyces lactis can be distinguish from

Saccharomyces cerevisiae by two conditions of mitochondria. *K. lactis* need mitochondrial DNA to perform their regulatory system whereas *S. cerevisiae* can live without it. Furthermore, it was stated under the same study that during anaerobic environment, *K. lactis* will not grow due to limited glucose repression as they only can grow by glycolysis which is the fermentation of glucose as carbon source. It seems different in *S. cerevisiae* which they can undergo respiratory metabolism by glucose repression due to the ability to use wider range of carbon source (Zivanovic et al. 2005).

The cell division processes of yeast that distinguish it from human is by the presence of septins as the key component. It was found that septins was present in both *K. lactis* and *S. cerevisiae.* Thus the cytokinesis is similar between the yeast with the presence of KlChs2 and KlCdc10 septins genes (Rippert et al.2017). In the research done by Ishtar et al.(2006), it was found that *Kluyveromyces lactis* unable to grow in anaerobic condition due to lack of sterols that required by oxygen as biosynthetic pathways. Based on the research they found that if *K. lactis* able to be altered in engineering processes, the lactose that produced as the product can be a carbon source for *Saccharomyces cerevisiae* and they can produce even more ethanol by full fermentation that will be huge finding in industrial production.

In addition, *K. lactis* generally known as petite-negative ascomycetes yeasts due to the presence of petite colonies that can be observed in part of *S. cerevisiae* as the presence of mitochondrial DNA and cells usually simpler, smaller, tRNA genes minimal, less frequent of introns and absence of endonuclease gene in rRNA intron (Zivanovic et al.2005).In research studied by Breunig et al.(2003) *K. lactis* has been proved suitable as a host for recombinant protein production due to low protease levels, high folding and secretion capacity, and the absence of a Crabtree effect. Thus, this make them act as one of important roles in biotechnology industry.

3. Application of *Kluyveromyces lactis* in industrial process

K. lactis is considered as probiotic yeast. In the sceening of probiotic yeast, the strains will be examined on their ability to colonize the intestine by adhering to the intestinal epithelium. This ability been examined by using Caco-2 cells that investigated *in vitro* and it was found that *K. lactis* possess highest adherence among probiotic yeast toward the Caco-2 cells that usually bounded by probiotic bacteria. The capability to adhere toward the intestinal epithelium makes it dependable to reduce the pathogenic bacteria inside the gastrointestinal tract (Spohner et al. 2016; Fadda et al.2017). The exogenous lactase obtained from the probiotic yeast of *Kluyveromyces lactis* allow the efficient absorption of gastrointestinal process through break down of lactose to glucose and galactose (Deng et al.2015).

In industrial manufacturing of protein production, *Kluyveromyces lactis* is the best alternative since decades due to its ability to secrete high level of protein that applicable in producing metabolic and heterologous proteins (Demain et al.1998). There are over than 40 proteins that successfully expressed by *K. lactis* making it one of alternative in yeast expression system (Van Ooyen et al. 2006). Based on the high capacity of *K. lactis* to produce enzymes, it has been applied for β-galactosidase production as biocatalyst for the production of lactose-free dairy products and prebiotic galacto-oligosaccharides (GOS). In addition, this type of yeast was also used a biofactory for heterologous protein for recombinant chymosin production which is widely used in cheese production based on its biocatalytic activity for flavour compounds production such as terpenes, esters, and lactones (Demain et al.1998;Spohner et al. 2016). In addition, there are also several applications of *K. lactis* in pharmaceutical area which are treatment of autoimmune disorders by the production of human interleukin 1-β, cancer therapy from the recombinant interferon-α, acute and chronic treatment of hepatitis B and C, fight the disorders of hematopoietic system. Moreover, the enzyme produced by *K. lactis* has ability to mimic human glycosylation just like previously carried out by *P. pastoris* and *S. cerevisiae*and since 1960s, dried and inactivated *Kluyveromyces lactis* has become a protein supplement in food (Van Ooyen et al. 2006; Spohner et al. 2016).

4. Metabolite production profile of *Kluyveromyces lactis*

The probiotic effect and industrial processes of *Kluyveromyces lactis* can be linked by their metabolite production profile.

4.1. The strain design

Van Ooyen et al. (2006) stated that there are varieties of *Kluyveromyces lactis* strains that provide different genome sequenced result in different efficacy in heterologous protein production. For example, usually in research purposes, the strain that being used is CBS 2359 whereas in producing high yield of protein secretion GG 799 is being used and even more commercialize in industry. But recently, the genome can be modified by transforming desired DNA to obtain the desired genome for industrial purposes.In the Crabtree effect that relate with the protein production, *Kluyveromyces lactis* producing biomass and the hexoses inside it pathway that metabolized by pentose phosphate pathway (Spohner et al. 2016).

The expression of heterologous genes that yields high value of biomass production of *K. lactis* is due to the strength of their LAC4 promoter which one of two LAC genes found in the probiotic yeast that absence in *Saccharomyces cerevisiae* with the purpose of lactose assimilation (Krijger et al.2012). The inducible LAC4 promoter can result in yield high production of toxic proteins that important in industrial processes of *K. lactis*

by separate the growth and expression phases. Moreover, if the lactose is absence, this lactase promoter cannot completely induce because it usually depends on inducible lactose to be functioned.

The gene expression and secretion of yeast can be applicable by the presence of promoter and signal sequences. Proteins production by the promoter is usually accumulate inside the cytoplasm but also can be discharged through the culture medium. The cytoplasm accumulation usually produce high biomass production but in downstream process, the protein purification processes need to be added into account. On top of that, the production of pigment pulcherrimin, cause the colonies of *K. lactis* colored cream to pink. Their growth can be observed in between 25°C and 35°C that usually being isolated from dairy products (Spohner et al. 2016).

In the same research, during scaling up industrial process in fed batch fermentation, *Kluyveromyces lactis* will provide highest protein production. This process using the bioreactor system, high oxygen level, and provide the optimized culture medium for their growth that can result in high production of biomass up to 10 m^3 and reduce the time-consuming processes. There are examples of recombinant proteins produced in this research which are chymosin, brazzein, fructosyltransferase and β-galactosidase.The active form of bovine chymosin which is one of the recombinant proteins can be produced by *Kluyveromyces lactis* in food industry whereas the inactive form usually produced by *S. cerevisiae*. This recombinant protein usually applicable in the cheese production and undergo the batch fermentation to optimize the highest yields.Sweet tasting recombinant protein produced by *K. lactis* is called brazzein and YPGal medium is used to produce 114 mg/L sweet tasting protein brazzein which composed 90-95% of total secretory protein of *K. lactis*(Spohner et al. 2016; de Albuquerque et al. 2021; Lambré et al. 2022).

4.2. Crabtree effect

Adenosine triphosphate (ATP) is the important compound needed for the cell growth, biosynthesis, transport and motion in cellular energy metabolism. There are two different ways yeast produce ATP from sugars which are fermentation and respiration. High productions of ATP are produced by respiration while fermentation produce less ATP and without require the presence of oxygen. Crabtree negative yeasts exclusively produce ATP by respiration while the alteration of fermentation and respiration will be held by Crabtree positive yeast (Pfeiffer et al. 2014).

Advance in molecular genetics tools development with the lower degree of genetic redundancy cause the milk yeast *Kluyveromyces lactis* become alternative model to investigate the basic cellular processes of cells as explained in regulation studies of cytokinensis. The mitochondrial function of *K. lactis* in production of energy is mainly based on respiratory metabolism and lower amount of glucose respiration that make it presented as crabtree-negative yeast (Rippert et al.2017). Thus, *K.lactis* do not undertake the anaerobic alcoholic fermentation that make them reliable by the industry to produce high amount of biomass production in aerated cultures without using extra carbon for unwanted production of alcohol (Wagner et al. 2016).

4.3. Exopolysaccharides (EPS)

Exopolysaccharides (EPS) composed of monosaccharide and substituent of non- carbohydrate those also known as extracellular polysaccharides with high molecular weight polymers that can give cell adhesion and protect the cells during stressful conditions. In recent studies, exopolysaccharides structural found to be fundamental in food as emulsifier, stabilizer, viscosifier, and also moister retention (Yildiz and Karatas, 2018). Moreover, exopolysaccharides that commonly produced by kefir, become fundamental in food, medicine and pharmacy industrial applications due to the ant anaemic and antibiofilm properties that make them important as immunostimulating and antitumor agents (Chen et al. 2016). The advance understanding of exopolysaccharides mechanism in biosynthesis of microbes also will enhance their genetic production that becomes another potential application in industry (Madhuri and Prabhakar, 2014).In another research, the presence of microorganism inside the kefir has been studied using RAPD markers and rRNA gene sequencing that found it composed of bacteria and yeasts. Furthermore, the research has been extended by using culture independent analysis (PCR-DDGE) analysis that specified several bacteria like *Lactobacillus paracasei and Lactobaciilu sparabuchneri, Lactobacillus kefiri*and *Acetobacter lovaniensis* including yeast such as *Saccharomyces cerevisiae* and *Kluyveromyces lactis* were kind of microbes that presence in kefir (Fiorda et al.2017)

In the manufacturing of fermented dairy products, exopolysaccharides will be synthesized in varies condition which are in terms of the amount production, chemical composition, molecular size, the size chains presence, and the rigidity of the molecules which result in firmness and creaminess of the dairy products. Moreover, EPS also provide therapeutic effect toward the gut health and improve the theology of fermented dairy products (Duboc et al.2001). In the research done by Simova et al. (2000), *Kluyveromyces lactis* has been used by co-cultivation process in enhancing the production of exo polysaccharides of *Rhodotorula glutinis* that usually produced by lactose hydrolysis. This is due to the function of lactose that acts as substrate in synthesizing exopolysaccharides. The strain producer *Rhodotorula glutinis* usually assimilate glucose, galactose and lactic acid and it is lactose-negative yeast. Thus, the possibility of this strain to produce exopolysaccharides by lactose as the carbon source will only happen by co-cultivation with *K. lactis* that produce ß-galactosidase into glucose and galactose in cheese whey unfiltrate.It was found that co-

cultivation will improve the efficacy of fermentation in which there are studies that involve two different cultures in one reactor and the resistance for contamination can be reduced as well improve the production of biomass (Muller, 2009).

5. Industrial Cultivation of *Kluyveromyces lactis*

5.1. Fermentation Process Optimization Strategy

Scaling up the production of probiotic is a huge finding in industrialization of microbial processes of manufacturing technology (Figure 1).

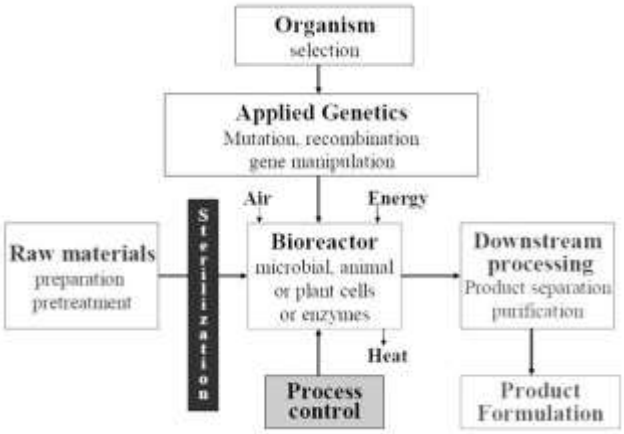

Figure 1: Schematic overview of fermentation process (Adapted from Huang et al.2007).

The processes include planning the suitable medium and design the reliable cultivation system. Challenges in scaling up processes are the amount of oxygen transfer, mixing, consumption of power, heat transfer and sterilization cause the effectiveness of the process that give the large yield of productivity, affect the cell density, final product concentration and also the purity of the product. There are also certain issues that need to cope which are end product inhibition, foaming, contamination and the stability of the product that need to take into account and ensure all the processes can reduce or prevent the issues (Xia et al. 2021).On top of that, there are three common ways to grow microorganisms which are batch, fed batch and continuous cultures. For batch fermentation, inoculate the microbes' cells into fresh media without any further nutrient is added until the end product produced (Huang et al. 2007).In the illustration below shows the main technical procedure for fermentation process that composed of media selection and genetic manipulation, raw materials and sterilization of the material, bioreactor outline and operation as well the product recovery downstream process, purification and formulation.

In batch and fed-bath fermentation, glucose possess as carbon source that promote the cell growth as well as protein production. Moreover, glucose also serves as important factor in structuring and maintenance cells. It was studied that *K. lactis* have ability to clone and express xylanese enzyme with the present of 2g/L glucose and 80% oxygen by fermentation. In contrast, the exceed presence of glucose will result in oxygen depletion and give negative impact toward the protein expression. Fed-batch fermentation that usually attain high cell density cultivation and improves the ancient ways fermentation in substrate inhibition, low cell concentrations, glucose impact, catabolite repression, auxotrophic mutants as well as the high viscosity of broth culture. The important factor that fundamental in fed batch fermentation are pH, lactose concentration and temperature (Huang et al.2007).

Optimization of Culture Condition

In obtaining high yield of enzyme production, optimization of culture medium condition by Response Surface Methodology (RSM) had been take into taken as the most effective method. This method apparently uses to overcome the traditional method such as "One-factor-at-a-time" technique that is time consuming and unable to correlate with current global optimal condition. Furthermore, RSM also usually use to obtain the optimal growth, enzyme and metabolite production that made up from four steps which first the movement into optimum region, then behaviour of response optimum region, optimal condition estimation and finally verification (Unni et al. 2019). In the research studied by Feng et al. (2011), temperature that being used in production of enzyme recombinant calf chymosin for 24h incubation is 30°C under agitation of 180 rpm.

Biotechnological industrial process that includes fermentation process can be divided into two groups which are aerobic and anaerobic. The aerobic fermentation usually faster but they need higher power inputs for agitation, aeration and cooling capacity. This will be reduced the capability of scaling up in bioreactor process (Huang et al. 2007).At the beginning of fermentation process, aerobic fermentation will take action. This process designed to be shorter and more intense than anaerobic fermentation. The limitation of this fermentation is the oxygen limitation due to its low solubility with water. In this type of fermentation, the dissolved oxygen (DO) concentration needs to be maintained as high as possible by increase the oxygen transfer rate (OTR) to make sure the culture is at high density, high agitation and aeration rate. In current research for industrial processes, the utilized of oxygen pressure have been applied to enhance the OTR or DO level during fermentation to produce the metabolite desired products (Huang et al. 2007).

Anaerobic fermentation designed to occur in fermentation vessel with replacing oxygen with N_2 and CO_2 which result on the slower process of fermentation. The chambers specifically for anaerobic fermentation were invented in 1960s and 1970s that allow the cultivation of the anaerobic organisms. The bright side of anaerobes is they are able to retain the unfavourable conditions and minimize the contamination during fermentation due to the

unusual enzymes and catabolic pathways. This result in successfulness of high yield production and more carbon can easily be converted to the end product. Thus, the overall cost of fermentation process can be reduced and make it one of important part in industrial fermentation (Huang et al.2007). However, one of main problem occur in large scale fermentation is foaming that result in asepsis problems, loss of fermenting broth, increase the pressure drop, stuck adequate circulation of gas and liquid in bioreactors and might terminate the whole process. It might also be a route for contaminating the cells and damage the whole production of cells. Foaming usually occur due to the usage of complex media or high protein level media. Thus, surfactants act as antifoam agents that reduce foaming but they will reduce the oxygen transfer rate and inhibit the cell growth (Huang et al. 2007).

5.3. Fermentation Medium Optimization

Suitable medium composition is necessary to obtain higher yield of the fermentation product. The media culture has nutrients such as carbon, nitrogen, hydrogen, oxygen, phosphorus, sulphur, trace elements, vitamins, growth factors and metabolic precursors that helps to maintain cell growth and products generation. There are two type of media culture which is defined media that contain more certain amount of pure chemical compounds and composition whereas another is enriched media that consist of natural compounds that doesn't have certain chemical composition. Enriched media is more expensive than define media but provide a better control for fermentation process (Parrou et al. 1999; Huang et al. 2007).

Carbon source for cultivation process which will be the main material cost usually depend on the cultures. For the *K. lactis* can utilize cheap carbon source such as cheese whey that make them competent for industrial production (Huang et al. 2007). They are usually differs than *Saccharomyces cerevisiae* in context of carbon source metabolism is the absence of formation aerobic ethanol that make *K. lactis* become more benefitted in industrial scale due to the ability to produce higher yields in protein production (Breuniget al.2003). Genes contained in *K. lactis,* LAC4 and LAC12, able them to grow on lactose and galactose medium as carbon source. In order to generate ATP, *K. lactis* will utilize sugar that act as carbon source and the most preferred sugar usually is glucose but they can also grow in lactose, cellobiose, melezitose, xylitol, mannitol, lactate, succinate, butane-2,3-diol and several amino acids (Breunig et al.2003).The presence of nitrogen source will affect the metabolite production. There are two types of nitrogen source which are inorganic and complex nitrogen source. The nitrogen source includes ammonium sulphate, peptone, yeast extract and casein (Rao et al. 2007). Gethins et al.(2015) studied that the production of alcohols 2-phenylethanol and isoamyl alcohol on *Kluyveromyces marxianus*is the highest when using yeast extract.

The composition ratio of carbon and nitrogen source or dissolved oxygen (DO) will cause impact in the protein production, cell growth and fermentation product in *Kluyveromyces lactis.* As DO concentration give impact toward the density of cell cultivation, before the fed batch fermentation, the glucose and DO concentration will be optimized under the batch fermentation. Response Surface Methodology (RSM) is a statistical method that used to analyze the effect of initial glucose level and DO concentration toward the activity of recombinant protein production (Fuzi*et al.* 2014).There are few parameters can be examined by using RSM in order to optimize the production of *Kluyveromyces lactis.* Based on studies by You et al.(2017) that optimize the production of β-galactosidase three variables were used in five different level which are nitrogen source (YE), pH and temperature at -2,-1,0,+1,+2 which was made in six replicates. They used fed batch 7L fermented with pH control as the optimized production thus the fed batch strategy found it can easily being control and more applicable in scaling up the production.

5.4. Kinetics of cell growth

During the research one optimizing the culture medium, the kinetic cell growth will be examined. In stationary phase, the cell mass will gradually decrease along the time in very low rate until the end of cultivation time. Whereas, in exponential growth of cells, the quality inoculums is high, the production medium is rich with nutrients and the conditions of cultivation are at optimum state. Furthermore, at the cell growth phase, the concentration of glucose in the culture will be decrease gradually. Due to the acid production in the culture, the pH will be dropped and this phase can be seen during the exponential phase and lactic acid production phase (Elmarzugiet al.2010).

6. Probiotic yeast products developments

Yeasts are unicellular eukaryotic microorganisms which result in cooperation between genetic manipulation and polypeptide expressions of eukaryotic process (Gellissen et al.1997). They are able to grow in high cell densities with wide variety of carbon sources and perform variety post translational modifications, compartmentalize the reactions in the organelles, high secretions capacity and lack of the presence infectious agents. The main probiotic that commonly used is *Saccharomyces cerevisiae* which is due to the well annotated genome and historically dominated the arena of probiotic yeast in manufacturing of bioprocess engineering (Staniszewski et al. 2021). Despite of that, due to the work out done in synthetic biology research there are few non-conventional probiotic yeast taking part into the industries which are *Hansenula polymorpha (syn. Ogataea polymorpha), Kluyveromyces lactis, Pichia pastoris (syn. Komagataella pastoris),* and*Yarrowia lipolytica.* The new non-conventional yeast presents different advantages,

commonalities and diversity compared to *S. cerevisiae* that make them reliable in biotechnological studies (Wagner et al. 2016; Staniszewski et al. 2021).

6.1. Microencapsulation of the Microbial Cells

A coating process of particles is changing the diameter from a few nm to a few mm in which entrap one substance to another substance can also be defined as encapsulation and has been review to encapsulate wide range of products in the pharmaceuticals, volatile oils, plant extracts, enzymes and flavours industries (Rathore et al. 2013). The encapsulated substance usually being called core materials whereas the substance that encapsulating will be called coating substance. Moreover, there are several requirement that decide the most efficient coating process which are the encapsulation process, cost, stability while storage, legal or religious constraints and the efficacy of final product (Mokhtari et al. 2019). During probiotic development, the encapsulation of probiotic is necessary to ensure that the microbe can stay active and provide its growth process under harmful environment stress (Song et al. 2013). Furthermore, microencapsulation of probiotic can be used to improve the viability during the industrial processes for commercials products as well to ensure that it can retain stressful environment when it going through the gastrointestinal tract and also retain the shelf life of the probiotic under constant change of storage condition (Anal et al. 2007; Mokhtari et al.2019).

In certain functional foods that are demanding in the bioprocess industry microencapsulation result in stabilizing the functional foods and maintain their functional properties during processing and storage. Thus, the viability of this functional food will retain until it being consumed. The appropriate method to encapsulate the bioactive compounds usually chosen based on the component need to be encapsulated and the matrix that will protect the bioactive compound (Duboc et al.2001). This process also will ensure that the structure of bacteria will remain before degrading and dissolve in the intestine of gastrointestinal tract by immobilize the bacteria into polymer matrix (Cook et al.2012). Materials that usually used to encapsulate the probiotic are mainly based on naturally produced polysaccharides and proteins which are sugars, cyclodextrins, maltodextrins, modified starches, gums, proteins or liposomes whereas the technology that has been used to encapsulate are spray drying, freeze drying, fluidized bed coating, extrusion, emulsification, coacervation and electrostatic methods (Mokhtari et al.2019) due to their features as lack of cytotoxicity which can not harm the bacteria and host, safe biodegradation and able to perform function without usage of organic solvents in preparing the microcapsules (Cook et al. 2012). The usage of polysaccharides in coating is due to mechanical properties which serve as good oxygen, odour and oil barriers but the moisture permeability reduces their capability because of their hydrophilic properties. The combination of polysaccharides and protein need the presence of plasticizers which act as film forming materials that reduce the glass transition temperature. Thus improve the flexibility of the film. The common use plasticizers are glycerol,sorbitol,sucrose and polyethene glycol. Compared from polysaccharides that are more moisturize, protein are poor water resistance but they have better character in mechanical and barrier (Pavli et al. 2018). However, the important part in microencapsulation is selecting the suitable encapsulation materials. It must be chemically compatible, unreactive polymers with the presence of encapsulating component with coating properties based on the strength, permeability, stability and flexibility (Castro-Rosas et al.2017).

6.2. Coating material for probiotic encapsulation

Microencapsulation known as a procedure that ensure the bioactive materials coated with the protection mixtures to prevent the exposition of core materials from the environmental stresses such as oxygen, high acidity, gastric condition that will affect their survivability (Vidhyalakshmi et al. 2009; Arslan et al.2015).

Different type of material being used in encapsulating the cells is needed in order to scale up the viability cells and is usually depend on the microcapsule functionality and the technique that will be used in the encapsulating process. The materials that involve in this process in order to encapsulate the cells are alginate, cellulose, acetate phthalate, Arabic gum, k-carrageenan, modified starch, chitosan, gellan, xanthan and animal proteins such as milk and gelatine (Arslan et al.2015).

Alginate is one of material that use in microencapsulation which made from extraction of brown sea weeds that improve the viscosity and ability to bind with water that will reduce syneresis in stirred yogurts (Huq et al. 2013).The probiotic encapsulation using alginate is commonly used due to the biocompatibility, cost-effectiveness, simplicity, non-toxic and easy to digest but the usage is limited due to low physical disability and anti-gelling cations presence. At low pH, alginate molecules will be degrade by cross linked alginate materials, reduce the molecular weight and cause the release of entrapped active materials become faster. There are few researchers found that the encapsulated cells with alginate can retain the acidic stress condition make them survive in stomach and pursue their journey to the intestine. Probiotic survival in gastric conditions was found in the combination of 3% sodium alginate, 1% pancreatic digested casein and 3% sodium alginate(Vidhyalakshmi et al. 2009).The N-deacetylated product from polysaccharide chitin known as chitosan is one of important coating material in the industry which being isolated from the shells of crustacean, insect cuticles and the membrane of fungi. They composed of β (1,-4)-linked glucosamine unites along with the part of *N*-acetyl glucosamine units. It was found that this material

able to protect the probiotic from harsh environment such as acidic pH and maintain the stability of cells during storage at 4 and 22°C. the morphology and the particle size will be influenced by the stirring rate, gelling agent level, temperature, concentration of surfactant polymer and the viscosity in every phase (Iravani et al.2015).

6.3.Encapsulation techniques

Encapsulation technique mainly can be done chemically or mechanically. The chemical encapsulation composed of simple and complex coacervation while mechanical encapsulation composed of spray drying, extrusion, freeze drying or fluidized bed (Castro-Rosas et al. 2017).One of the techniques in microencapsulation process is drying method. This method will dry the encapsulate mixture until cell powders and granules form (Huq et al.2013).The manufacturing process in industry, it was known that spray drying usually been used in food industry for the encapsulation process by transferring heat and mass simultaneously from air to atomized droplets. On top of that, it is a low-cost process that is a single unit process for particle formation and drying. Due to the high temperature used in this process, spray drying gives detrimental effect to the cells and decrease the survival amount of microorganism (Arslan et al. 2015).

CONCLUSION

Kluyveromyces lactis is emerging probiotic yeast that can be used in the food industry, in biotherapy or as a cell factory for the production of proteins. It is well suited to industrial processes, however an optimization of the fermentation process, from the strain, to the fermentation stage (conditions and medium of culture) and product development is necessary in order to increase the yield and ensure a process sustainability. Therefore, this yeast will not only high potential application when used as living cells (as probiotic) or when used as biofactory of bioactive metabolites (enzymes), but also is considered as one of the high potentials biofactory for recombinant high value protein production in biopharmaceutical industries beside the traditionally used yeast biofactories such as *Saccharomyces cerevisiae,* and *Pichia pastoris,* and *Yarrowia lipolytica.*

CONFLICT OF INTEREST

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

ACKNOWLEDGEMENT

The authors would like to express their gratitude to UniversitiTeknologi Malaysia (UTM) for Contract research grant number R.J130000.7609.4C463 and R.J130000.7609.4C359 that have supported this research.

AUTHOR CONTRIBUTIONS

AI, AZ, DJ, SZ, HE involved in data collection, writing the manuscript and designed the work. IC, DS, HE reviewed the manuscript. All authors read and approved the final version.

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