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Review

Kluyveromyces marxianus: An emerging yeast cell factory for applications in food and biotechnology

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ABSTRACT

Several yeasts, which are eukaryotic microorganisms, have long been used in different industries due to their potential applications, both for fermentation and for the production of specific metabolites. *Kluyveromyces marxianus* is one of the most auspicious nonconventional yeasts, generally isolated from wide-ranging natural habitats such as fermented traditional dairy products, kefir grain, sewage from sugar industries, sisal leaves, and plants. This is a food-grade yeast with various beneficial traits, such as rapid growth rate and thermotolerance that make it appealing for different industrial food and biotechnological applications. *K. marxianus* is a respiro-fermentative yeast likely to produce energy by either respiration or fermentation pathways. It generates a wide-ranging specific metabolites and could contribute to a variety of different food and biotechnological industries. Although *Saccharomyces cerevisiae* is the most widely used dominant representative in all aspects, many applications of *K. marxianus* in biotechnology, food and environment have only started to emerge nowadays; some of the most promising applications are reviewed here. The general physiology of *K. marxianus* is outlined, and then the different applications are discussed: first, the applications of *K. marxianus* in biotechnology, and then the recent advances and possible applications in food, feed and environmental industries. Finally, this review provides a discussion of the main challenges and some perspectives for targeted applications of *K. marxianus* in the modern food technology and applied biotechnology in order to exploit the full potential of this yeast which can be used as a cell factory with great efficiency.

1. Introduction

Yeasts, a heterogeneous group of eukaryotic fungi that mostly exists as a unicellular organism, has had diversified applications and spectacular impact on industrial, biotechnological, medial and environmental arena from the rise of ancient civilizations until today. Yeasts seem to have many advantageous phenotypes, such as high secretion capacity, high growth rate on a wide variety of carbon sources, potential to compartmentalize reactions in organelles, ability to carry out many post-translational modifications, easy to cultivate in small vessels or large bioreactors, easy product purification, and an absence of susceptibility to infectious agents like bacteriophages. Those attractive traits make them specially suitable for extensive applications in different sectors (Wagner and Alper, 2016). Consequently, the number of the described yeast species is increasing every year; however, biotechnological or industrial applications are limited to a small number of species, mostly belonging to *Saccharomyces cerevisiae* (*S. cerevisiae*) and its associated synonymous species (e.g., *S. uvarum*, and *S. carlsbergensis*), *Candida utilis* (*C. utilis*) and its sexual form (*Hansenula jadinii*), *Kluyveromyces marxianus* (*K. marxianus*) including its asexual forms (e.g., *K. fragilis*, *K. bulgaricus*, *C. pseudotropicalis*, and *C. Kefyr*), *Yarrowia lipolytica* (*Y. lipolytica*), and *Pichia pastoris* (Lane and Morrissey, 2010; Wagner and Alper, 2016). Among them, *S. cerevisiae* has a dominating position, as it is a widely employed and mostly used microbe in the field of biotechnology, due to its availability, genetic tractability, well-annotated genome, well-known physiology, and overall ease of use. Furthermore, this model yeast has traditionally occupied the field of modified yeasts for industrial processing (Wagner and Alper, 2016). Other than that of conventional yeast, there are various non-conventional yeast species with beneficial characteristics for industrial bioprocesses application, some of which are also getting importance, which includes *Y. lipolytica*, *P. pastoris*, *K. lactis*, and *H. polymorpha* (Gellissen et al., 2005; Wagner and Alper, 2016). Among the non-conventional yeasts, the species of the genus *Kluyveromyces* were observed to have potential for many industrial applications. Particularly, *K. lactis* was the first species after *S. cerevisiae*, to be given Generally

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Regarded As Safe (GRAS) status (Bonekamp and Oosterom, 1994), a crucial precondition to be used for biotechnical applications.

Recently, the yeast *K. marxianus*, a sister species of *K. lactis*, has piqued particular research interest, due to its beneficial traits that render it exceptionally suitable industrial application. These features mainly include the ability to utilize a broad range of sugars including lactose and inulin, thermotolerance, secretion of lytic enzymes, the highest growth rate than other eukaryotes, and the production of fuel ethanol by fermentation (Lane and Morrissey, 2010; Varela et al., 2017). *K. lactis* has been the prevalent research species within the general of *Kluyveromyces*, primarily for the studies on lactose metabolism, but later as a model for nonconventional yeasts (Fukuhara, 2006; Spohner et al., 2016). However, in contrast to *K. lactis*, *K. marxianus* has widely been adopted by industries, mainly because it owns some special features of industrial interest that are not present in *K. lactis* (Lane et al., 2011; Lane and Morrissey, 2010) such as its ability to produce inulinase enzyme to hydrolyze complex plant fructans (Arrizon et al., 2011). Moreover, it has a rapid growth compared to *K. lactis*, even at high temperatures (> 40 °C) (Rouwenhorst et al., 1988); however, the underlying reasons for the faster growth, remained to be determined (Blank et al., 2005). In addition, it has also achieved Qualified Presumption of Safely (QPS) and GRAS status in European Union and United States respectively, due to its long history in safe association and use with regular dairy products; which makes it particularly suitable to produce pharmaceuticals and food-grade proteins.

In contrast to *S. cerevisiae,* the majority of the strains of *K. marxianus* are apparently Crabtree-negative or an aerobic-respiring characteristic and as such, do not undergo aerobic alcoholic fermentation. This can be a beneficial phenotype for industrial production of those compounds whose titer are linked to biomass formation (i.e., biomass-directed applications) since ethanol formation as a toxic or unintended byproducts under aerobic condition could be avoided (Wagner and Alper, 2016). Moreover, *K. marxianus* can metabolize variety of low-cost substrates including cheese whey or other dairy wastes due to its unique physiological characteristics and capability of producing heterologous proteins as a diligent host. This capacity makes it an indispensable candidate for commercial applications in the pharmaceutical, food, and feed industries (Löser et al., 2013; Morrissey et al., 2015). However, *K. marxianus* has not been studied at the same extent as *K. lactis*, in spite of its extensive biotechnological applications including the bioethanol production from cheese whey, biomass accumulation, valuable enzyme production such as inulinase and *β*-galactosidase, and so on (Lane and Morrissey, 2010).

Increasing consumer demand for biologically synthesized molecules to be used in the foods and other products, creates a unique opportunity of exploiting the potential of *K. marxianus* in the food and environmental biotechnology applications. The main setback to its advancement has been limited fundamental knowledge of its physiology and genetics, however, this scenario is changing quickly (Morrissey et al., 2015). Most of the improvements have centered on the optimization of growth conditions and the fermentation processes. Further development of new strains by applying evolutionary or rational engineering approaches is still required. Consequently, the possibility of exploiting *K. marxianus* for diversified applications and the necessity for further development of its traits for biotechnological production of different biologically synthesized products has motivated researchers to study its physiology, metabolism mechanism and genomic sequences in more details.

In this review, we set out to explore the possible applications and future potential of *K. marxianus* in biotechnology, food industry and environmental engineering. We begin our review with a short discussion of the physiology, followed by an outline on current applications including its future application strategies and challenges. Thus, it will provide a broad insight into mechanism and limitations of current applications, and the challenges for further development in future application strategies. After all, the comprehensive understanding of its diversified applications will underpin future developments in physiology, genetics, evolutionary engineering strategies, and other important molecular tools of *K. marxianus*.

2. Taxonomy and physiology of *K. marxianus*

K. marxianus was first identified as yeast belonging to the genus *Saccharomyces* and was named as *S. marxianus*. This microorganism was isolated from beer wort and from grape. The species was then transferred to the genus *Kluyveromyces* and since then, 45 species have been classified to this genus. The closest relative of *K. marxianus* is the yeast *Kluyveromyces lactis*, which is used in the dairy industry for the production of fermented milk such as kefir and kumis. Moreover, both *Kluyveromyces* and *Saccharomyces* are considered as a part of the *Sacchromyces* complex, subclade of the Saccharomycetes. By using the 18S rRNA gene sequencing technique, it was suggested that *K. marxianus*, *K. aestuarii*, *K. dobzhanskii*, *K. lactic*, *K. wickerhamii*, *K. blattae*, *K. thermotolerans*, and *K. waltii* collectively constituted a distinct clade of separate ancestry from the central clade in the genus *Kluyveromyces*. Within this complex, two categories are defined based on the presence in certain taxa of a whole-genome duplication event: the two clades are referred to as pre-Whole Genome Duplication (WGD) and post-WGD. *Kluyveromyces* species are affiliated with the first of this clades while species of *Saccharomyces* belong to the latter. Separation of these clades based on the presence of the WGD event explains why, even though the two species are closely related, fundamental differences exist between them (Lane et al., 2011; Lane and Morrissey, 2010).

K. marxianus is well known due to the frequent connection with traditional dairy products like fermented milk, kefir, yoghurt, cheese and so on (Coloretti et al., 2017). It is a dairy yeast that has also been previously described by synonyms in scientific literature, notably *K. fragilis* and *S. keyfr* (Morrissey et al., 2015; Reed, 2012). *Kluyveromyces* is a genus within hemiascomycetous yeasts and contains six described species abide by the reclassification into monophyletic genera based on 26S rDNA sequence, from which two species, *K. lactis* and *K. marxianus* are very prominent (Lachance, 2007). The main popular traits of *K. marxianus* and *K. lactis* is their ability to assimilate lactose and to utilize this sugar as carbon source. This is one of the most important features that distinguish them from *S. cerevisiae*, which lacks this phenotype (Lane and Morrissey, 2010). As a result, they are considered as lactose positive yeast and they do carry *LAC12-LAC4* gene pair that is accountable for uptake and subsequent cleavage of lactose into galactose and glucose (Vigneault et al., 2007). Although lactose utilization is one of the long considered distinguishing traits of *K. marxianus*; however, it was interesting that most, but not all, stains of *K. marxianus* were reported to consume lactose efficiently. This is due to the polymorphisms in the *LAC12* gene, that is responsible for encoding a permease to transport lactose molecules into the cell (Varela et al., 2017). Generally, the *LAC12* encodes a lactose permease which is essential to uptake lactose into cell, while *LAC4* encodes a β-galactosidase, which is responsible for hydrolyzing lactose to the monomers, galactose and glucose. Although the evolutionary history of *LAC12-LAC4* gene pair is not clear enough, their regulation is well-incorporated with the *Gal4p/Gal80p* system, which is comprehensively explored in *S. cerevisiae* (Lane and Morrissey, 2010). The important features of *K. marxianus* have been compared with *K. lactis* and *S. cerevisiae* as presented in Table 1.

K. marxianus is a yeast whose metabolism is of the respiratory-fermentative type, similar to that of *S. cerevisiae* which is widely used in brewery and bread making. Thus, *K. marxianus* produces energy by the TCA cycle following oxidative phosphorylation, as well as by the fermentation of sugars into ethanol. In reference to *S. cerevisiae*, it strongly follows the pathway towards fermentation in high sugar concentration even under aerobic conditions, implying that the cell preferentially directs pyruvate to the production of ethanol. This phenomenon is called Crabtree effect. Because of the strong 'Crabtree-positive' characteristics of *S. cerevisiae*, it usually needs a regulated supply of the

Table 1
The important physiological characteristics of *K. marxianus* in comparison with *K. lactis* and *S. cerevisiae* as model yeasts.

Features	***S. cerevisiae***	*K. lactis*	*K. marxianus*	References
Energy metabolism	Alcoholic fermentation (Crabtree-positive)	Respiration (Crabtree-negative)	Respiro-fermentative, Respiration [a] & fermentation [b] (Crabtree-negative)	(Lane and Morrissey, 2010; Madeira-Jr and Gombert, 2018; Rodrussamee et al., 2011)
Generation time [c]	80 min	80 min	70 min	(Rodicio and Heinisch, 2013)
Glucose utilization route	Mostly glycolysis	Pentose phosphate (PP) pathway and glycolysis	-Embden–Meyerhof–Parnas pathway (EMP) -Preferentially, energy generation either via direct metabolism towards TCA cycle by oxidative phosphorylation or by fermentation to ethanol -Capable of carrying out simultaneous fermentation and respiration	(Blank et al., 2005; Lane and Morrissey, 2010; Rodicio and Heinisch, 2013)
Sensibility to glucose repression	High sensibility	Low sensibility	Low sensibility, since the fermentative and oxidative metabolism can occur simultaneously. This trait is variable across *K. marxianus* strains.	(Lane et al., 2011; Silveira et al., 2005)
Genome structure and Ploidy	Haploid, diploid or/even polyploid	Mainly haploid and can mate to form an unstable diploid which reverts to the haplo-phase immediately	Less clear yet but both haploid and diploid lifestyle have been reported. Triploidy has been identified in *K. marxianus* .	(Lane and Morrissey, 2010; Ortiz-Merino et al., 2018; Rodicio and Heinisch, 2013)
Ploidy (Haploid lifestyle)	Stable and readily mate to form diploid (*MAT*a/*MATα*)	Stable (*MAT*a or *MATα*)	Stable (*MAT*a or *MATα*)	(Lane and Morrissey, 2010; Rodicio and Heinisch, 2013)
Ploidy (Diploid lifestyle)	Stable and easily be induced to undergo meiosis to reform haploid progeny	Semi-stable	Stable	(Lane and Morrissey, 2010; Rodicio and Heinisch, 2013)
Mendelian genetics (crossing and tetrad analysis)	Yes	Yes	Yes	(Cernak et al., 2018; Lane and Morrissey, 2010; Rodicio and Heinisch, 2013; Yarimizu et al., 2013)
Vectors, selection and transformation classification	Available	Available	Available	(Lane and Morrissey, 2010; Nurcholis et al., 2020; Rodicio and Heinisch, 2013)
Homologous recombination	Excellent	Good [d]	Generally considered as poor. Some studies referred as good.	(Choo et al., 2014; Lane and Morrissey, 2010; Rodicio and Heinisch, 2013)
Genome sequence	Available (post-WGD)	Available (no-WGD) [e]	Available (pre-WGD)	(Inokuma et al., 2015; Jeong et al., 2012; Lane and Morrissey, 2010; Lertwattanasakul et al., 2015; Rodicio and Heinisch, 2013)
Deletions of ORE	Completely collected by Berkeley, EUROSCARE	Partial and scattered[f]	Partial, scattered, though some heterogenous protein generation systems become available	(Lane and Morrissey, 2010; Rodicio and Heinisch, 2013; Rodrussamee et al., 2011)
Beneficial Phenotype	-High bioethanol production -High homogenous recombination capacity -Established physiology and genomics -Advanced synthetic biology tools	-Ability to grow on lactose -Capacity of high protein secretion	-Ability to grow on wide-range of sugars -Faster growth capability -Thermotolerance -High ethyl acetate production -Capacity of protein secretion	(Löbs et al., 2017; Varela et al., 2017)

[a] Energy release (respiration): $C_{12}H_{22}O_{11} + H_2O + 12O_2 \overset{Yeast}{\rightarrow} 12CO_2 + 12H_2O + \Delta E_1$
[b] Energy release (fermentation): $C_{12}H_{22}O_{11} + H_2O \overset{Yeast}{\rightarrow} 4C_2H_5OH + 4CO_2 + \Delta E_2$.
[c] Approximate generation times for laboratory with a standard deviation of ± 10 min at 30 °C.
[d] Homologous recombination could be upgraded using a *Klku80* deletion background.
[e] Whole Genome Duplication (WGD) event could not happen in *K. lactis* resulting in less redundancy of genes encoding iso-enzymes.
[f] Very limited collection of deletion mutants and usually each group working on a special pathway disposes of its own.

carbon source to evade fermentative metabolism, which is extremely unwelcomed in biomass-directed applications. By contrast, *K. marxianus* and *K. lactis* are categorized as Crabtree-negative by tradition, meaning an incompetence to efficiently ferment sugars to ethanol (Fonseca et al., 2008; Lane et al., 2011). In this context, it is worth mentioning that that there are some contradictory reports in the literature of the 'Crabtree status' of *K. marxianus* and *K. lactis* (Merico et al., 2009). The prevalent reports of application in ethanol production suggest that both species do carry the genes required for ethanol production and could adopt the fermentation life style under certain conditions (Merico et al., 2007). However, it has been demonstrated that *K. marxianus* exhibits a strong 'Crabtree-negative' property and this was supported by the fact that it does not produce ethanol, contrary to what is largely observed in the case of *S. cerevisiae* known to be a big ethanol producer or *K. lactis* to a lesser extent (Merico et al., 2009; Merico et al., 2007). This apparent conflicting finding is probably due to the strain variability, as most of the yeast comparative studies utilized only one representative strain of each species and the level of physiological variation within a species was not evaluated. It can be concluded that a high degree of intra-species disparity exists for this yeast, not only in terms of its genetics, but also of its physiology (Lane et al., 2011).

3. Applications in biotechnology

3.1. Native enzymes production

K. marxianus is considered as a favorable host for producing extracellular proteins due to its capacity to grow on various low-cost substates such as spent sulphite liquor, molasses, and whey (Fonseca et al., 2008; Hensing et al., 1994). Moreover, *K. marxianus* is capable to grow on several polysaccharides including pectin, and inulin (Hensing et al., 1994). *K. marxianus* possess natural ability to secrete enzymes, since all these aforementioned complex materials are hydrolyzed extracellularly to monomers (Chi et al., 2011). The growth of *K. marxianus* on feedstocks such as sucrose and inulin proceeds through the activity of extracellular enzymes, particularly inulinase (*β-1,2-D*-fructan fructanohydrolase) which is excreted in the culture medium but also retained in the cell wall (Rouwenhorst et al., 1988). The enzyme that retained in the cell wall is a tetramer whereas the secreted enzyme in the culture fluid is a dimer (Hensing et al., 1994). The latter portion comprises more than 60% of total excreted proteins in the culture fluid for certain *K. marxianus* strains, indicating that the inulinase is expressed from a strong promoter and its secretion is instigated by an effective signal sequence (Zhou et al., 2018). Furthermore, lactose degradation by excreting *β*-galactosidases is very promising due to the simultaneous production of bioingredients, biomass, and enzymes of industrial interest (Chi et al., 2011; Fonseca et al., 2008). The schematic representations in Fig. 1 show the uptake mechanism and metabolism in the yeast cells. The enzyme expressing efficiency of different *K. marxianus* strains is summarized in Table 2 and described below.

3.1.1. Inulinase production

Inulinase has gained intertest in recent time, as it is widely used to hydrolyze inulin (Jerusalem artichoke, chicory roots, dahlia tubers) to produce bioethanol, fructose, and fructo-oligosaccharides, all of which are important ingredients in food and pharmaceutical industry (Gao et al., 2009; Hoshida et al., 2018). The enzymes, inulinases (EC 3.2.1.7) have been produced from different microbes such as yeast *Cryptococcus aureus* (Sheng et al., 2007), *K. marxianus* (Selvakumar and Pandey, 1999), filamentous fungi *Aspergillus niger*, *Aspergillus fumigatus* (Kango, 2008), *Penicillium* sp. (Moriyama and Ohta, 2007), *Rhizopus* sp. (Ohta et al., 2002), and bacteria *Streptomyces* sp., *Bacillus* sp. (Gao et al., 2009), *Staphylococcus* sp. (Selvakumar and Pandey, 1999), *Xanthomonas*, and *Pseudomonas* (Kalil et al., 2005). However, the strains of *A. niger* and *K. marxianus*, have been described as the most promising microbes among the diverse kinds of microbial strains for inulinase production (Zhang et al., 2012).

Nevertheless, *K. marxianus* is considered as the most potential to produce inulinase enzyme at a commercially acceptable yields (Kalil et al., 2005; Zhou et al., 2014). A study with fourteen yeast strains of some genera like *Kluyveromyces*, *Debaryomyces*, *Candida*, and *Schizosaccharomyces* revealed that the strain *K. marxianus* ATCC 36907 possess high potential to produce inulinase. The enzymes produced by this microbe were shown to be stable for 60 min at low pH (4.0) and high temperature (45 °C) (Passador-Gurgel et al., 1996). In another study, *K. marxianus* CBS 6556 exhibited superior properties than other strains in terms of high temperature, substrate specificity, and inulinase production (Rouwenhorst et al., 1988). The highest enzyme production was encountered at temperatures between 37 and 42 °C, corresponded to the optimal temperature for growth of *K. marxianus*. The high temperature for the optimum growth is particularly interesting for commercial application as this enables cooling during large-scale fermentation process where heat transfer is a limiting factor. In a recent study, higher inulinase production on xylose medium was observed with higher agitation rate in the culture media using *K. marxianus* DMKU3–1042, indicating that the oxygen supply affects the inulinase production (Hoshida et al., 2018). However, this effect of higher agitation on inulinase production probably specific for xylose medium because the inulinase production by *K. marxianus* var. *bulgaricus* ATCC16045 did not enhance by increased oxygen supply in sucrose medium (Silva-Santisteban et al., 2009). Nevertheless, Singh and Bhermi (2008a) observed that a higher agitation and aeration decreased the inulinase production by *K. marxianus* YS-1 using inulin as carbon source (Singh and Bhermi, 2008b).

Apart from that the inoculum size is also important in fermentation process to produce inulin because a lower inoculum size may result in an insufficient biomass production and permit the growth of unexpected organisms while a higher inoculum density can produce too much biomass and reduce the nutrients required for production formation (Selvakumar and Pandey, 1999). Selvakumar and Pandey (1999) observed that the inulinase production by *K. marxianus* reached a maximum value of 116.43 U/gds with a 4% inoculum size at 72 h. Cruz-Guerrero et al. (1995) reported that *K. marxianus* CDBB-L-278 was a hyperproducing strain for inulinase. This strain demonstrated a high resistance to 2-deoxyglucose, however, inulinase production was under catabolic repression. The catabolic repression was created when glucose or fructose were enhanced from 0.25 to 1% in the medium. Furthermore, a batch fermentation with a 4% inulin and 4% glucose confirmed that the stain CDBB-L-278 was under catabolic repression because inulinase was not produced until the level of glucose were low enough (Cruz-Guerrero et al., 1995). Kushi et al. (2000) achieved the highest yield of inulinase with sucrose as substrate in carbon and energy-limited continuous cultures. Nevertheless, the lower enzyme activities were observed with higher the higher concentrations of residual substrate, indicating that the enzyme synthesis is regulated by catabolic repression/residual sugar concentration in the medium (Kushi et al., 2000; Rouwenhorst et al., 1988). It is possible, however, to produce pyruvate and acetate while *K. marxianus* subjected to excess sugar. This tendency may be a problem during large-scale fermentation because of the presence of sugar gradients in the reactor (Rouwenhorst et al., 1988). The distribution of enzyme is also influenced by the growth parameters. Indeed, it has been reported that important content of enzyme in the growth medium after centrifugation with a concomitant decrease in the amount of cell wall enzyme was obtained when growth (production) temperatures below the optimal temperature interval were used. The inverse situation was observed when growth temperatures higher than the optimal values were used. Also, it has been anticipated that growing of *K. marxianus* at higher pH values can contribute to enhance the relative amount of enzyme excreted into the culture medium. This was explained by the fact that the increased pH stimulated the process of inulinase release from the yeast cell wall (Rouwenhorst et al., 1988). Moreover, carbon-limited growth on

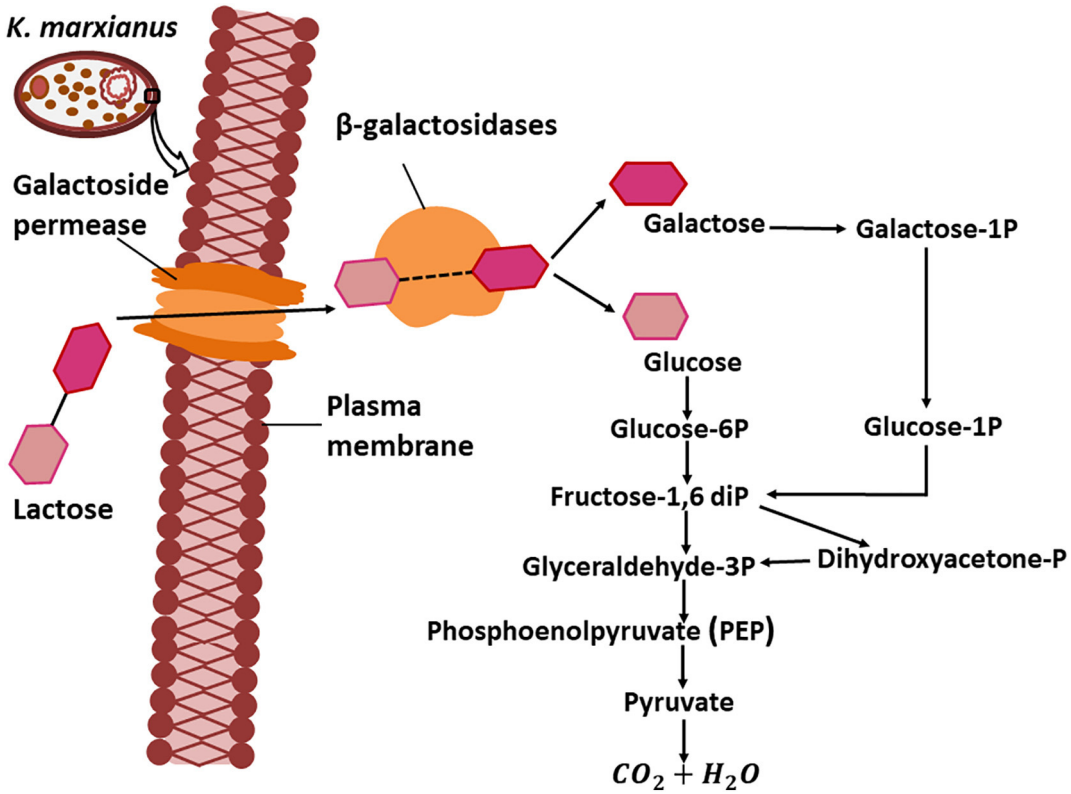

Fig. 1. Schematic diagram of the catabolic pathway of lactose and galactose by *K. marxianus*.

glucose or lactose or nitrogen-limited growth on sucrose resulted in the higher percentages of inulinase production in the culture medium and low amounts of enzyme in the cell wall (Rouwenhorst et al., 1988).

The inulinase production from *Staphylococcus* sp. RRL-1 and *K. marxianus* ATCC 52466 was investigate by Selvakumar and Pandey (1999) using agro-industrial wastes such as rice bran, wheat bran, corn flour, and coconut oil cake via solid-state fermentation. It was found that bacterial culture took a relatively shorter time (48 h) than the yeast culture (72 h) to attain the maximal yield (90.53 U/gds), however, the yeast culture produced comparatively higher yield (106.37 U/gds) of enzyme at 37 °C (Selvakumar and Pandey, 1999). Furthermore, *K. marxianus* var. *bulgaricus* ATCC16045 produced higher amounts of inulinase while grown on glucose, sucrose, fructose, inulin, and raffinose as carbon sources (Kushi et al., 2000). Nevertheless, they observed that the inulinase activity was significantly higher for the inulin from Dahlia tubers than that of other carbon sources. Similarly, Gao et al. (2009) reported that the inulin was the best carbon source (inulin > lactose > fructose > mannitol > glucose > sucrose) to produce inulinase using *Bacillus smithii T7*, *K. marxianus*, *Cryptococcus aureus G7a*; which indicated that the inulinase is an inducible enzyme. They also demonstrated that the enzyme activity was increased (69.5 IU/mL) with increase of inulin concentration up to 2% and then decreased in activity was noticed. Furthermore, the inulinase production was enhanced to 104.3 IU/mL by using $(NH_4)H_2PO_4$ up to 0.5% (w/v) as nitrogen source, however, the greater concentration of $(NH_4)H_2PO_4$ had an inhibitory effect on inulinase synthesis (Gao et al., 2009).

Although it has been reported by several authors (Gao et al., 2009; Grootwassink and Hewitt, 1983; Parekh and Margaritis, 1986) that the production of inulinase was inducible, but the inulinase production was partially constitutive for the strains *K. marxianus* CDBB-L-278 and *K. marxianus* NCYC-1429, since the inulinase (up to 20% of the total activity attained with inulin) was produced using glycerol as carbon source without inulin, which does not present neither induction nor catabolite repression (Cruz-Guerrero et al., 1995). Glucose and fructose, on the other hand, acted as both inducers and repressors (Gao et al., 2009; Grootwassink and Hewitt, 1983; Parekh and Margaritis, 1986). It has been generally known that the expression of most of the inulinase genes in a native producer was repressed by glucose and fructose and induced by inulin and sucrose (Liu et al., 2013). The inulinase activity by *K. marxianus* KM-0 was increased when glucose concentration increased to 20 g/L from 10 g/L, however, the activity was decreased for further increment of glucose to 80 m/L (Zhou et al., 2014). This attributed to the fact that the biosynthesis and secretion of inulinase in this strain was, indeed, repressed by glucose. The transcriptional repressor *Mig1* encoded by *MIG1* played as a central component for glucose repression. However, *K. marxianus* KM-69 with the disrupted *MIG1* gene, produced higher inulinase (101.7 U/mL) compared to the wild type strain *K. marxianus* KM-0 (84.3 U/mL) (Zhou et al., 2014). They also showed that the overexpression of the native inulinase gene from the wild type strain KM-0 into the disruptant KM-69 could further enhance the inulinase activity to 119.3 U/mL using a recombinant strain, namely *K. marxianus* KM-526 (Zhou et al., 2014). The secretory expression of inulinase enzyme in *K. marxianus* could be further improved by increasing the efficiency of the inulinase-encoding gene (*INU1*) promoter and signal sequence engineering (Zhou et al., 2018). The lower Michaelis–Menten constant (Km) value (3.04 mM) of the purified inulinase from *K. marxianus* CDBB-L-278 (Cruz-Guerrero et al., 1995) compared to inulinases from other microorganisms, including *C. aureus* G7a (20.06 mg/mL) (Sheng et al., 2008), *B. subtilis* 430A (8 mM) (Uzunova et al., 2001), and *B. smithii* T7 (4.17 mM) (Gao et al., 2009), could make *K. marxianus* a better candidate for inulin hydrolysis. Moreover, the enzymes from *K. marxianus* have a good thermal stability up to 50–55 °C (Cruz-Guerrero et al., 1995; Kushi et al., 2000). Therefore, the inulinases produced by *K. marxianus* could be broadly used in biotechnology, pharmaceutical, food, feed, chemical and biofuel industries (Chi et al., 2011).

Table 2
The efficacy of different *K. marxianus* strains with enzyme expressing activity.

Native enzymes	Strains	Cultivation medium	Specific enzyme activity [a]	References
Inulinase	*K. marxianus* YS-1	Liquid culture	55.40	(Singh et al., 2007)
	K. marxianus ATCC 16045	Liquid culture	121	(Silva-Santisteban and Maugeri Filho, 2005)
	Kluyveromyces sp. Y-85	Liquid culture	59.50	(Xiong et al., 2007)
	K. marxianus var. *bulgaricus* ATCC 16045	Continuous cultivation	107	(Kushi et al., 2000)
	K. marxianus NRRL Y-7571	Solid-state fermentation	391.9 U/ g of dry fermented bagasse	(Bender et al., 2006)
	K. marxianus NRRL Y-7571	Liquid culture	8.87 U g_{ids}^{-1} h^{-1}	(Mazutti et al., 2006)
	K. marxianus S120	Solid-state fermentation	409.8 U g_{ids}^{-1}	(Xiong et al., 2007)
	K. marxianus NRRL Y-7571	Solid-sate fermentation	436.7 ± 36.3 U g_{ids}^{-1}	(Mazutti et al., 2010)
	K. marxianus CBS 6556	Liquid culture	52 U/ mg cell dry weight	(Rouwenhorst et al., 1988)
	K. marxianus ATCC 52466	Solid-sate fermentation	122.88 U/ g of dry fermented substrate	(Selvakumar and Pandey, 1999)
	K. marxianus CBS 6556	Liquid culture (Fed-batch)	> 2 g/ L	(Hensing et al., 1994)
	K. marxianus NRRL Y-7571	Solid-sate fermentation (Fixed-bed bioreactor)	219 U/mg	(Golunski et al., 2011)
	K. marxianus CBS 6556	Liquid culture	2714 U/mg	(Zhang et al., 2012)
	K. marxianus KM-526	Liquid culture	133.5	(Zhou et al., 2014)
	K. marxianus DMKU 3–1042	Liquid culture	330	(Hoshida et al., 2018)
	K. marxianus YS-1	Liquid culture	420 IU/mg	(Singh et al., 2017)
β- Galactosidase	*K. marxianus* KM-15	Liquid culture	121	(Zhou et al., 2013)
	K. marxianus CBS 6556	Liquid culture	1400 U/OD_{600}	(Martins et al., 2002)
	K. marxianus CBS 712	Liquid culture	333.8 U_{ONPG}/g lactose	(Rech et al., 1999)
	K. marxianus CBS 6556	Liquid culture	129.7 U_{ONPG}/g lactose	(Rech et al., 1999)
	K. marxianus NRRL Y-1109	Liquid culture	2800 ± 250 U/g, 32,700 ± 2000 U/L	(Cortés et al., 2005)
	K. marxianus MTCC 1388	Liquid culture	1.14 U/mg	(Bansal et al., 2008)
	K. marxianus CCT 7082	Liquid culture	463 U/g	(Manera et al., 2011)
	K. marxianus ATCC 16045	Submerged cultivation	10.4	(Braga et al., 2012)
β- Xylosidase	*K. marxianus*	Liquid culture	333 IU/g cells on xylose	(Rajoka, 2007)
Pectinase	*K. marxianus* CCT 3172	Liquid culture	21.69 μmol galacturonic acid/μg protein/ min	(Schwan et al., 1997)
	K. marxianus P 5656	Liquid culture	0.78	(Harsa et al., 1993)
Lipolytic enzyme	*K. marxianus* CECT 1018	Liquid culture	80	(Deive et al., 2003)
Endo-poly-galacturonase	*K. marxianus* CCT 3172	Continuous production packed bed reactor (PBR)	7.82	(Almeida et al., 2003).
	K. marxianus CCT 3172	Continuous stirred tank reactor (CSTR)	1.01	(Almeida et al., 2003).
	K. marxianus ATCC 36907	Liquid culture	1 UE/mg	(Dinnella et al., 1996)
Polygalacturonase	*K. marxianus* 166	Liquid culture	14.2 μmol of galacturonic acid/μg protein/ min	(da Silva et al., 2005)
Protein phosphatases	*K. marxianus* (strain not indicated)	Liquid culture	437.62 nmol/min/mg	(Jolivet et al., 2001)
Carboxypeptidases	*K. marxianus* (own isolate)	Liquid culture	5.43 U/mg	(Ramírez-Zavala et al., 2004)
Aminopeptidases	*K. marxianus* (own isolate)	Liquid culture	22.53 U/mg	(Ramírez-Zavala et al., 2004)
Endo-*β*-1,4-glucanase	*K. marxianus* NBRC 1777	Liquid culture	1.6	(Hong et al., 2007)
β-glucosidase	*K. marxianus* NBRC 1777	Liquid culture	83 m U/mL	(Hong et al., 2007)
Cellobiohydrolase	*K. marxianus* NBRC 1777	Liquid culture	60 μU/mL	(Hong et al., 2007)
Lactate dehydrogenase	*K. marxianus* KM-1	Liquid culture	8.4 U/OD_{600}	(Pecota et al., 2007)
α- Galactosidase	*K. marxianus* CBS 6556	Liquid culture	153	(Bergkamp et al., 1993)
Cu/Zu super-oxide dismutase	*K. marxianus* NBIMCC 1984	Liquid culture (batch)	996 U/mg of protein	(Nedeva et al., 2009)
Serine protease	*K. marxianus* IFO 0288	Liquid culture	4.47 I U/mg	(Foukis et al., 2012)

[a] U/mL unless otherwise mentioned; Initial dry substrate (ids).

3.1.2. *β-galactosidases production*

β-galactosidases are the mostly used enzymes in the food processing industries. *β*-galactosidases (EC 3.2.1.23), generally well-known as lactase, that catalyzes the hydrolysis of lactose, and producing a mixture of galactose and glucose. In particular, *β*-galactosidases have significant applications in the food and pharmaceutical industries as it is used for saccharification of whey and, in the treatment of milk for reducing lactose content (Singh et al., 2016). The latter use is mainly related to the populations with the genetic deficiency for lactose metabolism, such as the black populations in Brazil, United States, and Central Africa (Bayless et al., 2017; Belem and Lee, 1998). Various strains of *Kluyveromyces* were reported as efficient for the industrial production of *β*-galactosidases (Hensing et al., 1994; Oliveira et al., 2011). Various approaches with different cultivation strategies were used to produce *β*-galactosidases from industrial media like molasses (Morrissey et al., 2015) and cheese whey (Padilla et al., 2015; Rech et al., 1999). The hydrolytic and transgalactosylic activities, which are indispensable in food processing, usually carried out using commercial *β*-galactosidases (Pivarnik et al., 1995). The hydrolytic activity is used to reduce lactose content in milk in the food industry, while the transgalactosylation activity is performed to synthesize the galactose and galacto-oligosaccharides containing chemicals (Oliveira et al., 2011). Recently, Padilla et al. (2015) described that the *β*-galactosidases from *K. lactis* and *K. marxianus* could be successfully used for the production of lactulose oligosaccharides through isomerization of transgalactosylated whey permeate.

The regulation of *β*-galactosidases expression in *K. marxianus* occurred by its natural inducers, galactose and lactose. However, the production of *β*-galactosidases was dependent on substrate concentration. A repressive mechanism was superimposed to the inducing effect of the substrate while *K. marxianus* were exposed to a higher concentration of lactose or D-glucose, consequently, the *β*-galactosidases

activity was decreased (Martins et al., 2002). This might be due to the accretion of intermediate glycolysis metabolites when microbial cells consumed D-glucose or lactose at a high rates (Martins et al., 2002). In a previous study, Zhou et al. (2013) reported that the biosynthesis of β-galactosidase suppressed by glucose, however, its production was derepressed after removal of the *MIG1* gene. They also found that, after disrupting the *MIG1* gene of *K. marxianus* KM, the disruptant (*mig1* mutant) *K. marxianus* KM-15 strain achieved more β-galactosidase than *K. marxianus* KM. A galactosidase activity of 121 U/mL was obtained by the disruptant KM-15 in 60 h under the optimal conditions. Likewise, Bergkamp et al. (1993) achieved a high β-galactosidase activity of 153 mg/L and secretion efficiency of 99% by addressing the *INU1* promoter and signal sequence in *K. marxianus*.

Besides the substrate concentration, other operational parameters including pH, incubation time, temperature, inoculum size, and age significantly influenced the β-galactosidases activity. The optimum growth and higher β-galactosidases activity were noticed at a temperature of 37 °C and a pH of 5.5. using *K. marxianus* CBS6556 and CBS712 in cheese whey (Rech et al., 1999). Panesar (2008) obtained the maximum activity (1580 IU/g dry weight) with an optimal conditions of temperature 30 °C, pH 5.0, inoculum size 6% (v/v) having 20 h age, shaking 100 rpm after 28 h of incubation time using *K. marxianus* NCIM 3465 in whey. Gupte and Nair (2010) observed maximum β-galactosidase activity of *K. marxianus* NCIM 3551 at 25 °C temperature, pH 5.0, and inoculum size of 10% after a 20 h of incubation time. An incubation period extending up to 30 h was also reported for *K. marxianus* MTCC 1388 (Bansal et al., 2008). However, a decrease in the enzyme activity was noticed with further increase in the incubation time (24 h to 36 h), attributed to the fact that the growth of culture reached the stationary phase (Gupte and Nair, 2010; Panesar, 2008). The earlier workers reported that the enzyme activity probably started after the lag phase and highest yield obtained at the beginning of the stationary phase of growth (Pinheiro et al., 2003). The enzyme activity remained quasi-constant during the stationary phase, thereafter the yield of the enzyme decreases (Gupte and Nair, 2010; Panesar, 2008; Rech et al., 1999). Temperature also strongly influenced the enzyme activity and an optimum temperature of 28–31 °C has been mostly used in the earlier studies (Gupte and Nair, 2010; Panesar, 2008).

The enzyme activity was increased with the agitation mode attributed to the uniform distribution of yeast culture in the medium resulting in oxygen transfer rate (OTR) and nutrient availability (Panesar, 2008). According to some authors (Barberis and Gentina, 1998; Pinheiro et al., 2003), the expression of β-galactosidases enzyme was correlated to the OTR because the growth of aerobic cultures could be enhanced by air pressure raise in limited OTR (Belo and Mota 1998). However, in many cases, improved oxygen partial pressure can be toxic to the aerobic cultures and inhibits growth and product formation because of the formation of reactive oxygen species (ROS) (Onken and Liefke, 1989). *K. marxianus* possess several defensive mechanisms such as induction of antioxidant enzyme superoxide dismutase (SOD) in air pressure raise (Dellomonaco et al., 2007; Pinheiro et al., 2003). Pinheiro et al. (2003) investigated the impact of total air pressure increase on cell growth and β-galactosidases activity of *K. marxianus* CBS 7894 in batch cultures. They demonstrated that the specific β-galactosidase productivity was increased from 5.8 to 16.9 U/g/h using a 6-bar air pressure instead of air at atmospheric pressure Therefore, the rise in air pressure up to 6-bar could be an alternative for preventing the oxygen limitation in β-galactosidase production. The influence of the oscillating dissolved oxygen tension (DOT) on the metabolism of *K. marxianus*, in particular, on the β-galactosidases production was also investigated by Cortés et al. (2005). They observed that the faster oscillations of DOT can increase the final volumetric and specific enzyme activity. The findings of their study imply that the β-galactosidase production by *K. marxianus* in industrial scales would be more robust in respect to the oxygen variations (Cortés et al., 2005). Furthermore, the extraction methods also have an influence on the activity of β-galactosidase since it is an intracellular enzyme. SDS-Chloroform technique was observed to be the best method followed by Toluene-Acetone, sonication, and homogenization with glass beads out of the four methods for extraction (Bansal et al., 2008). In addition, the thermostable characteristics of β-galactosidases produced from *K. marxianus* was studied by Tomáška et al. (1995). The results of this study suggest that *Kluyveromyces* β-galactosidase was thermostable at a temperature of more than 45 °C and could be further improved by confinement and stabilization inside the cells or by combining with immobilization technique.

3.1.3. Pectinase production

Endo-polygalacturonases (EC 3.2.1.15), commonly known as pectinases, hydrolyze pectins. Pectins are heteropolysaccharides comprising the main structural elements of the plant cell walls. Pectinases have been used in the juice and wine manufacturing because of their ability to degrade the cell wall (Alimardani-Theuil et al., 2011). These enzymes are mostly produced by plants and different microorganisms including bacteria, yeasts, and filamentous fungi. Among them, yeast pectinases are of great interest for last one decade (Alimardani-Theuil et al., 2011). Based on the environmental ang genetical background, the pectolytic yeasts can produce different kinds of enzymes. Four species of yeast, namely *Torulopsis kefir*, *S. fragilis*, *S. cerevisiae*, *K. marxianus* have been widely exploited for pectolytic activity. They produce polygalacturonases (PG), pectinlyases (PL), pectinesterase (PE) or pectate lyase (PaL), depending on the temperature, pH, and substrate availability. For instance, *Candida, Sacharomyces,* and *Kluyveromyces* produce PG (mainly endopolygalacturonase), whereas *Rhodotorula* produces both PG and PE (Alimardani-Theuil et al., 2011; Blanco et al., 1999)

K. marxianus was reported as the prominent pectinolytic yeast with 85% of total secreted protein, containing of a constitutive endopolygalacturonase (*endo*-PG), while compared with 12 cocoa pulp-degrading yeasts (Schwan et al., 1997). Moreover, a simple one-stage purification scheme attained more than 90% recovery of a highly purified PG enzyme from *K. marxianus* fermentation process (Harsa et al., 1993).In a study, Garcia-Garibay et al. (1987) observed that *K. marxianus* can produce endo-PG using whey as carbon source. They observed that the dissolved oxygen significantly influenced the biomass production and endo-PG synthesis by *K. marxianus*. The production of biomass was very low under anaerobic condition whereas the enzyme activity was maximum at an intermediate level of aeration as enzyme activity associated with growth. However, the enzyme was fully repressed when *K. marxianus* was cultivated under the highest aeration conditions. Although the enzyme was repressed at the high aeration levels, significant amounts of PG produced under such conditions when pectin was complemented as inducer. Under these conditions, the productivity was 4 times higher than the anaerobic fermentation in absence of the inducer. Some other studies exhibited endo-PG activity in glucose as the carbon source, however, PG activity was increased by replacing glucose with galactose in the culture medium (Radoi et al., 2005). Moreover, the transcription of *PGU1* was also improved in the yeast strains when PG activity intensified on galactose compared to glucose (Louw et al., 2009).

The synthesis of microbial enzymes at the industrial scale typically requires highly productive strains to reduce the cost and to enhance the efficacy, however, the regulatory mutants have rarely been exploited. Most of the pectinases were induced by pectins and exposed to catabolic repression, however, the PG of *K. marxianus* is interesting because the production was constitutive and not repressed by carbohydrates. A maximum yield was obtained in a 10% (w/v) glucose under self-induced anaerobic conditions (Schwan and Rose, 1994). Furthermore, PG activity could be increased by adding pectins or their degradation products on fermentation medium (Radoi et al., 2005). Oliveira et al. (2012) demonstrated that *K. marxianus* CCMB 322 can use pectin as a carbon source. Although glucose can considerably affect the regulation

of the PG synthesis, and the product of the citrus pectin hydrolysis (galacturonic acid) was the most effective product for induction. Furthermore, the extracellular PG activity was increased (from 0.2126 to 0.7457 μmol/mL/min) while the strain was cultivated in the media combined with glucose (1%) and pectin substances (1%) compared to the individual substrates. This fact suggests that the production of PG using *K. marxianus* CCMB322 was partially constitutive. Likewise, Wimborne and Rickard (1978) demonstrated that the enzyme secretion efficiency *K. marxianus* could be increased to 100% by adding pectin in the culture medium. Low PG activity (except for galacturonic acid) were produced in the absence of glucose suggesting that the product of citrus pectin hydrolysis (galacturonic acid) was the most efficient for PG production by *K. marxianus* CCMB322, although glucose had an impact on the regulation PG synthesis (Oliveira et al., 2012). However, different results were previously reported by McKay (1988), who demonstrated that the PG activity was constitutive during the growth of *K. marxianus* NCYC 587 on glucose, which unable to grow on polygalacturonic acid as sole carbon source. Likewise, Schwan and Rose (1994) did not observe any significant change in PG activity by addition of pectin, pectic acid or polygalacturonic acid to a medium containing 1% (w/v) glucose using *K. marxianus* CCT 3172. To sum up, the potential effect of carbon sources on PG activity seems to be strain dependent and is associated with other complex means of regulation.

Beside medium composition, the excretion and synthesis of PG enzymes depend on the variables of fermentation and culture medium such as temperature, pH, inoculum size, incubation time, or type of microbial strain. For example, the pectinolytic enzyme secretion capacity was highly efficient for *K. marxianus* isolated from tropical fruits (da Silva et al., 2005). Also, Schwan et al. (1997) observed that the optimum PG activity can be obtained at a pH of 5.0 and a temperature of 40 °C using *K. marxianus* CCT 3172. However, the optimum pectinolytic activity was achieved at a pH of 7.36 and a temperature of 70 °C using *K. marxianus* CCMB 322 after 48 h of incubation, and 93% of its original activity can be retained for 50 min at 50 °C (Oliveira et al., 2012). Moyo et al. (2003) revealed that the PG from *K. wickerhamii* had an optimum pH and temperature of 5.0 and 32 °C, respectively. The results imply that the optimum temperature and pH of *K. marxianus* CCMB 322 were higher than those of *K. wickerhamii* for PG activity. Interestingly, most of the microbial PG enzymes showed optimum activity in the acidic region (pH 4–5). The most interesting factor for biotechnological applications, is the optimum temperature for the activity of these enzymes because some yeast PG can act over a wide range of temperature (0–60 °C) (Barnby et al., 1990). However, Cordeiro and Martins (2009) revealed that the PG of *Bacillus* sp. SMIA-2 maintained only 70% of activity after heating for 120 min at 50 °C. Whereas, the PG produced by *K. marxianus* CMB 322 displayed more thermal stability (~93% at 50 °C), thus it can hydrolyze polygalacturonic acid at the usual commercial temperature (50 °C) that render this enzyme from *K. marxianus* particularly interesting for use in the fruit juice industry (Oliveira et al., 2012). Furthermore, Sieiro et al. (2014) observed that the *endo*-PG from *K. marxianus* KMPG enhanced the total compounds responsible for the aroma in white wines compared to the commercial pectic enzyme. In addition, endo-PG enzyme from *K. marxianus* CCT3172 causes a drastic reduction in the pectin viscosity, is responsible for the depolymerization of pectin, and therefore, has industrial importance in the wine, juice, vegetable and animal feed industry (de Mansoldo et al., 2019). Indeed, the yeast pectinases could potentially be used in various kinds of industries such as, in vegetables and fruits processing, citrus processing, wine making, tea and coffee fermentation, textile processing, and paper making industries (Alimardani-Theuil et al., 2011).

3.1.4. Lipase production

The term lipolytic enzymes refers to the lipases and carboxylic ester hydrolases. The lipases, also known as triacylglycerol-acyl-hydrolases (EC 3.1.1.3) are hydrolytic enzymes that catalyze both the hydrolysis and the synthesis of esters. They are generally accountable for the hydrolysis of acyl-glycerides, which are indispensable for the bioconversion of lipids (triacylglycerol) in nature (Vakhlu, 2006). Lipases possess several unique features, which includes the stereospecificity, substrate specificity, regiospecificity, and the ability to catalyze a heterogenous reaction at the interface of water insoluble and soluble systems (Sharma et al., 2002). Like the carbohydrases and proteases, the lipases of microbial origin enjoy greater industrial importance as they are more stable than the animal or plant lipases. Moreover, they can be produced in bulk at a low cost (Vakhlu, 2006). In addition, the lipases from microbial origin are more beneficial because of a wide ranging catalytic activities available, rapid growth of microbes on low cost media, absence of seasonal fluctuations, and ease of genetic manipulations (Šiekštelė et al., 2015). Microbial lipases are mostly extracellular and obtained from bacterial and fungal species. Among them, the fungal lipases are extensively exploited due to their exclusive characteristics such as pH and thermal stability, economical extraction process, substrate specificity, and efficient activity in organic solvents (Sarmah et al., 2018).

Lipase production is prevalent among yeasts, however, only few species can produce lipases with suitable traits and in sufficient amounts to be industrially useful. *C. rugosa*, *Y. lipolytica*, *C. antarctica*, *C. utilis*, and *Saccharomyces* were reported as the most promising lipase producing yeasts (Sarmah et al., 2018). Deive et al. (2003) investigated the ability of *K. marxianus* to produce extracellular lipolytic enzymes and to observe the effect of several lipidic compounds and surfactants on enzyme secretion. They observed that *K. marxianus* showed lipolytic activity in a complex liquid medium with several potential inducers such as triacylglycerols, fatty acids. However, the tributyrin and oleic acid were identified as the best inducers in their study. The highest extracellular lipolytic enzyme production (about 80 U/mL in 3 d) was obtained while the medium was supplemented with a 2 g urea/L plus 5 g tributyrin/L. However, addition of surfactants did not improve production. Furthermore, the enzymes demonstrated high thermal stability in aqueous solution (73% residual activity after 9 d at 50 °C; 16 min half-life time at 100 °C), good tolerance to organic solvents (70% residual activity, after 2 d in n-hexane of cyclohexane) and stability at acidic pH (> 85% residual activity after 24 h of incubation time at 25 °C for pH 2.0–8.0, 100% activity at pH 4.0). However, the lipase produced by *K. marxianus* was sensitive to alkaline pH and nearly to be inactivated after 24 h at pH above 8 (Deive et al., 2003). The stability at acidic pH could be of some commercial interest, since most lipases from some other microbial strains were rapidly inactivated in this condition (Corzo and Revah, 1999; Sharma et al., 2002).

Apart from the physicochemical factors such as temperature, pH, and dissolved oxygen, the production of lipases from *K. marxianus* was significantly affected by medium composition. In respect to this, Stergiou et al. (2012) optimized several process parameters influencing the extracellular lipase production using *K. marxianus* IFO 0288. They observed the productivity was increased by18-fold with an optimized nutritional (0.5% olive oil) and cultivation (pH 6.5, 35 °C, 150 rpm) conditions during the 65 h of fermentation of olive oil as substrate. Recently, a bioinformatics analysis of the genes of lipases from *K. marxianus* L-2029 was also performed to analyze biochemical characteristics, properties, and phylogeny of the extracellular lipases from *K. marxianus* L-2029 (Martínez-Corona et al., 2019). The phylogenetic analysis of *K. marxianus* lipases showed evolutionary affinities with the lipases from abH15.03, abH23.01, and abH23.02 families. Furthermore, Cardoso et al. (2015) observed that *K. marxianus* 83F showed higher lipolytic activity in traditional Serro Minas cheese; hence, it could be used as a good starter culture in cheese production due to their effects on the sensory properties. Indeed, microbial lipases are very versatile enzymes having many promising applications in different industries, such as detergent industry, baking and food industry, organic synthetic industry, dairy and flavor industry, paper manufacturing industry, medical and pharmaceutical industry, biosurfactant synthesis

cosmetics and perfumery, leather industry; oleochemical and agrochemical industry, biosensors, and bioremediation (Sarmah et al., 2018).

3.1.5. Cell factory applications of K. marxianus

Many recent studies highlighted the potential of *K. marxianus* to be used as effective cell factory to produce valuable metabolites by means of engineered manipulations. For example, Kim et al. (2014) reported a study in which 2-Phenylethanol, which is an aromatic alcohol with a rose scent, was successfully produced by using genetically modified yeast strains by the Ehrlich pathway. In the study of Kim et al. (2014), *K. marxianus* was genetically engineered in order to be able to overproduce 2-Phenylethanol by using glucose as carbon source. To achieve this objective, the authors induced an overexpression of phenylpyruvate decarboxylase and alcohol dehydrogenase genes of *S. cerevisiae in the used K. marxianus strain*. Thus, approximately 1.0 g/L of 2-Phenylethanol was produced. A similar level of 2-Phenylethanol was also produced from evolved *K. marxianus*, which was genetically engineered by using specific genes from *Klebsiella pneumoniae*. This modification allowed an overexpression of 3-deoxy-D-arabino-heptulosonate-7-phosphate synthase in the used *K. marxianus*. Thus, by using this cell engineering technique, the authors reported a yield of 1.3 g/L of 2-Phenylethanol when a 20 g/L glucose solution without addition of phenylalanine to the medium was used (Kim et al., 2014). In another study, Zhang et al. (2020) used different sugars as carbon source such as glucose, xylose, and fructose for glycerol production. They reported that a *TPI1* gene encoding triose phosphate isomerase was deleted from *K. marxianus* NBRC1777 and that this procedure allowed the newly engineered *K. marxianus* to be able to grow with glucose, fructose, and xylose as sole carbon sources. Thus, under aerobic conditions at a temperature of 42 °C, the engineered *K. marxianus* YZB115 strain fermented 80 g/L glucose, fructose, and xylose solutions, yielding 40.32, 41.84, and 18.64 g/L glycerol without by-product, respectively (Zhang et al., 2020). In a study reported by Zhang et al. (2017), *K. marxianus* was used as cell factory to produce fructose from inulin. They hypothesized their research on the fact that in yeast, the hexose assimilation is started at hexose phosphorylation. However, in *K. marxianus*, the hexokinase and glucokinase genes were not identified. Thus, in the study they reported, the *KmHXK1* and *KmGLK1* genes were over-expressed in different *K. marxianus* strains. They showed that glucose and fructose assimilation ability decreased significantly in the *KmHXK1* genre disrupted *K. marxianus* YLM001 strain. However, this ability was not changed obviously in *KmGLK1* disrupted *K. marxianus* strain YLM002. In the case of over-expressing *KmGLK1* in YLM001, only the glucose assimilation ability was recovered in the engineered *K. marxianus* YLM005 strain. They also showed the engineered strains by the KmHXK1 gene could phosphorylate glucose and fructose, and that the KmGLK1 gene induced only a glucose phosphorylation. The authors were also able to obtain a thermo-tolerant *K. marxianus* YGR003 strain which produced glucose-free fructose solution from inulin in one step (Zhang et al., 2017).

3.2. Cell proteins production

Single cell proteins (SCPs) are known as dietary single-cell microorganisms whose biomass or protein extracts are derived from pure or mixed microalgae, yeasts, mushrooms or bacterial cultures. These microorganisms can be used as protein-rich foods or food ingredients or dietary supplements for human and animal consumption (Ritala et al., 2017). Therefore, large-scale production of microbial biomass could be advantageous for replacing proteins of agricultural origin (plant and animal proteins) for food or feed due to the high multiplication rate and a high protein content (30–80% protein in terms of dry weight) of microorganisms, the ability to utilize the large number of different low-cost carbon sources including waste materials (Karimi et al., 2018). Furthermore, SCPs could be obtained in a shorter time compared the proteins from agricultural origin due to short life cycle of the microorganisms and relatively smaller amount of space and labor needed. More importantly, it does not need huge arable land, thus the conflicts with world food production could be avoided (Karim et al., 2018).

Among the SCP producing microorganisms, yeasts have been considered as more suitable candidate due to the high protein content, small particle size, ease handling and relatively low production costs. *K. marxianus* is a candidate of great interest for SCP production and being used as feed organism in various countries. *K. marxianus* (*K. fragilis*) was found to have a higher specific growth rate than that of *S. cerevisiae* in continuous production of a novel yeast diet. *K. fragilis* showed a maximum biomass yield of 4.81 g/L/h in an aerobic continuous fermentation of 2.5% fructose medium for producing a high nutritious protein diet with a protein content of 50–55% (Kim et al., 1998). Yadav et al. (2014) used *K. marxianus* GQ 506972 for SCP production and concurrent COD removal of cheese whey and obtained a biomass production of 6 g/L with a 55% COD removal efficiency after 36 h of fermentation in batch system at 40 °C and pH 3.5. Moreover, an increase in the inoculum concentration resulted in the increase of biomass production of 15 g/L with a COD removal of 80%. In another study, whey permeate was fermented by *K. marxianus* to produce SCP under batch and aerobic condition at a low pH of 4.5 and a temperature of 35 °C where ammonium sulphate was added as nitrogen source to increase biomass yield (Yadav et al., 2014a). Also, Yadav et al. (2014b) evaluated the potentiality of co-culture to obtain improved quality SCP and to enhance the COD removal during the batch and continuous fermentation of whey at extreme culture conditions (low pH, 3.5 and high temperature, 40 °C). The batch system of mixed culture (*K. marxianus* and *C. krusei*) resulted in a 19% higher biomass yield with 33% increased productivity and simultaneously 8.8% higher removal of COD than the monoculture. In addition, the SCP obtained from mixed culture was enriched with required protein content and all necessary amino acids including lysine. The results revealed that the mixed culture of thermo-tolerant and acid resistance yeasts can be a potential approach to produce SCP and concurrent removal of COD from wastewater under extreme conditions (Yadav et al., 2014a, 2014b). In another study, the co-culture of *K. marxianus* and *Trichoderma reesei* was more efficient for production of SCP (51%) from beet pulp compared to a monoculture of *T. reesei* (49%) and the protein contained all essential amino acids (Ghanem, 1992). In another study, a mixed culture of *S. cerevisiae* and *K. marxianus* was used for producing food-grade SCPs at at 30 °C and pH 6.5. The result showed that 92% of total whey proteins was recovered by the co-culture while 84% by monoculture under these optimized conditions (Yadav et al., 2016).

Furthermore, several microorganisms (*S. cerevisiae, K. marxianus* and *C. kefir*) with industrial interest were grown in the food wastes through solid state fermentation, and *K. marxianus* was found to contain the highest protein and fat concentration (59.2% w/w dm), thus it could be utilized for its high fat content and livestock feed enrichment (Aggelopoulos et al., 2014). The essential amino acid composition of SCP from *K. marxianus* using sugar cane molasses was found to be similar with other yeast species (Anderson et al., 1988). Øverland et al. (2013) evaluated the performance of *C. utilis, K. marxianus* and *S. cerevisiae* yeasts as protein sources in diets for Atlantic salmon (*Salmo salar*).

3.3. Single cell oil and fatty acids production

Single cell oil (SCO) or microbial lipids are considered as potential substitutes of vegetable oils and animal fats to produce non-fossil biofuels (i.e., biodiesel) and oleochemicals as the oil properties are similar in types, structure, and composition of fatty acids. Some microorganisms attain a higher oil content compared to vegetable oils, but they do not need fertile land, thus avoiding conflicts associated with the food vs. fuel issue. Furthermore, they are not disturbed by the climate and the seasons (Karim et al., 2019).

K. marxianus CBS 6556 was capable of producing SCOs using deproteinized whey (sweet and sour) concentrates as carbon source and showed an increase in quantity of essential amino acids (Schultz et al., 2006). *K. marxianus* NCYC 1424 was most efficient for conversion of whey to SCO while an investigation with several organisms was conducted by Willetts and Ugalde (1987). The oxygen availability in the medium was found to have a great influence in the conversion efficiency of whey to biomass (Willetts and Ugalde, 1987). Arous et al. (2017) observed that *K. marxianus* CC1 was the most efficient to assimilate and to ferment a wide variety of carbon sources and, showed a strong lipolytic activity to grow on fats. It obtained 6 g/L of biomass comprising 12.9% w/w lipids after 72 h fermentation at an initial medium pH of 6 ± 0.1 and at 28 °C temperature. This quantity was considerably higher than that of reported by Fonseca et al. (2007) in *K. marxianus* ATCC 26548 (=CBS 6556) (5.2% w/w lipids). However, the lipid accumulation was higher for *S. cerevisiae* (7% wt/wt lipids) compared to *K. marxianus* in batch culture (Fonseca et al., 2007). These variations in lipid production capabilities might be ascribed to the specific physiological behavior of each microorganism or to the differences in the culture conditions.

Saccharomyces cerevisiae is a yeast that played a central role in human society due to its use in food production such as bread, beer, and wine. In modern scientific research, this yeast is also widely used as a model microorganism to perform various genetic manipulations and achieve biotechnological goals that require microbial engineering (Cernak et al., 2018). However, various studies have shown that *S. cerevisiae* is genetically difficult to manipulate to develop new strains capable of metabolizing unconventional carbon sources to obtain specific metabolites. This yeast also requires fairly specific conditions for optimum growth and tolerates little specific industrial applications where it is difficult to work under the optimum conditions for its growth. Also, it has been reported that yeasts capable of solving many of these problems specific to *S. cerevisiae* remain difficult to manipulate genetically, hence the interest of *K. marxianus* as a potential substitute of *S. cerevisiae* as a biotechnological tool (Cernak et al., 2018).

In this context, Cernak et al. (2018) successfully designed the thermotolerant yeast *Kluyveromyces marxianus* as a novel platform for microbial engineering and synthetic biology. They used the CRISPR-Cas9 technique and showed that wild strains of *K. marxianus* can be made heterothallic for sexual crossing. By selecting two mating-type *K. marxianus* strains, these authors were able to combine three complex traits: thermotolerance, lipid production, and easy transformation with exogenous DNA into a single host. This has made it possible through microbial engineering to use *K. marxianus* as a cell plant for the production of lipids at temperatures exceeding those of other fungi, opening up highly potential prospects for large-scale industrial applications. These results showed that *K. marxianus* can be used as a substitute for *S. cerevisiae*, as it exhibits more robust metabolic characteristics with potential for the industrial production of ingredients of high nutritional and functional value such as fatty acids and antimicrobial peptides. In addition, unlike *S. cerevisiae*, these authors concluded that the yeast *K. marxianus* can easily grow at high temperatures while being able to utilize a wide range of carbon sources, making it a promising microorganism for industrial biotechnology and the production of specific metabolites from renewable raw materials such as plant biomass (Cernak et al., 2018).

3.4. Bioethanol production

Bioethanol production through fermentation at a high temperature has received attention nowadays since rapid fermentation at elevated temperature can reduce cooling cost and continuous change from fermentation to distillation, decrease the risk of contamination, carry out simultaneous saccharification and fermentation, and be used in tropical countries (Fonseca et al., 2008). The high temperature optimum for growth (P_{max} of 0.86/h at 40 °C) of *K. marxianus*, is particularly interesting because this facilitate cooling during large-scale fermentations for which heat transfer is proven to be limiting factor. Currently, industrial ethanol production mostly depends on conventional strains of *S. cerevisiae* because of its high production rate and better tolerance to high ethanol titers (upwards of 120 g/L), however, the suitable temperature of this strain is comparatively low (only 25 to 30 °C) (Qiu and Jiang, 2017). In this regard, there has been a significant interest in the yeast species which are able to produce ethanol at high temperature, and the isolates of *K. marxianus* appear to be particularly promising (Madeira-Jr and Gombert, 2018). *K. marxianus* species can grow at 47 °C (da Silva et al., 2018; Nonklang et al., 2008), 49 °C (Hughes et al., 1984), and even 52 °C (Banat et al., 1992) and can produce ethanol at temperature more than 40 °C (Banat et al., 1992; Madeira-Jr and Gombert, 2018). In addition, *K. marxianus* can utilize a broad range of low-cost substrate such as corn silage juice (Hang et al., 2003), molasses (Martínez et al., 2017), whey permeate (Ozmihci and Kargi, 2007; Zafar and Owais, 2006) to produce ethanol. Considering these advantages, *K. marxianus* is currently being promoted as a feasible alternative to *S. cerevisiae* as an ethanol producer.

Recently, it was revealed that bioethanol production at high temperatures (~48 °C) using *K. marxianus* NCYC 3396 from sugarcane can decrease contamination levels, cooling costs, use of antibiotic, use of H_2SO_4 in cell recycling, water usages, and energy use in distillation; which reduced ultimate cost of bioethanol production in Brazilian biorefineries (Madeira-Jr and Gombert, 2018). They obtained a similar yield (0.40 g ethanol/g glucose) using *K. marxianus* at 48 °C to those displayed by *S. cerevisiae* CEN.PK113-7D at 37 °C temperature. Although the ethanol production was similar (0.43 g/g glucose) by *K. marxianus* K213 and *S. cerevisiae* using glucose at 30 °C, however, *S. cerevisiae* almost lost its ability to produce ethanol at 45 °C whereas *K. marxianus* K213 still maintained same conversion efficiency (0.43 g/g glucose) (Yan et al., 2015). An optimization study showed that the temperature (32.5–35 °C) was the most significant factor for ethanol production from cheese whey using *K. marxianus* URM7404, followed by pH (4.8–5.3) and lactose concentration (61–65 g/L) (Murari et al., 2019). A recent study by Suzuki et al. (2019) revealed that the recombinant *K. marxianus* DMB13 strain converted xylose to ethanol rapidly, particularly after depletion of glucose, and achieved the maximum ethanol yield (0.402 g/g) in a xylose/glucose co-fermentation at 40 °C.

Furthermore, *K. marxianus* is of particular interest because of its ability to utilize xylose as a carbon source at temperatures as high as 45–52 °C, at which the fermentation efficiency was similar to that of at *S. cerevisiae* 30 °C (Suzuki et al., 2019). Therefore, *K. marxianus* could be advantageous for second generation bioethanol production, which uses lignocellulosic biomass (LCB) as substrates, because the temperature is typically higher for the saccharification process of LCB than usual fermentation temperatures and the hydrolytic enzymes have their optimum activity at these high values (Madeira-Jr and Gombert, 2018). In another study, *K. marxianus* DSMZ-7239 was the most suitable strain for ethanol production (3.3%, v/v) using whey permeate (50 g/L) as substrate (Ozmihci and Kargi, 2007). In addition, *K. marxianus* CCT7735 showed a higher efficiency compared to *K. lactis*, due to its high ability to express LAC4 gene (*β*-galactosidase) and *RAG6* gene (pyruvate decarboxylase) and, genes of Leloir pathway under hypoxia and high lactose concentrations (Diniz et al., 2012). Nevertheless, it is still not evident whether the oxygen concentration regulates the expression of the lactose transport in the *Kluyveromyces* genera. However, *K. marxianus* is preferred to produce ethanol from both LCB (mainly, xylose) and whey (mainly, lactose) at high temperature (> 40 °C), however, in contrast to *S. cerevisiae*, *K. marxianus* cannot endure high ethanol concentrations which is the major drawback of this strain to be used at industrial level as its growth is strongly inhibited by an ethanol concentration higher than 6%. This is because the low membrane stability and down regulation of some gene-encoding enzymes of the ergosterol biosynthesis pathway under high ethanol stress (Diniz et al.,

2017).

Nowadays, different techniques including the pre-treatment techniques for substrates, simultaneous saccharification and fermentation (SSF), co-production of bioethanol and other bioproducts, coculture inoculum, immobilization technique, and evolutionary engineering, have introduced to improve the productivity of bioethanol production using *K. marxianus*. For example, SSF of taro waste exhibited higher bioethanol productivity (2.23 g/L/h) and maximum ethanol concentration (48.98 g/L) after 22 h when 5% of *K. marxianus* K21 inoculum was employed at 40 °C (Wu et al., 2016). The SSF of *Agave teuilana* fructans (ATF) using *K. marxianus* strains has been proved as a potential approach for industrial application due to the production of specific *exo*-fructanhydrolase activity for ATF hydrolysis, and simultaneous production of bioethanol as well (Flores et al., 2013). Furthermore, the ethanol production (7.34 g/L) was 1.78-fold higher for *K. marxianus* K213 at 42 °C than *S. cerevisiae* at 30 °C using $NaOH/H_2O_2$ -pretreated water hyacinth (Yan et al., 2015). Moreover, *K. marxianus* ATCC36907 produced 7.53 g/L ethanol using alkaline pretreated Carnauba straw residue through SSF, which was 3-fold higher than *S. cerevisiae* CAT-1 (2.3 g/L) at 45 °C (da Silva et al., 2018). In addition, the coproduction of bio-oil and bioethanol by *K. marxianus* through valorizing the steam-exploded wheat straw displayed it's potentiality for a biorefinery approach (Tomas-Pejo et al., 2017). Nevertheless, the coculture of *K. marxianus* and *S. cerevisiae* resulted in higher ethanol production (21.12 g/L) and better sugar consumption (88%) than monoculture from enzymatic rice waste hydrolysates (Saratale et al., 2017). Recently, a coculture of immobilized *K. marxianus* and *S. cerevisiae* resulted in a higher hydrolysis of whey and produced higher ethanol than the single cultures during the batch fermentation (Beniwal et al., 2018). Moreover, the development of high lactose utilizing osmotolerant yeast strain and its further use to ferment lactose rich whey has gained interest for higher ethanol titer and to lower energy consumption because expression of GPD1, TPS1and TPS2 upregulated in lactose adapted *K. marxianus* MTCC 1389 strain and it accumulated the trehalose and glycerol in response to lactose stress. Consequently, the osmotolerant *K. marxianus* cells leads to efficient conversion of whey lactose into bioethanol. More recently, the strain *K. marxianus* MTCC 1389 (ATCC64884) was reported as more resistant to osmotic and oxidative stresses than *S. cerevisiae* MTCC170 because the genes related to glutathione biosynthesis and glycerol synthesis were upregulated and, resulted in high glutathione level (6.8 μg/mg protein) and high intracellular glycerol (2.2 g/g cell dry weight) in the presence of osmotic and oxidative stress (in 150 g/L lactose), that is indicating the outcome of stress protectants at the transcriptional level (Saini et al., 2017).

4. Applications in food and feed industry

4.1. Bioemulsifier-mannoprotein production

Bioemulsifiers (biosurfactants) are surface-active molecules or proteins, lipoproteins, lipopolysaccharides, amphipathic polysaccharides, or complex mixtures of these biopolymers synthesized by different microorganisms. Increasing environmental concern about chemical emulsifiers prompts attention to the bioemulsifiers mainly due to their environmental-friendly nature, biodegradability, and low toxicity (Nitschke and Costa, 2007). Presently, the bioemulsifiers are mostly used in the remediation of pollutants, however, the interest in these compounds have considerably been increasing as substitute to the chemical surfactants such as sulphonates, carboxylates, and sulphate acid esters in the food, pharmaceuticals, and oil industries, specially to be used as emulsifiers, solubilizers, wetting, foaming, antiadhesive and antimicrobial agents (Gudiña and Rodrigues, 2019). Although the bacteria have been reported as a major source of various microbial emulsifiers in several literatures (Shekhar et al., 2015), many emulsifiers from bacterial sources are not recommended to use in the food industry because of pathogenic nature of the producers (Shepherd et al., 1995). In contrast, yeasts have widely been used to produce the emulsifiers. Generally, bioemulsifier producing strains include *C. petrophilum* (Iguchi et al., 1969), *C. tropicalis* (Käppeli and Fiechter, 1977), *Torulopsis petrophilum* (Cooper and Paddock, 1984), *C. lipolytica* (Cirigliano and Carman, 1985) and *C. bombicola* (Brakemeier et al., 1998). However, commercial application of bioemulsifiers from these species is unappealing due to the requirements of water-immiscible substrates (i.e., oils and alkanes) for facilitating metabolism, difficulties in isolation, foam fractionation, requirement of enzyme digestion, and repetitive extraction process with solutions of methanol-chloroform (Cameron et al., 1988); moreover, the yields of these complex procedures were very low (Cameron et al., 1988). Although the cell wall proteins of *S. cerevisiae* were reported as a bioemulsifier in foods (Shekhar et al., 2015), more understanding is required to evaluate their potentials as food ingredients.

Recently, the interest on other nonconventional yeast species including *K. marxianus, K. lactis, K. fragilis* etc. have been increasing due to their high ability to utilize low-cost substrates and high biomass production, which could ultimately lead to high bioemulsifier yields (Lukondeh et al., 2003). However, only a few studies have been conducted on protein production from *Kluyveromyces* sp. so far (Akanni et al., 2015; Galinari et al., 2018; Hajhosseini et al., 2020). Lukondeh et al. (2003) revealed that the emulsification properties of mannoprotein extracted from *K. marxianus* FII 510700 cell wall were like to those mannoprotein obtained from the cell walls of traditional source, *S. cerevisiae*. The optimization study of Hajhosseini et al. (2020) demonstrated that the carbon and nitrogen concentrations, fermentation time, and medium pH significantly influenced the mannan production as bioemulsifier by *K. marxianus* IBRC-M 30114. They observed that a mannan yield of 245.98 mg/100 mL could be obtained at the optimized conditions (pH: 4.99, glucose: 55.15 g/L, yeast extract: 9.35 g/L, and fermentation time: 168 h). Galinari et al. (2018) described that the cell wall polysaccharides such as α-D-mannan fractions from yeast *K. marxianus* CCT7735 showed hydroxyl-radical scavenging, superoxide radicals scavenging, copper- and iron-chelating activities, and reducing power as well as total antioxidant capacity. Thus, *K. marxianus* can be considered as an ideal source renewable and natural polysaccharides with pharmacological properties (antioxidant, antiproliferative, and immunostimulatory properties).

4.2. Baker's yeast and biomass for bread production

Yeast are considered as one of the most important single cell nutrition sources because they contain low amounts of nucleic acids but all essential amino acids especially lysine in higher amount compared to algae and bacteria. *S. cerevisiae* is the most common food grade yeast, also called baker's yeast, which has been used worldwide to produce bread and baking products. It contains 35–45% of carbohydrates (high β-glucan), 40–58% of proteins, 6.5–9.3% of nitrogen, 5–7.5% of minerals, 4–6% of lipids, various kinds of vitamins, and high volumes of glutathione depending on its types and growth conditions (Öztürk et al., 2017). Several efforts have been made to utilize the low-cost substrate whey to produce baker's yeast, however, *S. cerevisiae* is not able to use lactose as carbon source (Caballero et al., 1995). Therefore, several methods have been proposed to overcome this limitation, such as hydrolysis of lactose (Reed, 2012), or its conversion to lactic acid through an addition fermentation step (Champagne et al., 1990). In the contrary, using native lactose fermenting yeast could be an attractive approach for bread production. Caballero et al. (1995) carried out an experiment with *K. marxianus* stains since this yeast possess high growth in whey without any previous treatment. The efficiency of two *K. marxianus* strains (NRRL-Y-1109 and NRRL-Y-2415) as baker's yeast were assessed and compared with the strains of *S. cerevisiae,* which were isolated from compressed yeast and active dry yeast, respectively. The dough proofing activity (both in rich and lean doughs) and sensory

evaluation of breads were tested and observed that both *K. marxianus* stains displayed a higher proofing activity in the rich doughs (prepared with whey or lactose) than the commercial baker's yeast strains (Caballero et al., 1995). The improved aroma of breads was attained by applying *K. marxianus* (IFO 288) as starter culture for making sourdough bread. Furthermore, the use of mixed cultures (*K. marxianus* and *Lactobacillus delbrueckii* ssp. *bulgaricus*, or L. *helveticus*) as starter cultures to make sour dough bread could lead to have longer shelf-life and better sensorial qualities of sourdough breads (Plessas et al., 2008).

On the other hand, the reduction of oligo-, di-, and monosaccharides and polyols (FODMAPs) levels in whole wheat bread is imperative because a diet low in FODMAPs could reduce the abdominals symptoms to about 70% of the patients suffering from irritable bowel syndrome (Struyf et al., 2018). The main source of FODMAPs in our diet is usually from wheat bread since wheat contains relatively high levels of fructans. Recently, Struyf et al. (2017) proposed a yeast-based approach to reduce the FODMAPs in wheat bread, and the strains of *K. marxianus* showed a superior efficiency compared to the control *S. cerevisiae.* They observed that the fructan levels in the final products could be reduced to 90% using an inulinase secreting *K. marxianus* in dough fermentation whereas only 56% reduction was achieved by the control *S. cerevisiae*. This is might be due to the higher inulinase enzyme secretion capacity of *K. marxianus* having both secreted form and cell wall associated form of inulinase production capacity, while the invertase produced by *S. cerevisiae* is preserved in the cell wall instead of secreting into the dough (Caballero et al., 1995). As a result, the wheat grain fructans (that present in the dough) were more accessible to *K. marxianus* inulinase compared to *S. cerevisiae* invertase; and subsequently, a higher degradation of fructans might be obtained by *K. marxianus* inulinase (Struyf et al., 2017). Moreover, *K. marxianus* CBS6014 produced different levels of five volatile flavor compounds than the conventional bakery strain *S. cerevisiae* during the fermentation of whole meal breads low in FODMAPs (Struyf et al., 2018). Furthermore, a coculture of *S. cerevisiae* and *K. marxianus* could efficiently be used in bread preparation since the breads prepared by the coculture had a very low fructan level ($\leq$0.2% dm) and the volume of loaf was almost same as control. Thus, the reduction of FODMAPS level in bread using K. marxianus could be an attractive approach.

4.3. *K. marxianus as a probiotic and its stimulation by prebiotics*

The live microbes, which when administrated in sufficient amounts to confer a health benefit on the host is called probiotics (Hill et al., 2014) and mostly includes bifidobacterial, clostridia, lactobacilli, *Faecalibacterium*, enterococci, and recently, propinonibacteria (Altieri, 2016). Although *S. cerevisiae* var. *boulardii* has been described as a probiotic for a long time (Moré and Swidsinski, 2015), there is an increasing interest towards the probiotic potential of the other non-conventional yeasts in the recent years. It is verified that some strains of yeasts can produce the bile salt hydrolase (BHS) enzyme like the probiotic bacteria, and subsequently, deconjugating the bile salts (Liu et al., 2012). This capacity can increase the secretion of endogenous cholesterol, which may stimulate the hepatic bile salts synthesis. Consequently, the amount of absorbed cholesterol would be reduced and the development of micelles will be compromised (Kumar et al., 2012). *K. marxianus* is very promising to be used as a probiotic due to the capacity of modifications in the cell immunity, adhesion, and human gut microbiota; and having the antioxidative, anti-inflammatory, and hypocholesterolemic properties (Cho et al., 2018; Xie et al., 2015).

K. marxianus may have the ability of surviving in the digestive tract to safely reach the intestines and to function as prebiotics because of its resistance to acid and bile that could be encounter in the gastrointestinal environment. The capacity of surviving in the acid and bile, and a higher ability to adhere to the Caco-2 cells suggested that it might have higher antioxidant activities (Cho et al., 2018). *K. marxianus* isolated from kefir showed cholesterol lowering ability Cho et al. (2018) observed that *K. marxianus* isolated from kefir showed cholesterol lowering ability and the strain KU140723-04 reduced 30% of cholesterol, which was even higher than *S. cerevisiae* ATCC 6037 and *K. lactis* ATCC 34440. Liu et al. (2012) revealed that the amount of eliminated cholesterol from culture media was proportional to the BSH enzyme activity of *K. marxianus* strains. In another study, the impacts of *K. marxianus* on diet-induced hypercholesterolemia in rats were studied, which revealed that *K. marxianus* M3 isolated from Tibetan mushrooms had a protective effect in hyperlipidemic rats (Xie et al., 2015). Furthermore, the synergistic effects of *K. marxianus* KU140723-02 isolated from kefir and polyphenol rich grape seed extracts (GSE)/grape seed flour (GSF) on radical scavenging ability suggested that *K. marxianus* performed as a probiotic while GSE/GSF as probiotics. *K. marxianus* performed as a probiotic and GSF/GSE as prebiotics. Thus, *K. marxianus* together with the GSE/GSF would be used as an efficient functional food ingredients to enhance the anti-oxidant activity in the gut (Cho et al., 2018). Furthermore, *K. marxianus* AS4, isolated from traditional dairy products like yogurts and cheese, displayed an outstanding tolerance to high bile salts (with a survival rate of 83%) and low pH (with a survival rate of 71%), a high antipathogenic activity, a satisfactory antifungal susceptibility. Besides, it exhibited a higher anticancer activity in gastric cancer cells (~54% mortality) due to the secreted metabolites, downregulated Bcl-2 gene, and upregulated BAD and CASP9 gene expression system (Saber et al., 2017). Nevertheless, *K. marxianus* displayed an increase in HDL-cholesterol and a reduction in serum TC, LDC-cholesterol and TAG concentration (Xie et al., 2015).

Furthermore, the strains of *K. marxianus* (VM003, VM004, VM005), isolated from whey, were able to survive under gastrointestinal conditions and they showed weak auto-aggregation and co-aggregation with pathogenic bacteria (*Escherichia coli, Serratia* sp., *Salmonella* sp., and *Salmonella typhimurium*) (Díaz-Vergara et al., 2017). Fadda et al. (2017) found that artisanal cheese-derived *K. marxianus* stains have significant functional characteristics and lack of undesirable properties, consequently, it could be used as a suitable probiotic. Recently, the strain *K. marxianus* B0399, isolated from milk, was reported as a potential probiotic strain as it was also able to survive in gastrointestinal track, retaining its vitality and fermentation capability (Tabanelli et al., 2016). This strain has also exhibited its capability to increase the bifidobacterial concentration in the colonic model system, to affect the colonic microbiota, and to induce the formation of higher quantities of short chain carboxylic acids, acetate and propionate. In addition, it is highly adhesive to human enterocyte such as Caco-2 cells and can modulate immune response; and hence, fermented milk containing *Bifidobacterium animalis* sp., *K. lactis* BB12, *K. marxianus* B0399 was suggested for the patients with irritable bowel syndrome in some studies. Thus, *K. marxianus* can be introduced as a potential probiotic yeast (Maccaferri et al., 2012).

A substrate that is selectively utilized by host microorganism conferring a health benefit is called a prebiotic (Gibson et al., 2017). To be considered as a prebiotic, a substance must have the ability to manipulate the host microbiota for some beneficial health effects. Currently, the fructans (fructo-oligosaccharides and inulin) and the glactans (galacto-oligosaccharides or GOS) are being considered as dominating prebiotics as evidenced by several studies on their prebiotic effects (Collins and Reid, 2016). *K. marxianus* can produce high value-added bioingredients such as oligosaccharides (OS), that is used as prebiotics to increase the growth of *Bifidobacterium* sp. in the human and animal intestines; oligonucleotides (ON), usually used as enhancer of flavors in food products; and oligopeptides (OP), added to dairy products as immuno-stimulators (Belem and Lee, 1998). When added to foods, these compounds (i) act as immunopotentiators; (ii) lower the low density lipoprotein-cholesterol (a risk for cardiovascular diseases); (iii) promote protection against bacterial infections; (iv) enhance food flavors; and (v) stabilize food emulsions (Collins and Reid, 2016).

It is proven that the mortality from acute myocardial infarction (heart attack) could be reduced by lowering the plasma cholesterol

levels since hypercholesterolemia is a risk factor for the coronary heart disease (Inzucchi et al., 2015); hence, the demand for biological ingredients that is able to lower plasma cholesterol is rising in the field of medicine, food, and nutrition. Nowadays, the heat inactivated dried yeasts are used as nutrition supplement because whole yeast cells are rich in protein, vitamin B, and dietary fiber (Inzucchi et al., 2015). It was seen from some previous reports that the yeasts or constituents of yeasts possess hypocholesterolemic activity (Nicolosi et al., 1999), antitumor activity (Mifuchi et al., 1969) or immuno-stimulation activity (Williams et al., 1992), or can prevent constipation (Nakamura et al., 2001) in animals or humans (Inzucchi et al., 2015). However, not all but only a few species are widely used as dried yeasts including *S. cerevisiae*, *C. utilis*, and *K. marxianus* (Inzucchi et al., 2015; Yoshida et al., 2004). Recently, Yoshida et al. (2004) investigated the hypocholesterolemic activities of some yeasts (81) strains from different species in rats fed, a high cholesterol diet. In this study, some yeasts species such as brewer's and baker's yeasts, that are predominantly used for food, did not show the hypocholesterolemic activity even when administered at a high concentration of 10%. In contrast, the highest potentiality in hypocholesterolemic activity of *K. marxianus* YIT 8292 was observed. Moreover, plasma total cholesterol as well as liver total cholesterol were significantly reduced by this strain when introduced as a dietary admixture at a concentration of 3%. Therefore, *K. marxianus* could be used as a novel food supplement with the ability to prevent hypercholesterolemia.

4.4. Fructose and fructo-oligosaccharides production

Fructose could be an alternative sweetener to sucrose because of its higher sweeting capacity (1.5–2 times than sucrose) and can increase iron absorption in children, whereas fructo-oligosaccharide (FOS) could be a promising source of dietary fiber with a bifidogenic effect. Interestingly, both compounds can be obtained through the enzymatic hydrolysis of inulin. However, the production of FOS and fructose typically performed at a high temperature (around 60 °C) because inulin shows a limited solubility at room temperature. Therefore, the isolation and characterization of thermostable inulinases are of great interest to hydrolyze inulin at high temperatures (Flores-Gallegos et al., 2015).

FOS are used as food ingredients because of its health benefits like inducing proliferation of intestinal bifidobacterial community (probiotics), promoting a good balance in the intestinal flora. Moreover, oligosaccharides have recently achieved 'GRAS' status by Food and Drug Administration (Flores-Gallegos et al., 2015). Inulin could be an efficient feedstock to produce the inulo-oligosaccharides using the *endo*-inulinases; but insolubility of inulin in cold water or slightly soluble (only 5%) in water at 55 °C remained the main challenge to hydrolyze it. Only a few inulinases can be found that own an optimal temperatures of 50 °C or higher (Gao et al., 2009), thus exploring of thermostable inulinase procedures are of interest for industrial applications. In this regard, *K. marxianus* can be a promising candidate to produce thermostable inulinases. Furthermore, the development of new techniques to produce fructose syrups has received more attention because it is less viscous, highly soluble and less cariogenic than sucrose, and can be utilized by diabetics (Flores-Gallegos et al., 2015; Vandamme and Derycke, 1983). However, the production of fructose by conventional methods using starch required several enzymatic stages, such as α-amylase, amylo-glucosidase, and glucose isomerase activity; but yielding only a maximum of 45% fructose solution. In contrast, fructose production by acid hydrolysis is not suggested because of the undesirable coloring of the inulin hydrolysate and the formation of di-fructose anhydride without any sweetening property. Therefore, the enzymatic hydrolysis of inulin using microbial enzymes could be alternative method to produce fructose syrups containing 95% fructose (Vandamme and Derycke, 1983).

Microbial inulinase enzymes from various organisms (yeasts, fungi) are known to split up the β-(2,1)-fructofuranosidic bonds of inulin (Chi et al., 2009). Yeasts are preferred for fructose production because the inulinases produced by yeasts were capable of exohydrolysis of inulin (Liu et al., 2013). These inulinases commonly obtained from several nonconventional yeast species including *K. marxianus* or *K. fragilis* (Holyavka et al., 2016), *C. kefyr* (Negoro and Kito, 1973), *Debaryomyces antarelli* (Beluche et al., 1980). Among them, *K. marxianus* can grow on fructans such as inulin, thus inulinases might have the ability to saccharify the fructans of plant origin (Chi et al., 2011; Hensing et al., 1994). A large volume of inulin is generally found in the tubers of many plants like chicory, dahlia, yacon, and Jerusalem artichoke. The inulinases can hydrolyze the fructo-oligosaccharides and inulin into fructose by breaking down the glycosidic linkages of their molecules (Holyavka et al., 2016). The pure inulin and raw inulin (from roots of *Asparagus racemosus*) were hydrolyzed by using an extracellular exoinulinase produced by *K. marxianus* YS-1 to produce a high-fructose syrup. The exoinulinase successfully hydrolyzed the pure inulin (84.8%) and raw inulin (86.7%) for production of 43.6 and 41.3 mg/mL of fructose in a batch system, respectively (Singh et al., 2007a). The fructose production through enzymatic hydrolysis of poly- and oligosaccharides of plant extracts by immobilized inulinases of *K. marxianus* would be an efficient and advantageous approach for commercial sugar production (Holyavka et al., 2016). The extracellular exoinulinase (from *K. marxianus* YS-1) was immobilized on Duolite A568 after partial purification and the immobilized biocatalyst was used to produce a high-fructose syrup, which yielded 40.2 and 39.2 g/L of fructose in 4 h using pure and raw inulin (from roots of *A. racemosus*), respectively (Singh et al., 2007b). Furthermore, the inulinases produced by *K. marxianus* using xylose medium as carbon source could be another promising options to produce high concentration fructose syrup at industrial level (Hoshida et al., 2018).

4.5. Aroma compounds production

Production of flavor and fragrance compounds through biotechnological process plays a significant role in several industries including food, pharmaceuticals, cosmetics and chemical industries because of the growing demand for natural food additives and other products of biological origin (SÁ et al., 2017). Two aromatic alcohols, namely 2-phenyethanol (2-PE) and 2-phenyethyl acetate (2-PEA) having a rose like flavor, are widely used in the food and cosmetics industries. Therefore, the attention is renewed to the microbial production of 2-PE and 2-PEA (Hoşoğlu, 2018; SÁ et al., 2017). The mostly used biotechnological route of producing 2-PE and 2-PEA is the bioconversion of *L*-phenylalanine (L-phe) using food-grade yeasts via Ehrlich pathway (Etschmann et al., 2004). In this process, L-phe is transformed into phenylpyruvate (an intermediate metabolite), that is later decarboxylated to phenylacetaldehyde and then reduced to 2-PE though dehydrogenation. Thereafter, 2-PE can be transesterified to 2-PEA (Martínez et al., 2018a). Therefore, different yeast strains are receiving increased interest in catalyzing the bioconversion of L-phe to L-PE for developing an efficient biotechnological production process (Güneşer et al., 2016). Among various yeasts, several *Kluyveromyces* strains are promising candidate to synthesis significant amounts of aromatic compounds, particularly *K. marxianus* possess high potential to produce different aroma compounds such as 2-PE, alcohols, furanones, fruit esters, ketones, carboxylic acids, and aromatic hydrocarbons (Güneşer et al., 2016; Morrissey et al., 2015).

Martínez et al. (2018a) achieved a maximum 10.21 mg/g of 2-PE and 8.20 mg/g of 2-PEA through the solid-state fermentation process of sugarcane bagasse supplemented with *L*-phenylalanine using *K. marxianus* as inoculum. In another study, *K. marxianus* CCT7735 was the most outstanding strain among 267 strains to produce the maximum 2-PE (3.44 g/L) titer under optimized conditions of 30 °C temperature, 3.0 g/L of glucose, and L-phe concentrations of 4.0 g/L (De Lima et al., 2018). The growth of *K. marxianus* CCT7735 was impaired by the concentration of 2-PE in the medium, however, this effect was less

Table 3
Flavor compounds produced by *K. marxianus* in different medium during fermentation.

Aroma compounds	Flavors	Substrate used	Applications	References
2-Phenylethanol (2-PE)	Rose-like smell	Whey	Food industry: fruit formulas, ice cream, candy, soft drinks, gelatins, puddings, rubber gum; Pharmaceutical industry: antiseptic and local anesthetic; Perfumes and cosmetics	(Conde-Báez et al., 2017)
2-Phenethyl acetate (2-PEA)	Floral and rose-like odor	Sugarcane bagasse	Food, fragrance, cosmetic industries	(Martínez et al., 2018a)
Phenylethyl propanate	Caramel aromas	YPD medium	Flavors, fragrances	(Hoşoğlu, 2018)
Ethyl acetate	Fruity and sweet	Whey permeate	Manufacturing inks, adhesives, photoresists, coating formulations, and utilized as an extracting agent.	(Löser et al., 2013)
Isoamyl alcohol	Banana like smell	YPD medium	Flavors, fragrances	(Hoşoğlu, 2018)
Isoamyl acetate	Sweet aromatic, fruity smell like banana or pear	YPD medium	Flavors, fragrances	(Hoşoğlu, 2018)
2-phenylethyl-isobutyrate	Floral smell	Whey and pomaces	Flavoring and fragrance agent used in decorative cosmetics, fine fragrances, shampoos, toilet soaps, other toiletries, and non-cosmetic products	(Güneşer et al., 2016)

pronounced compared to that of reported for certain *S cerevisiae* strains (De Lima et al., 2018). The growth inhibition of *K. marxianus* was observed while the concentration of 2-PE reached a critical value of ~1.4 g/L (Fabre et al., 1998). Table 3 shows several flavor compounds produced by *K. marxianus* during fermentation.

Garavaglia et al. (2007) observed that the yield of 2-PE was significantly affected by medium pH, temperature, L-phe concentration, and oxygen concentration. An optimum yield of 2-PE (0.59 g/L) was achieved using *K. marxianus* CBS6556 on grape must at a pH of 7.0, L-phe concentration of 3.0 g/L, temperature of 37 °C, and oxygen mass transfer of 2.0/h. In a recent study, Gethins et al. (2015) noticed that the carbon and nitrogen source had a pronounced effect on the yield of volatile and flavor metabolites production using *K. marxianus*. The highest levels of the 2-PE and isoamyl alcohol obtained while yeast extract used as supplementary nitrogen source, however, ammonium showed a repressing effect on the 2-PEA production (Gethins et al., 2015). In contrast, nitrogen source did not show any influence on isoamyl acetate or ethyl acetate production, attributed to the fact that more than one alcohol acetyl transferase activity was present in *K. marxianus*. Moreover, the lower production of all acetate esters in a growth medium containing lactose as a unique carbon source compared to glucose or fructose, indicates a lower pool of the acetyl-CoA (Gethins et al., 2015). The ethyl acetate is derived from the esterification of acetyl-CoA and ethanol by the action of alcohol acyltransferase enzyme which catalyzes the esterification reaction of aliphatic and aromatic alcohols and acyl-CoA into esters. Thus, probably *K. marxianus* DSM5422 synthesized the ethyl acetate from acetyl-CoA. However, it was observed that impairing the activity of the TCA cycle by limiting the availability of iron (Fe) or copper (Cu), the production of ethyl acetate can be triggered (Löser et al., 2012). This might be attributed to the diversion of acetyl-CoA to ester synthesis from the tricarboxylic acid cycle in a Fe-limiting condition because of the reduced activity of aconitase which catalyzes the stereo-specific isomerization of citrate to isocitrate, and succinate dehydrogenase (both enzymes depend on Fe) as well as for a limited oxidation of NADH in the respiratory chain because the electron transferring proteins depend on Fe and Cu (Löser et al., 2015). Recently, Löbs et al. (2018) demonstrated that the alcohol acetyl transferase *Eat1* is the critical enzyme for ethyl, isoamyl, and phenylethyl acetate production by using *K. marxianus* and that high ester biosynthesis is contingent on *Eat1* mitochondrial localization.

Lately, the efficiency of fermentation was enhanced using cell immobilization technique while *K. marxianus* was cultivated on apple/chokeberry, which resulted in higher yield of aroma compounds by immobilized *K. marxianus* stain (Wilkowska et al., 2015). Furthermore, delignified cellulosic supported *K. marxianus* strain demonstrated to be suitable for whey fermentation at high temperature. The whey after fermentation by *K. marxianus* observed to have a low level of amyl alcohols and an improved aroma with an alluring flavor compared to unfermented one (Kourkoutas et al., 2002). In addition, the higher concentration of volatile metabolites such as 2-phenylethyl isobutyrate, phenylethyl acetate, phenyl ethyl alcohol, ethyl acetate, isoamyl acetate, isovaleric acid, and isoamyl alcohol could be synthesized by a strain of Na-alginate entrapped *K .marxianus* LOCK0024 using agro-industrial wastes such as pepper and tomato pomaces, grape, and acid whey as substrate (Güneşer et al., 2016).

5. Bio-environmental applications

5.1. Organic load reduction from agri-food industry wastewater

Environmental pollution caused by extensive industrial wastewater generation is a serious issue and it cannot be avoided due to population growth, industrialization, and food production practices (Karim et al., 2019). As such, the agro-industries including dairy industry, brewery industry, apple industry, etc. generate a huge amount of waste in North America, especially, in Canada. Although there are different kinds of

agro-industries in Canada, the large amounts of wastewater generation are related to the dairy industries. Generally, these dairy industries are associated with the transformation of the raw milk to the milk, yoghurt, cheese, ice cream, butter, milk powders, and other milk products by distinct kinds of production processes (Davarnejad et al., 2018). The dairy effluents are considered as high strength wastewater as they contain a higher degree of chemical oxygen demand (COD), biochemical oxygen demand (BOD), and various kinds of nutrient compounds, mainly phosphorus and nitrogen. According to Ghaly and Singh (1989), the higher level of lactose concentration in dairy wastewater might be responsible for the high COD, for example, a lactose content of 50 g/L corresponds to a COD of 40,000–60,000 ppm and, which can interfere with the biological process of sewage disposal plants. For years, the disposal of wastewater has been problematic and often discharged into local water or fields (Price, 2019; Yousuf et al., 2017). Therefore, the effective treatment of effluents must be required, not only to minimize the environmental burden but also for the purpose of water recovery from industrial processes (Davarnejad et al., 2018). Apart from lactose, the whey also contains lipids, soluble proteins, some nitrogenous compounds, vitamins and detergents, and various kinds of mineral salts. Consequently, these abundant, inexpensive, micronutrient rich substrates could potentially be used as alternate carbon sources for bioprocessing.

The bioconversion of wastewater by using yeast could be a promising technique due to the higher sugar contents of whey (Kourkoutas et al., 2002; Zafar and Owais, 2006). Whey, a wastewater from dairy industry, contains a number of minerals and vitamins in addition to the basic sugar lactose, which may efficiently enhance the physiological activity of the cells. The bioconversion of whey to ethanol using the yeasts, particularly, *Kluyveromyces* species (Zafar and Owais, 2006) appears to be potential through the efficient bioremediation of plant effluents (Kourkoutas et al., 2002). *K. marxianus* URM 7404 showed a reduction of 78.94% COD from cheese whey during the bioethanol production (0.50 g/g, 2.57 g/L/h) (Murari et al., 2019). Moreover, *K. marxianus* is a suitable species which was able to produce alcohol and fodder yeast by fermentation of deproteinized and concentrated whey due to its special physiological characteristics. It can uptake and hydrolyze lactose to the monomers, glucose and galactose by a lactose permease and a β-galactosidase enzyme, respectively. A combined approach of biomass harvesting (such as SCO or SCP or others) and pollution potential reduction (e.g., COD reduction) could be employed using deproteinized whey as a substrate for *K. marxianus*. Schultz et al. (2006) reported that 80% COD was reduced by *K. marxianus* using deproteinized sour and sweet cheese whey as substrates to produce SCP. In another study, 42% total COD, 65% soluble COD, 53% total solids, and 90% ammonium nitrogen were reduced by successful fermentation of the whey permeates. Nevertheless, the significant reduction of suspended solids and organic nitrogen were also achieved to 60% and 17%, respectively (Ghaly and Singh, 1989).

Furthermore, the maximum COD reduction of 80.20% was achieved at a retention time of 24 h through a high biomass productivity (0.17 g/L/h) by a coculture of *K. marxianus* and *C. krusei* using whey as carbon source (Yadav et al., 2014a; Yadav et al., 2015). The removal efficiency of COD for several *K. marxianus* strains via biomass production from different substrates is presented in Table 4. Nevertheless, *K. marxianus* is also able to treat the highly acidic food processing waste effluents by removing the organic pollutants from wastewater. The lactic acid could be rapidly removed by a flocculent strain of *K. marxianus* from sauerkraut processing effluents (Hang et al., 2003; Nowak and Hang, 2003). The biodegradation of lactic acid by *K. marxianus* from sauerkraut brine was studied by Nowak and Hang (2003) and they found that the lactic acid concentration reduced by 81.2 to 90.17% after 48 h of fermentation. They observed that *K. marxianus* was the best candidate to produce a higher amount of biomass over other microbial cultures used (over 13 g of CDW per liter substrate) and, significantly consumed the organic pollutants (acetic acid, lactic acid, and ethanol) from the corn silage juice (Hang et al., 2003).

5.2. Heavy metals recovery from agri-food industry wastewater

The wastewater with the presence of heavy metals or/and radionuclides from different industrial effluents poses serious environmental threat due to the metal toxicity effects, non-degradability; and moreover, their accretion through the food chain may lead to terrible health and ecological crises. There are various kinds of physical and chemical methods to treat the wastewater for heavy metals; however, remediation through physiochemical methods is not cost effective, high energy consuming (Pal et al., 2009), and less feasible for the effluent containing complex organic matters (Aksu and Dönmez, 2000; Fonseca et al., 2008). Therefore, the bioremediation (biotechnological approaches) of heavy metals and other pollutants has been getting renewed attention in the recent time because of its potential application in many industries (Fonseca et al., 2008; Islam et al., 2018). Several microorganisms including *S. cerevisiae*, *C. utilis*, *K. marxianus* have much potential for removing metals from wastewater either by active or passive uptake mechanisms. There are two ways of bioremediation by microorganisms, bioaccumulation (a metabolism-dependent slow uptake) and biosorption (a metabolism-independent rapid surface reaction) (Aksu and Dönmez, 2000). Yeast cells show a better performance over other microorganisms due to their special characteristics of metal uptake (Pal et al., 2009), faster growth rate on cheap media, ease of cultivation at large scale, and their capability of accumulating a wide range of heavy metals under different external conditions (Aksu and Dönmez, 2000; Karim et al., 2018).

Aksu and Dönmez (2000) proposed a microbiological approach to remove copper (II) or Cu^{2+} ions using *K. marxianus* in molasses. They observed that, at a constant initial Cu^{2+} concentration (100 mg/L), *K. marxianus* efficiently removed copper ions with a maximum uptake of 8.0 and 9.8 mg/g of dry biomass at an initial sucrose concentration of 5 and 20 g/L, respectively. Increase in the biomass biomass production, Cu^{2+} accumulation, and specific Cu^{2+} uptake was observed with increasing initial sucrose concentration attributed to the defense mechanism of *K. marxianus* cells, namely acclimation to toxicity. Furthermore, the rate of Cu^{2+} bioaccumulation was also observed to increase (4.3 to 55.0 mg/g) with increasing initial Cu^{2+} concentration (from 50 to 500 mg/L); however, the growth rate was decreased from 0.082 to 0.038 h^{-1} at same initial sucrose concentration (5 g/L). This might be due to the inhibitory effects of excess Cu^{2+} concentration in the medium. The maximum growth and bioaccumulation by *K. marxianus* were determined at an optimum pH of 4.0. It achieved a maximal Cu^{2+} uptake capacity of 63.6 mg/g at the end of the exponential phase, while the initial Cu^{2+} and initial sucrose concentrations were 512.2 mg/L and 20.4 g/L, respectively. However, the Cu^{2+} concentration was only decreased by 48.4% after 8 days of biomass growth. The overall results of that study imply that *K. marxianus* possess a high level of copper (II) resistance, which probably facilitated by constitutive production of metallothionein as well as synthesis of other copper-binding proteins. Moreover, they could have enough energy reserves for active transport of Cu^{2+} metal that eventually deposited into the vacuoles, which become expanded with the increasing time of exposure to the Cu^{2+} solution.

Similarly, Dönmez and Aksu (1999) reported that the performance of accumulating copper (II) and the ability of microbial growth were mostly dependent on the initial concentration of Cu^{2+} and the pH of medium. The maximum Cu^{2+} accumulation was obtained at the optimal pH vales of 5.0, 4.0, 4.0, and 4.0 for *K. marxianus*, *S. cerevisiae*, *Chizosaccharomyces pombe*, and *Candida* sp., respectively. *K. marxianus* and *Candida* sp. were more efficient than *S. cerevisiae* and *C. pombe* in respect to heavy metal resistance and bioaccumulation of Cu^{2+} at higher Cu^{2+} concentrations without losing their biological activities. The maximum removal efficiency of Cu^{2+} was achieved to 25, 72.6, 74.2, and 90.3% for *S. pombe*, *Candida* sp., *S. cerevisiae*, and *K.*

Table 4
COD removal efficacy and simultaneous biomass (SCP) production performances of several *Kluyveromyces* species from waste effluents.

Strains	Substrates	Objectives	Initial COD (gL^{-1})	Removal efficiency (%)	Conversion rate (%)	Process conditions	References
K. marxianus GQ 506972	Diluted cheese whey	COD removal and biomass (SCP) production	50	55–78.50	12–19	Batch fermentation with high cell densities and continuous process	(Yadav, Bezawada, Elharche, et al., 2014)
K. marxianus CBS 6556	Deproteinized sweet whey	COD removal and SCP production	193	90	52	Batch fermentation	(Schultz et al., 2006)
K. marxianus CBS 6556	Deproteinized sour whey	COD removal and biomass (SCP) production	150	83	48	Batch fermentation	(Schultz et al., 2006)
K. marxianus	Deproteinized cheese whey	COD removal and SCP production	–	96.26	30	Batch, $(NH_4)_2$. SO_4 as nitrogen supplementation	(Anvari and Khayati, 2011)
K. fragilis	Deproteinized sweet cheese whey	SCP production	–	–	55	Batch, Enriched matrix	(Kebbouche-Gana and Touzi, 2001)
K. marxianus	Whey permeate, 4.5% (*w/v*) solution	SCP production	~48	75.60	84	Batch fermentation, 0.22% (w/v) urea as nitrogen source	(Yadav et al., 2016)
K. fragilis	Acid cheese whey	COD removal and biomass (SCP) production	74.22	42.98	40.98	Batch, without supplementation	(Ghaly and Kamal, 2004)
K. marxianus CHY 1612	Diluted cheese whey	COD removal and SCP production	30	78	26	Batch fermentation, 0.15% (w/v) urea as nitrogen source	(Yadav, Bezawada, Ajila, et al., 2014)
K. marxianus ATCC 36907	Cassava wastewater	COD removal and and 2-phenylethanol	16.09	80.42	13.08	Batch, glucose and phenylalanine as supplement	(de Oliveira et al., 2013)
K. marxianus + *S. cerevisiae*	Whey permeate, 4.5% (w/v) solution	SCP production	~52	73.30	92	Batch fermentation, 0.22% (w/v) urea as nitrogen source	(Yadav et al., 2016)
Serratia marcescens + *K. fragilis*	Fresh sweet whey	Protease production and COD removal	88	76	46	Batch fermentation	(Ustáriz et al., 2007)
K. marxianus + *C. krusei*	Diluted cheese whey	COD removal and biomass (SCP) production	30	86.80	31	Batch fermentation, 0.15% (w/v) urea as nitrogen source and TSB media	(Yadav, Bezawada, Ajila, et al., 2014)
K. marxianus + *C. krusei*	Diluted cheese whey	COD removal and SCP production	30	26–80.2	17–27	Continuous process, 6 h to 24 h HRT	(Yadav, Bezawada, Ajila, et al., 2014)
K. marxianus + *C. krusei*	Diluted cheese whey	COD removal and SCP production	30	80.20	37.20	Batch fermentation, without any supplement	(Yadav et al., 2015)

Table 5
Valorization of different low-cost waste by-products of the food and agricultural industries by *K. marxianus*.

Strains	Substrates	Process	Purpose	References
K. marxianus K21	Agro-industrial waste -Taro waste	Simultaneous saccharification and fermentation	Bioethanol production	(Wu et al., 2016)
K. marxianus IFO 0288	Dairy waste -Cheese whey	Hybrid fermentation–enzymatic bioprocess	Ethanol and lactic acid produced in fermentations were esterified to ethyl lactate	(Koutinas et al., 2014)
K. marxianus IMB3	Agro-industrial wastes -Dairy industry (Cheese whey) -Sugar industry (Molasses) -Solid wastes (brewer's spent grains and malt spent rootlets) -Vegetable and fruit processing wastes (potato and citrus)	Solid-state fermentation	Single cell protein, aroma volatiles and fat production	(Aggelopoulos et al., 2014)
K. marxianus LOCK0024	Agro-industrial wastes (tomato, pepper, grape, and acid whey)	Liquid fermentation	Ethanol and volatile metabolites (flavor compounds) such as ethyl acetate, isoamyl alcohol, isoamyl acetate, 2-phenylethyl isobutyrate, phenylethyl acetate, and phenylethyl alcohol production	(Güneşer et al., 2016)
K. marxianus NRRL Y-8281	Agro-industrial wastes -Olive pomace	Solid-state fermentation	Tannin acyl hydrolase (tannase, E.C.3.1.1.20) and gallic acid production	(Mahmoud et al., 2018)
K. marxianus var. *marxianus* CBS 712.	Dairy industry wastes -Whey effluent -Scotta effluent	Semi-continuous fermentations	Bioethanol production	(Zoppellari and Bardi, 2013)
K. marxianus ATCC 10022	Agro-industrial wastes -Sugarcane bagasse -Sugar beet molasses	Solid-state fermentation (Batch, intermittent mixing and fed batch)	Aroma compounds production	(Martínez et al., 2018b)
K. marxianus KCTC 7118	Vegetable waste -Chinese cabbage	Liquid fermentation	Microbial biomass production	(Choi and Park, 2003)
K. marxianus BY25569	Agricultural wastes -Rice waste biomass	Solid-state fermentation	Bioethanol production	(Saratale et al., 2017)
K. marxianus Y01070	Industrial wastes -Old corrugated cardboard -Paper sludge	Simultaneous saccharification and fermentation	Bioethanol production	(Kádár et al., 2004)
K. marxianus	Dairy industry -Whey	Liquid fermentation	Biomass production	(Koutinas et al., 2009)
K. marxianus (own isolates)	Agro-industrial waste -Overripe mango pulp	Liquid fermentation	Bioethanol production	(Buenrostro-Figueroa et al., 2018)
K. marxianus (own isolates)	Agro-industrial waste -Cheese whey (raw whey, wastewater and swab samples)	Liquid fermentation	Bioethanol production	(Hesham et al., 2014)
K. marxianus CBS1555 (KCTC7001)	Lignocellulosic material -Empty palm fruit bunches (EFBs)	Simultaneous saccharification and fermentation	Bioethanol production	(Jung et al., 2015)
K. marxianus var. marxianus CBS 397	Dairy industry waste -ricotta cheese whey ("Scotta") -Raw cheese whey -Deproteinized whey	Liquid fermentation (batch bioreactor)	Bioethanol production	(Sansonetti et al., 2009)

marxianus, respectively. *Candida* sp. was highly resistant to Cu^{2+} concentration of 708 mg/L compared to *S. cerevisiae* (291 mg/L), *S. pombe* (101 mg/L), and *K. marxianus* (488 mg/L). On the contrary, an inhibitory effect on the growth of cell was observed for lead (II) uptake by *K. marxianus* from the contaminated molasses. However, the decline in biomass production did not lead to decrease lead (II) uptake; and the biosorption ability was greater at higher initial lead (II) concentrations (Fonseca et al., 2008). The capability of *K. marxianus* in biosorption of uranium was also reported by Bustard et al. (1997) where 120 mg U/g (dry weight basis) of biomass was achieved in the same time.

Furthermore, the bioaccumulation of metal cations such as Cu (II), Zn (II), Co (II) using both free and immobilized *K. marxianus* cells was studied by Yusef (1997). However, it was established that the main mechanism of metal accumulation in *K. marxianus* might be the absorption of metals by insoluble cellular materials (Yazgan and Özcengiz, 1994). The role of functional groups (i.e., carboxylic acids, amines, phosphates, sulfhydryl etc.) for biosorption of several heavy metals including lead, arsenic, cobalt, mercury, and cadmium using *K. marxianus* in different aqueous solutions was demonstrated and optimized by Pal et al. (2009) to ease the biosorption process in metal recovery process. Perhaps the most significant practical limitation to biological uptake is inhibition of cell growth in high metal ion concentration. Another important constraint can be the toxicity of wastewater to living cells such as extremes pH and high salt concentration. However, such limitations would not preclude the applications of living cells in the wastewater treatment processes through the bioremoval of heavy metals. The problem of metal toxicity may be overcome using a metal-resistant microbe like *K. marxianus*. Indeed, the tolerance and uptake capacities of a living organisms are the most preferential characteristics to be effectively utilized in a metal-ion removal process.

5.3. Paper waste and sludge treatment

Paper and pulp mill industries are responsible for the production of huge amount of wastes in many countries. The paper sludge is the solid waste stream, which generally contains short cellulose fibers, contaminants, and other paper making components such as clays and fillers (Chen et al., 2014). The disposing of this waste stream (highly

contaminated sludge or biosolids) makes the paper production costly; and hence, it provoked the entrepreneurs and the government to find out new options to use these biosolids (Chen et al., 2014; Kádár et al., 2004). However, paper sludge could be an attractive biomass source for the production of various value added products such as fuel ethanol, due to its high and easily accessible cellulose content of 50–60% (Kádár et al., 2004), low cost, and lack of special pretreatment requirement (Chen et al., 2014).

Kádár et al. (2004) investigated the efficiency of *K. marxianus* to utilize various paper mill substrates, such as paper sludge, old corrugated cardboard (OCC) waste, and Solka Floc in the SSF to produce ethanol. The result showed that *K. marxianus* was as good as *S. cerevisiae* in SSF at 40 °C using paper sludge and OCC, and the cellulose conversions of 55–60% were achieved for all substrates. No significant differences in the yield of ethanol production were observed between *K. marxianus* and *S. cerevisiae*, and the yield was 0.31 to 0.34 g/g cellulose added for both strains. These results showed that the conversion of lignocellulosic industrial wastes like paper sludge and OCC would efficiently be utilized to produce bioethanol in SSF. In another study, SSF experiments were performed at a higher temperature (32–45 °C) by utilizing Solka Floc (10%) as cellulose substrate, and the highest ethanol production (38 g/L) was achieved in 78 h using both *K. marxianus* and *K. fragilis* at a temperature of 42 °C (Ballesteros et al., 1991). As it was mentioned elsewhere, *K. marxianus* was not able to efficiently convert cellobiose to ethanol; however, this result demonstrated that *K. marxianus* can convert cellulose to ethanol in SSF batch system, even at 45 °C (Barron et al., 1995). Barron et al. (1995) showed that a maximum ethanol production (10 g/L; 39% of theoretical yield) can be achieved using milled paper as nutrient source (5% (w/v) cellulose with 0.75% (v/v) cellulase) at a high temperature (45 °C). The yield of ethanol production could be further increased by pretreating the milled paper with phosphoric acid (Nilsson et al., 1995), as the accessibility of substrate was improved after the pretreatment which resulting in a considerably higher ethanol yield. It is worth noting that *K. marxianus* was reported to utilize not only the paper wastes, but also a wide range of low-cost substrates from diversified sources as demonstrated in Table 5.

5.4. Biosorption of dyes

The textile industries have been discharging large volumes of wastewater into natural water bodies after dyeing process, which leads to serious environmental pollution. Generally, the effluents from textile industries contain huge amounts of toxic chemicals such as azo dyes, and reactive dyes. The excessive discharge of these effluents may adversely affect the natural resources, aquatic species, water quality, and soil fertility; and strongly disturb the integrity of ecosystems (Droste and Gehr, 2018). Discharging of effluents without adequate removal of these dyes might be responsible for severe environmental issues (Holkar et al., 2016). Therefore, the degradation of the dyes in textile effluents is indispensable to avoid toxicity. So far, various methods including physiochemical (such as coagulation, flocculation, flotation, ion exchange, irradiation, electrochemical destruction, adsorption, ozonation, precipitation, and chemical oxidation etc.) and biological methods were adopted for the reduction of azo dyes to achieve decolorization (Droste and Gehr, 2018; Holkar et al., 2016). Some of those methods have been proven to be efficient; however, the excess usage of chemicals, long time requirement, large amounts of sludge generation, high plant installment cost, and high operating and maintenance costs limited their application. The activated carbon has been reported to be a suitable dye absorbent but the manufacturing and regeneration of this absorbent is expensive (Priya and Selvan, 2017).

Recently, the microbial degradation and decolorization of textile effluents to detoxify the azo dyes has gained great attention from both industries and the scientists' community due to eco-friendly nature, general simplicity, and inexpensive technologies (Sudha et al., 2014). The toxicity and carcinogenicity of textile dyes is mostly due to the several toxic compounds including benzidine and other aromatic components, which could be successfully converted into simple compounds through microbial metabolism (Sudha et al., 2014). Therefore, biosorption technique could be a promising low-cost alternative to efficiently remove color from textile effluents. Certain types of microbial biomass displayed a strong biosorbent behavior towards some metallic ions and other contaminants, like the dyes from textile, as a function of the chemical make-up of the microbial cells of which the biomass consists (Mrudula et al., 2016). Furthermore, several charged pollutants such as acetamide groups of chitin; amido, amino, sulfhydryl and carboxyl groups in proteins; hydroxyl groups in polysaccharides; phosphate groups in nucleic acids could be attracted and sequestered by several chemical groups in biomass (Roane et al., 2015). According to Kakuta et al. (1998), several yeasts were found to be suitable as metal absorbent because of the ability to degrade the synthetic dyes. Meehan et al. (2000) observed that *K. marxianus* was capable of decoloring the solution of Remazol Black-B dye. The solution with Remazol Black-B was completely decolorized within 24 h by actively growing *K. marxianus* IMB3 cells under an aerobic condition and a maximum of 98% color removal was achieved at a temperature of 37 °C. They also demonstrated that the decolorization was mainly due to the physical adsorption of the dyes to cellular biomass, not due any chemical enzymatic activity (Meehan et al., 2000). However, the mechanism by which the cellular biomass takes-up the dye components was unexplained. In addition, the main challenge in biosorption based processes could be that, the use of biomass may lead to a large amount of sludge generation, which may require a further treatment like the use of solid-state fermentation. Therefore, the problem would be overcome using the microbes that capable of carrying out solid state fermentation including white rot fungi, *K. marxianus*, etc. which have been shown to be effective for textile dye decolorization (Senthilkumar et al., 2014).

6. Concluding remarks and future perspective

S. cerevisiae is a model yeast species which has been exploited for the applications in the biotechnology in addition to basic research in the fields of cell biology, biochemistry, and genetics. However, due to the advancement of biotechnological tools in addition to its intrinsically exceptional characteristics, *K. marxianus* has become one of the top interesting nonconventional yeasts, comparable to *S. cerevisiae*, at least for industrial applications (Nurcholis et al., 2020). Thermotolerance, high ethyl acetate production, utilization of a wide-ranging inexpensive carbon sources (e.g., inulin, lactose, and xylose), a shorter doubling time and faster growth rate make *K. marxianus* as a versatile tool in biotechnological, food and environmental applications. Moreover, the survivability of *K. marxianus* in the presence of relatively higher ethanol concentration and/or elevated temperature indicating its improved adaptation capacity. It was displayed in several literatures that *K. marxianus* possess tolerance to the different stressing conditions such as lower pH, elevated temperature, severe oxygen restraint condition, and higher concentration of ethanol which could be very beneficial for the biorefinery approach-based future industrial applications. In addition, the bioingredients such as flavor and fragrance molecules, SCPs, SCOs production capability and protein secretion performance of *K. marxianus* could be beneficial to the food-based industries by diversifying the product portfolio in future (Güneşer et al., 2016; Madeira-Jr and Gombert, 2018; Saini et al., 2017).

On the other hand, the metabolically engineered *K. marxianus* strains with inulin-assimilating capacity, can produce lactic acid by digestion of Jerusalem artichoke tuber powder (Bae et al., 2018). This approach to harvest optically pure L-lactic acid and/or D-lactic acid using recombinant stains could lead to the efficient production of bioplastics in a cost-effective way. However, most of the studies with *K. marxianus* dedicated on the liquid fermentation (LF) to date. The major setback of this technique was the inhibition of microbial cells due to a

Table 6
List of the equivalent strain repository numbers of *K. marxianus* strains (www.atcc.org).

ATCC strain of *K. marxianus*	Equivalent strain repository
ATCC 200963	HA 63 [NRRL Y-8281, CBS 712]
ATCC 22296, ATCC 56501	CBS 5671 [K.210, NRRL Y-8287, UCD 71-15]
ATCC 8554, ATCC 34439	CBS 5795, CCRC 21480
ATCC 2340	NRRL Y-6
ATCC 26548	NRRL Y-7571 [CBS 6556, KCTC 17555]
ATCC 26548	KCTC 17555, CBS 6556
ATCC 64885	NRRL Y-1175
ATCC 74080	pKD1
ATCC 200964	HA 732 [CBS 2231]
ATCC 28912	CCY 21-40-1
ATCC 46537, ATCC 56497, ATCC 56752	CBS 397 [CCRC 2147]
ATCC 36907	NCYC 587 [351]
ATCC 60480	SG 120 [4-67-2/4]
ATCC 200965	HA 729 [CBS 600]
ATCC 10022, ATCC 28126	NRRL Y-665 [CBS 6432, CCRC 21628]
ATCC 28244	5850 [VTT C-81111]

high volume of final product (such as 2-PE, bioethanol) concentration in the medium. Several strategies like stain development, cell immobilization, in situ product removal, and culture optimization were commonly employed using synthetic media as substrate to overcome this problem (Martínez et al., 2018a). Solid-state fermentation or dry fermentation (DF) using waste biomass as substrate can be of particular interest in this regard, over LF due to the smaller reactor capacity requirements, lower water content, no additional stirring and so on. However, the larger amounts of inoculum requirements and much longer retention time might be challenging in DF. Therefore, further study is required to engineer the proper techniques and appropriate reactor design for a large-scale application of DF. Nowadays, bioethanol production from LCB hydrolysates using *K. marxianus* has also been getting more importance due to the availability of LCB in nature. But then again, the fermentation of LCB might be difficult to digest due to the unique crystalline structure of LCB. The study with different kinds of pre-treatment methods, in this regard, could be attractive in future research to make the biomass more accessible for the microorganisms. Furthermore, the evolutionary adaptation and immobilization techniques of *K. marxianus* would be evaluated to increase the efficiency of various value-added product through economically viable fermentation processes (Table 6).

Considering all these aspects, it can be argued that the practical research on *K. marxianus* towards industrial application has come into a new era. On the contrary, the diversity of *K. marxianus* species at the genetic, metabolic, and physiological levels is relatively little explored, which would be designed via new engineering tools for producing valuable materials or productivity based on genomic and transcriptomic information and data from modeling of the metabolism (Nurcholis et al., 2020). For a fruitful commercial production platform, it is emergent to instigate the different aspects of strains and process development. In particular, the knowledge gaps around genes, pathways, enzymes and their regulation are sorely needed to fill for a comprehensive understanding of how *K. marxianus* produces relevant metabolites (Morrissey et al., 2015). To set the genetic and phenotypic diversity of *K. marxianus* into context, the metabolic pathway and its mechanism showed variation and functional diversities, hence there are still many opportunities for future study. Exploring of the relationship between genetic variation and functional diversities at species level could be the potential research opportunity for innovative and advanced knowledge. The pilot scale study as well as extensive lab-scale evolution of metabolic engineering would be employed to understand the mechanism of metabolism to justify the applicability in industrial level. Finally, it seems very likely that a wide range of applications of *K. marxianus* is exists but a greater depth of knowledge in the genetic diversity or population genetics is still required. This review on the diversified applications of *K. marxianus*, perhaps will pave the way for more in-depth studies in the biotechnological and environmental arena.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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References

Aggelopoulos, T., Katsieris, K., Bekatorou, A., Pandey, A., Banat, I.M., Koutinas, A.A., 2014. Solid state fermentation of food waste mixtures for single cell protein, aroma volatiles and fat production. Food Chem. 145, 710–716.
Akanni, G.B., du Preez, J.C., Steyn, L., Kilian, S.G., 2015. Protein enrichment of an Opuntia ficus-indica cladode hydrolysate by cultivation of Candida utilis and Kluyveromyces marxianus. J. Sci. Food Agric. 95, 1094–1102.
Aksu, Z., Dönmez, G., 2000. The use of molasses in copper (II) containing wastewaters: effects on growth and copper (II) bioaccumulation properties of Kluyveromyces marxianus. Process Biochem. 36, 451–458.
Alimardani-Theuil, P., Gainvors-Claisse, A., Duchiron, F., 2011. Yeasts: an attractive source of pectinases—from gene expression to potential applications: a review. Process Biochem. 46, 1525–1537.
Almeida, C., Brányik, T., Moradas-Ferreira, P., Teixeira, J., 2003. Continuous production of pectinase by immobilized yeast cells on spent grains. J. Biosci. Bioeng. 96, 513–518.
Altieri, C., 2016. Dairy propionibacteria as probiotics: recent evidences. World J. Microbiol. Biotechnol. 32, 172.
Anderson, P.J., McNeil, K.E., Watson, K., 1988. Thermotolerant single cell protein production byKluyveromyces marxianus var. marxianus. J. Ind. Microbiol. 3, 9–14.
Anvari, M., Khayati, G., 2011. Submerged yeast fermentation of cheese whey for protein production and nutritional profile analysis. Adv. J. Food Sci. Technol. 3, 122–126.
Arous, F., Azabou, S., Triantaphyllidou, I.E., Aggelis, G., Jaouani, A., Nasri, M., Mechichi, T., 2017. Newly isolated yeasts from Tunisian microhabitats: lipid accumulation and fatty acid composition. Engineering in Life Sciences 17, 226–236.
Arrizon, J., Morel, S., Gschaedler, A., Monsan, P., 2011. Purification and substrate specificities of a fructanase from Kluyveromyces marxianus isolated from the fermentation process of Mezcal. Bioresour. Technol. 102, 3298–3303.
Bae, J.-H., Kim, H.-J., Kim, M.-J., Sung, B.H., Jeon, J.-H., Kim, H.-S., Jin, Y.-S., Kweon, D.-H., Sohn, J.-H., 2018. Direct fermentation of Jerusalem artichoke tuber powder for production of L-lactic acid and D-lactic acid by metabolically engineered Kluyveromyces marxianus. J. Biotechnol. 266, 27–33.
Ballesteros, I., Ballesteros, M., Cabanas, A., Carrasco, J., Martin, C., Negro, M., Saez, F., Saez, R., 1991. Selection of thermotolerant yeasts for simultaneous saccharification and fermentation (SSF) of cellulose to ethanol. Appl. Biochem. Biotechnol. 28, 307–315.
Banat, I.M., Nigam, P., Marchant, R., 1992. Isolation of thermotolerant, fermentative yeasts growing at 52 C and producing ethanol at 45 C and 50 C. World J. Microbiol. Biotechnol. 8, 259–263.
Bansal, S., Oberoi, H.S., Dhillon, G.S., Patil, R., 2008. Production of β-galactosidase by Kluyveromyces marxianus MTCC 1388 using whey and effect of four different methods of enzyme extraction on β-galactosidase activity. Indian J. Microbiol. 48, 337–341.
Barberis, S., Gentina, J.C., 1998. Research note: effect of dissolved oxygen level on lactase production by Kluyveromyces fragilis. Journal of Chemical Technology & Biotechnology: International Research in Process, Environmental AND Clean Technology 73, 71–73.
Barnby, F.M., Morpeth, F., Pyle, D., 1990. Endopolygalacturonase production from Kluyveromyces marxianus. I. Resolution, purification, and partial characterisation of the enzyme. Enzym. Microb. Technol. 12, 891–897.
Barron, N., Marchant, R., McHale, L., McHale, A., 1995. Studies on the use of a thermotolerant strain of Kluyveromyces marxianus in simultaneous saccharification and ethanol formation from cellulose. Appl. Microbiol. Biotechnol. 43, 518–520.
Bayless, T.M., Brown, E., Paige, D.M., 2017. Lactase non-persistence and lactose intolerance. Current gastroenterology reports 19, 23.
Belem, M., Lee, B., 1998. Production of bioingredients from Kluyveromyces marxianus grown on whey: an alternative. Crit. Rev. Food Sci. Nutr. 38, 565–598.
Beluche, I., Guiraud, J., Galzy, P., 1980. Inulinase activity of *Debaromyces cantarelli*. Folia Microbiol. 25, 32–39.
Bender, J.P., Mazutti, M.A., De Oliveira, D., Di Luccio, M., Treichel, H., 2006. Inulinase production by Kluyveromyces marxianus NRRL Y-7571 using solid state fermentation. In: Twenty-Seventh Symposium on Biotechnology for Fuels and Chemicals. Springer, pp. 951–958.

Beniwal, A., Saini, P., Kokkiligadda, A., Vij, S., 2018. Use of silicon dioxide nanoparticles for β-galactosidase immobilization and modulated ethanol production by co-immobilized K. marxianus and S. cerevisiae in deproteinized cheese whey. LWT-Food Science and Technology 87, 553–561.
Bergkamp, R.J., Bootsman, T.C., Toschka, H.Y., Mooren, A.T., Kox, L., Verbakel, J.M., Geerse, R.H., Planta, R.J., 1993. Expression of an α-galactosidase gene under control of the homologous inulinase promoter in Kluyveromyces marxianus. Appl. Microbiol. Biotechnol. 40, 309–317.
Blanco, P., Sieiro, C., Villa, T.G., 1999. Production of pectic enzymes in yeasts. FEMS Microbiol. Lett. 175, 1–9.
Blank, L.M., Lehmbeck, F., Sauer, U., 2005. Metabolic-flux and network analysis in fourteen hemiascomycetous yeasts. FEMS Yeast Res. 5, 545–558.
Bonekamp, F.J., Oosterom, J., 1994. On the safety of Kluyveromyces lactis—a review. Appl. Microbiol. Biotechnol. 41, 1–3.
Braga, A.R.C., Gomes, P.A., Kalil, S.J., 2012. Formulation of culture medium with agroindustrial waste for β-galactosidase production from Kluyveromyces marxianus ATCC 16045. Food Bioprocess Technol. 5, 1653–1663.
Brakemeier, A., Wullbrandt, D., Lang, S., 1998. Candida bombicola: production of novel alkyl glycosides based on glucose/2-dodecanol. Appl. Microbiol. Biotechnol. 50, 161–166.
Buenrostro-Figueroa, J., Tafolla-Arellano, J.C., Flores-Gallegos, A.C., Rodríguez-Herrera, R., De la Garza-Toledo, H., Aguilar, C.N., 2018. Native yeasts for alternative utilization of overripe mango pulp for ethanol production. Revista Argentina de microbiologia 50, 173–177.
Bustard, M., Donnellan, N., Rollan, A., McHale, A., 1997. Studies on the biosorption of uranium by a thermotolerant, ethanol-producing strain of Kluyveromyces marxianus. Bioprocess Eng. 17, 45–50.
Caballero, R., Olguín, P., Cruz-Guerrero, A., Gallardo, F., García-Garibay, M., Gómez-Ruiz, L., 1995. Evaluation of Kluyveromyces marxianus as baker's yeast. Food Res. Int. 28, 37–41.
Cameron, D.R., Cooper, D.G., Neufeld, R., 1988. The mannoprotein of Saccharomyces cerevisiae is an effective bioemulsifier. Appl. Environ. Microbiol. 54, 1420–1425.
Cardoso, V.M., Borelli, B.M., Lara, C.A., Soares, M.A., Pataro, C., Bodevan, E.C., Rosa, C.A., 2015. The influence of seasons and ripening time on yeast communities of a traditional Brazilian cheese. Food Res. Int. 69, 331–340.
Cernak, P., Estrela, R., Poddar, S., Skerker, J.M., Cheng, Y.-F., Carlson, A.K., Chen, B., Glynn, V.M., Furlan, M., Ryan, O.W., Donnelly, M.K., Arkin, A.P., Taylor, J.W., Cate, J.H.D., 2018. Engineering Kluyveromyces marxianus as a robust synthetic biology platform host. Applied and Environmental Science 9, e01410–e01418.
Champagne, C., Goulet, J., Lachance, R., 1990. Production of bakers' yeast in cheese whey ultrafiltrate. Appl. Environ. Microbiol. 56, 425–430.
Chen, H., Han, Q., Daniel, K., Venditti, R., Jameel, H., 2014. Conversion of industrial paper sludge to ethanol: fractionation of sludge and its impact. Appl. Biochem. Biotechnol. 174, 2096–2113.
Chi, Z., Chi, Z., Zhang, T., Liu, G., Li, J., Wang, X., 2009. Production, characterization and gene cloning of the extracellular enzymes from the marine-derived yeasts and their potential applications. Biotechnol. Adv. 27, 236–255.
Chi, Z.-M., Zhang, T., Cao, T.-S., Liu, X.-Y., Cui, W., Zhao, C.-H., 2011. Biotechnological potential of inulin for bioprocesses. Bioresour. Technol. 102, 4295–4303.
Cho, Y.-J., Kim, D.-H., Jeong, D., Seo, K.-H., Jeong, H.S., Lee, H.G., Kim, H., 2018. Characterization of yeasts isolated from kefir as a probiotic and its synergic interaction with the wine byproduct grape seed flour/extract. LWT 90, 535–539.
Choi, M.H., Park, Y.H., 2003. Production of yeast biomass using waste Chinese cabbage. Biomass Bioenergy 25, 221–226.
Choo, J.H., Han, C., Kim, J.-Y., Kang, H.A., 2014. Deletion of a KU80 homolog enhances homologous recombination in the thermotolerant yeast Kluyveromyces marxianus. Biotechnol. Lett. 36, 2059–2067.
Cirigliano, M.C., Carman, G.M., 1985. Purification and characterization of liposan, a bioemulsifier from Candida lipolytica. Appl. Environ. Microbiol. 50, 846–850.
Collins, S., Reid, G., 2016. Distant site effects of ingested prebiotics. Nutrients 8, 523.
Coloretti, F., Chiavari, C., Luise, D., Tofalo, R., Fasoli, G., Suzzi, G., Grazia, L., 2017. Detection and identification of yeasts in natural whey starter for Parmigiano Reggiano cheese-making. Int. Dairy J. 66, 13–17.
Conde-Báez, L., Castro-Rosas, J., Villagómez-Ibarra, J., Páez-Lerma, J., Gómez-Aldapa, C., 2017. Evaluation of waste of the cheese industry for the production of aroma of roses (phenylethyl alcohol). Waste and Biomass Valorization 8, 1343–1350.
Cooper, D.G., Paddock, D., 1984. Production of a biosurfactant from Torulopsis bombicola. Appl. Environ. Microbiol. 47, 173–176.
Cordeiro, C.A.M., Martins, M.L.L., 2009. Produção de poligalacturonase, pelo termofílico Bacillus sp. e algumas de suas propriedades. Cienc. Tecnol. Aliment. 29, 135–141.
Cortés, G., Trujillo-Roldán, M.A., Ramírez, O.T., Galindo, E., 2005. Production of β-galactosidase by Kluyveromyces marxianus under oscillating dissolved oxygen tension. Process Biochem. 40, 773–778.
Corzo, G., Revah, S., 1999. Production and characteristics of the lipase from Yarrowia lipolytica 681. Bioresour. Technol. 70, 173–180.
Cruz-Guerrero, A., Garcia-Peña, I., Barzana, E., Garcia-Garibay, M., Gomez-Ruiz, L., 1995. Kluyveromyces marxianus CDBB-L-278: a wild inulinase hyperproducing strain. J. Ferment. Bioeng. 80, 159–163.
Davarnejad, R., Nikseresht, M., Ajideh, I., 2018. An efficient technique for dairy wastewater treatment. Int. J. Dairy Technol. 71, 532–538.
De Lima, L.A., Diniz, R.H.S., De Queiroz, M.V., Fietto, L.G., Da Silveira, W.B., 2018. Screening of yeasts isolated from Brazilian environments for the 2-phenylethanol (2-PE) production. Biotechnol. Bioprocess Eng. 23, 326–332.
Deive, F., Costas, M., Longo, M., 2003. Production of a thermostable extracellular lipase by Kluyveromyces marxianus. Biotechnol. Lett. 25, 1403–1406.
Dellomonaco, C., Amaretti, A., Zanoni, S., Pompei, A., Matteuzzi, D., Rossi, M., 2007. Fermentative production of superoxide dismutase with Kluyveromyces marxianus. J. Ind. Microbiol. Biotechnol. 34, 27–34.
Díaz-Vergara, L., Pereyra, C.M., Montenegro, M., Pena, G.A., Aminahuel, C.A., Cavaglieri, L.R., 2017. Encapsulated whey–native yeast Kluyveromyces marxianus as a feed additive for animal production. Food Additives & Contaminants: Part A 34, 750–759.
Diniz, R.H., Silveira, W.B., Fietto, L.G., Passos, F.M., 2012. The high fermentative metabolism of Kluyveromyces marxianus UFV-3 relies on the increased expression of key lactose metabolic enzymes. Antonie Van Leeuwenhoek 101, 541–550.
Diniz, R.H.S., Villada, J.C., Alvim, M.C.T., Vidigal, P.M.P., Vieira, N.M., Lamas-Maceiras, M., Cerdán, M.E., González-Siso, M.-I., Lahtvee, P.-J., da Silveira, W.B., 2017. Transcriptome analysis of the thermotolerant yeast Kluyveromyces marxianus CCT 7735 under ethanol stress. Appl. Microbiol. Biotechnol. 101, 6969–6980.
Dinnella, C., Stagni, A., Lanzarini, G., Laus, M., 1996. Immobilised pectinase efficiency in the depolymerisation of pectin in a model solution and apple juice. Prog. Biotechnol. 971–978 Elsevier.
Dönmez, G., Aksu, Z., 1999. The effect of copper (II) ions on the growth and bioaccumulation properties of some yeasts. Process Biochem. 35, 135–142.
Droste, R.L., Gehr, R.L., 2018. Theory and Practice of Water and Wastewater Treatment. John Wiley & Sons.
Etschmann, M., Sell, D., Schrader, J., 2004. Medium optimization for the production of the aroma compound 2-phenylethanol using a genetic algorithm. J. Mol. Catal. B Enzym. 29, 187–193.
Fabre, C.E., Blanc, P.J., Goma, G., 1998. Production of 2-phenylethyl alcohol by Kluyveromyces marxianus. Biotechnol. Prog. 14, 270–274.
Fadda, M.E., Mossa, V., Deplano, M., Pisano, M.B., Cosentino, S., 2017. In vitro screening of Kluyveromyces strains isolated from Fiore Sardo cheese for potential use as probiotics. LWT-Food Science and Technology 75, 100–106.
Flores, J.-A., Gschaedler, A., Amaya-Delgado, L., Herrera-López, E.J., Arellano, M., Arrizon, J., 2013. Simultaneous saccharification and fermentation of Agave tequilana fructans by Kluyveromyces marxianus yeasts for bioethanol and tequila production. Bioresour. Technol. 146, 267–273.
Flores-Gallegos, A.C., Contreras-Esquivel, J.C., Aguilar, C.b.N., 2015. Comparative study of fungal strains for thermostable inulinase production. J. Biosci. Bioeng. 119, 421–426.
Fonseca, G.G., Gombert, A.K., Heinzle, E., Wittmann, C., 2007. Physiology of the yeast Kluyveromyces marxianus during batch and chemostat cultures with glucose as the sole carbon source. FEMS Yeast Res. 7, 422–435.
Fonseca, G.G., Heinzle, E., Wittmann, C., Gombert, A.K., 2008. The yeast Kluyveromyces marxianus and its biotechnological potential. Appl. Microbiol. Biotechnol. 79, 339–354.
Foukis, A., Stergiou, P.-Y., Theodorou, L.G., Papagianni, M., Papamichael, E.M., 2012. Purification, kinetic characterization and properties of a novel thermo-tolerant extracellular protease from Kluyveromyces marxianus IFO 0288 with potential biotechnological interest. Bioresour. Technol. 123, 214–220.
Fukuhara, H., 2006. Kluyveromyces lactis–a retrospective. FEMS Yeast Res. 6, 323–324.
Galinari, É., Almeida-Lima, J., Macedo, G.R., Mantovani, H.C., Rocha, H.A.O., 2018. Antioxidant, antiproliferative, and immunostimulatory effects of cell wall α-d-mannan fractions from Kluyveromyces marxianus. Int. J. Biol. Macromol. 109, 837–846.
Gao, W., Bao, Y., Liu, Y., Zhang, X., Wang, J., An, L., 2009. Characterization of thermostable endoinulinase from a new strain Bacillus smithii T7. Appl. Biochem. Biotechnol. 157, 498–506.
Garavaglia, J., Flôres, S.H., Pizzolato, T.M., do Carmo Peralba, M., Ayub, M.A.Z., 2007. Bioconversion of L-phenylalanine into 2-phenylethanol by Kluyveromyces marxianus in grape must cultures. World J. Microbiol. Biotechnol. 23, 1273–1279.
Garcia-Garibay, M., Gómez-Ruiz, L., Bárzana, E., 1987. Studies on the simultaneous production of single cell protein and polygalacturonase fromKluyveromyces fragilis. Biotechnol. Lett. 9, 411–416.
Gellissen, G., Kunze, G., Gaillardin, C., Cregg, J.M., Berardi, E., Veenhuis, M., van der Klei, I., 2005. New yeast expression platforms based on methylotrophic Hansenula polymorpha and Pichia pastoris and on dimorphic Arxula adeninivorans and Yarrowia lipolytica–a comparison. FEMS Yeast Res. 5, 1079–1096.
Gethins, L., Guneser, O., Demirkol, A., Rea, M.C., Stanton, C., Ross, R.P., Yuceer, Y., Morrissey, J.P., 2015. Influence of carbon and nitrogen source on production of volatile fragrance and flavour metabolites by the yeast Kluyveromyces marxianus. Yeast 32, 67–76.
Ghaly, A., Kamal, M., 2004. Submerged yeast fermentation of acid cheese whey for protein production and pollution potential reduction. Water Res. 38, 631–644.
Ghaly, A., Singh, R., 1989. Pollution potential reduction of cheese whey through yeast fermentation. Appl. Biochem. Biotechnol. 22, 181–203.
Ghanem, K.M., 1992. Single Cell Protein Production from Beet Pulp by Mixed Culture.
Gibson, G.R., Hutkins, R., Sanders, M.E., Prescott, S.L., Reimer, R.A., Salminen, S.J., Scott, K., Stanton, C., Swanson, K.S., Cani, P.D., 2017. Expert consensus document: the International Scientific Association for Probiotics and Prebiotics (ISAPP) consensus statement on the definition and scope of prebiotics. Nature Reviews Gastroenterology and Hepatology 14, 491.
Golunski, S., Astolfi, V., Carniel, N., de Oliveira, D., Di Luccio, M., Mazutti, M.A., Treichel, H., 2011. Ethanol precipitation and ultrafiltration of inulinases from Kluyveromyces marxianus. Sep. Purif. Technol. 78, 261–265.
Grootwassink, J., Hewitt, G., 1983. Inducible and constitutive formation of-Fructofuranosidase (Inulase) in batch and continuous cultures of the yeast Kluyveromyces fragilis. Microbiology 129, 31–41.
Gudiña, E.J., Rodrigues, L.R., 2019. Research and production of biosurfactants for the food industry. Bioprocessing for Biomolecules Production 125–143.
Güneşer, O., Karagül-Yüceer, Y., Wilkowska, A., Kregiel, D., 2016. Volatile metabolites produced from agro-industrial wastes by Na-alginate entrapped Kluyveromyces

marxianus. Braz. J. Microbiol. 47, 965–972.
Gupte, A., Nair, J., 2010. β-Galactosidase Production and Ethanol Fermentation from Whey Using Kluyveromyces Marxianus NCIM 3551.
Hajhosseini, A., Doroud, D., Sharifan, A., Eftekhari, Z., 2020. Optimizing growth conditions of Kluyveromyces marxianus for Mannan production as a bioemulsifier. Applied Food Biotechnology 7, 115–126.
Hang, Y.D., Woodams, E.E., Hang, L.E., 2003. Utilization of corn silage juice by Klyuveromyces marxianus. Bioresour. Technol. 86, 305–307.
Harsa, S., Zaror, C., Pyle, D., 1993. Production of polygalacturonases from Kluyveromyces marxianus fermentation: preliminary process design and economics. Process Biochem. 28, 187–195.
Hensing, M., Vrouwenvelder, H., Hellinga, C., Baartmans, R., Van Dijken, H., 1994. Production of extracellular inulinase in high-cell-density fed-batch cultures of Kluyveromyces marxianus. Appl. Microbiol. Biotechnol. 42, 516–521.
Hesham, A.E.-L., Wambui, V., JO, H.O., Maina, J.M., 2014. Phylogenetic analysis of isolated biofuel yeasts based on 5.8 S-ITS rDNA and D1/D2 26S rDNA sequences. Journal of Genetic Engineering and Biotechnology 12, 37–43.
Hill, C., Guarner, F., Reid, G., Gibson, G.R., Merenstein, D.J., Pot, B., Morelli, L., Canani, R.B., Flint, H.J., Salminen, S., 2014. Expert consensus document: the International Scientific Association for Probiotics and Prebiotics consensus statement on the scope and appropriate use of the term probiotic. Nature Reviews Gastroenterology and Hepatology 11, 506.
Holkar, C.R., Jadhav, A.J., Pinjari, D.V., Mahamuni, N.M., Pandit, A.B., 2016. A critical review on textile wastewater treatments: possible approaches. J. Environ. Manag. 182, 351–366.
Holyavka, M., Artyukhov, V., Kovaleva, T., 2016. Structural and functional properties of inulinases: a review. Biocatalysis and Biotransformation 34, 1–17.
Hong, J., Wang, Y., Kumagai, H., Tamaki, H., 2007. Construction of thermotolerant yeast expressing thermostable cellulase genes. J. Biotechnol. 130, 114–123.
Hoshida, H., Kidera, K., Takishita, R., Fujioka, N., Fukagawa, T., Akada, R., 2018. Enhanced production of extracellular inulinase by the yeast Kluyveromyces marxianus in xylose catabolic state. J. Biosci. Bioeng. 125 (6), 676–681.
Hoşoğlu, M.İ., 2018. STUDY OF INCREASING THE PRODUCTION OF VOLATILE FLAVOR COMPOUNDS BY THE YEAST Kluyveromyces marxianus THROUGH OPTIMIZATION OF CARBON AND NITROGEN SOURCES. Food and Health 4, 112–123.
Hughes, D., Tudroszen, N., Moye, C.J., 1984. The effect of temperature on the kinetics of ethanol production by a thermotolerant strain of Kluveromyces marxianus. Biotechnol. Lett. 6, 1–6.
Iguchi, T., Takeda, I., Ohsawa, H., 1969. Emulsifying factor of hydrocarbon produced by a hydrocarbon-assimilating yeast. Agric. Biol. Chem. 33, 1657–1658.
Inokuma, K., Ishii, J., Hara, K.Y., Mochizuki, M., Hasunuma, T., Kondo, A., 2015. Complete genome sequence of Kluyveromyces marxianus NBRC1777, a nonconventional Thermotolerant yeast. Genome Announcements 3 (e00389-00315).
Inzucchi, S.E., Zinman, B., Wanner, C., Ferrari, R., Fitchett, D., Hantel, S., Espadero, R.-M., Woerle, H.-J., Broedl, U.C., Johansen, O.E., 2015. SGLT-2 inhibitors and cardiovascular risk: proposed pathways and review of ongoing outcome trials. Diabetes and Vascular Disease Research 12, 90–100.
Islam, M.A., Yousuf, A., Karim, A., Pirozzi, D., Khan, M.R., Ab Wahid, Z., 2018. Bioremediation of palm oil mill effluent and lipid production by Lipomyces starkeyi: a combined approach. J. Clean. Prod. 172, 1779–1787.
Jeong, H., Lee, D.-H., Kim, S.H., Kim, H.-J., Lee, K., Song, J.Y., Kim, B.K., Sung, B.H., Park, J.C., Sohn, J.H., Koo, H.M., Kim, J.F., 2012. Genome sequence of the thermotolerant yeast Kluyveromyces marxianus var. marxianus KCTC 17555. Eukaryot. Cell 11, 1584–1585.
Jolivet, P., Bergeron, E., Benyair, H., Meunier, J.-C., 2001. Characterization of major protein phosphatases from selected species of Kluyveromyces. Comparison with protein phosphatases from Yarrowia lipolytica. Can. J. Microbiol. 47, 861–870.
Jung, Y.R., Park, J.M., Heo, S.-Y., Hong, W.-K., Lee, S.-M., Oh, B.-R., Park, S.-M., Seo, J.-W., Kim, C.H., 2015. Cellulolytic enzymes produced by a newly isolated soil fungus Penicillium sp. TG2 with potential for use in cellulosic ethanol production. Renew. Energy 76, 66–71.
Kádár, Z., Szengyel, Z., Réczey, K., 2004. Simultaneous saccharification and fermentation (SSF) of industrial wastes for the production of ethanol. Ind. Crop. Prod. 20, 103–110.
Kakuta, T., Aoki, F., Okada, T., Shindo, H., Yoshizawa, K., Koizumi, T., Nojiro, K., 1998. Purification and properties of two different azoreductases from a yeast Candida curvata AN723. Sen'i Gakkaishi 54, 18–25.
Kalil, S.J., Maugeri-Filho, F., Rodrigues, M.I., 2005. Ion exchange expanded bed chromatography for the purification of an extracelular inulinase from Kluyveromyces marxianus. Process Biochem. 40, 581–586.
Kango, N., 2008. Production of inulinase using tap roots of dandelion (Taraxacum officinale) by Aspergillus niger. J. Food Eng. 85, 473–478.
Käppeli, O., Fiechter, A., 1977. Component from the cell surface of the hydrocarbon-utilizing yeast Candida tropicalis with possible relation to hydrocarbon transport. J. Bacteriol. 131, 917–921.
Karim, A., Yousuf, A., Islam, M.A., Naif, Y.H., Faizal, C.K.M., Alam, M.Z., Pirozzi, D., 2018. Microbial lipid extraction from Lipomyces starkeyi using irreversible electroporation. Biotechnol. Prog. 34 (4), 838–845.
Karim, A., Islam, M.A., Yousuf, A., Khan, M.M.R., Faizal, C.K.M., 2019. Microbial lipid accumulation through bioremediation of palm oil mill wastewater by Bacillus cereus. ACS Sustain. Chem. Eng. 7, 14500–14508.
Karimi, S., Mahboobi Soofiani, N., Mahboubi, A., Taherzadeh, M.J., 2018. Use of organic wastes and industrial by-products to produce filamentous fungi with potential as aqua-feed ingredients. Sustainability 10, 3296.
Kebbouche-Gana, S., Touzi, A., 2001. Valorisation du lactosérum par la production de levures lactiques avec les procédés de fermentation continue et discontinue.
Kim, J.K., Tak, K.-T., Moon, J.-H., 1998. A continuous fermentation of Kluyveromyces fragilis for the production of a highly nutritious protein diet. Aquac. Eng. 18, 41–49.
Kim, T.-Y., Lee, S.-W., Oh, M.-K., 2014. Biosynthesis of 2-phenylethanol from glucose with genetically engineered Kluyveromyces marxianus. Enzym. Microb. Technol. 61-62, 44–47.
Kourkoutas, Y., Dimitropoulou, S., Kanellaki, M., Marchant, R., Nigam, P., Banat, I., Koutinas, A., 2002. High-temperature alcoholic fermentation of whey using Kluyveromyces marxianus IMB3 yeast immobilized on delignified cellulosic material. Bioresour. Technol. 82, 177–181.
Koutinas, A.A., Papapostolou, H., Dimitrellou, D., Kopsahelis, N., Katechaki, E., Bekatorou, A., Bosnea, L.A., 2009. Whey valorisation: a complete and novel technology development for dairy industry starter culture production. Bioresour. Technol. 100, 3734–3739.
Koutinas, M., Menelaou, M., Nicolaou, E.N., 2014. Development of a hybrid fermentation–enzymatic bioprocess for the production of ethyl lactate from dairy waste. Bioresour. Technol. 165, 343–349.
Kumar, M., Nagpal, R., Kumar, R., Hemalatha, R., Verma, V., Kumar, A., Chakraborty, C., Singh, B., Marotta, F., Jain, S., 2012. Cholesterol-lowering probiotics as potential biotherapeutics for metabolic diseases. Exp. Diabetes Res. 2012.
Kushi, R., Monti, R., Contiero, J., 2000. Production, purification and characterization of an extracellular inulinase from Kluyveromyces marxianus var. bulgaricus. J. Ind. Microbiol. Biotechnol. 25, 63–69.
Lachance, M.-A., 2007. Current status of Kluyveromyces systematics. FEMS Yeast Res. 7, 642–645.
Lane, M.M., Morrissey, J.P., 2010. Kluyveromyces marxianus: a yeast emerging from its sister's shadow. Fungal Biology Reviews 24, 17–26.
Lane, M.M., Burke, N., Karreman, R., Wolfe, K.H., O'Byrne, C.P., Morrissey, J.P., 2011. Physiological and metabolic diversity in the yeast Kluyveromyces marxianus. Antonie Van Leeuwenhoek 100, 507–519.
Lertwattanasakul, N., Kosaka, T., Hosoyama, A., Suzuki, Y., Rodrussamee, N., Matsutani, M., Murata, M., Fujimoto, N., Suprayogi, Tsuchikane, K., Limtong, S., Fujita, N., Yamada, M., 2015. Genetic basis of the highly efficient yeast Kluyveromyces marxianus: complete genome sequence and transcriptome analyses. Biotechnology for Biofuels 8, 47.
Liu, H., Xie, Y.H., Xiong, L.X., Dong, R.T., Pan, C.L., Teng, G.X., Zhang, H.X., 2012. Effect and mechanism of cholesterol-lowering by kluyveromyces from tibetan kefir. Advanced Materials Research. Trans Tech Publ 1290–1298.
Liu, G.-L., Chi, Z., Chi, Z.-M., 2013. Molecular characterization and expression of microbial inulinase genes. Crit. Rev. Microbiol. 39, 152–165.
Löbs, A.-K., Schwartz, C., Wheeldon, I., 2017. Genome and metabolic engineering in non-conventional yeasts: current advances and applications. Synthetic and systems biotechnology 2 (3), 198–207.
Löbs, A.-K., Schwartz, C., Thorwall, S., Wheeldon, I., 2018. Highly multiplexed CRISPRi repression of respiratory functions enhances mitochondrial localized ethyl acetate biosynthesis in Kluyveromyces marxianus. ACS Synth. Biol. 7, 2647–2655.
Löser, C., Urit, T., Förster, S., Stukert, A., Bley, T., 2012. Formation of ethyl acetate by Kluyveromyces marxianus on whey during aerobic batch and chemostat cultivation at iron limitation. Appl. Microbiol. Biotechnol. 96, 685–696.
Löser, C., Urit, T., Stukert, A., Bley, T., 2013. Formation of ethyl acetate from whey by Kluyveromyces marxianus on a pilot scale. J. Biotechnol. 163, 17–23.
Löser, C., Urit, T., Keil, P., Bley, T., 2015. Studies on the mechanism of synthesis of ethyl acetate in Kluyveromyces marxianus DSM 5422. Appl. Microbiol. Biotechnol. 99, 1131–1144.
Louw, C., Young, P.R., Van Rensburg, P., Divol, B., 2009. Regulation of endo-poly-galacturonase activity in Saccharomyces cerevisiae. FEMS Yeast Res. 10, 44–57.
Lukondeh, T., Ashbolt, N.J., Rogers, P.L., 2003. Evaluation of Kluyveromyces marxianus FII 510700 grown on a lactose-based medium as a source of a natural bioemulsifier. J. Ind. Microbiol. Biotechnol. 30, 715–720.
Maccaferri, S., Klinder, A., Brigidi, P., Cavina, P., Costabile, A., 2012. Potential probiotic Kluyveromyces marxianus B0399 modulates the immune response in Caco-2 cells and peripheral blood mononuclear cells and impacts the human gut microbiota in an in vitro colonic model system. Appl. Environ. Microbiol. 78, 956–964.
Madeira-Jr, J.V., Gombert, A.K., 2018. Towards high-temperature fuel ethanol production using Kluyveromyces marxianus: on the search for plug-in strains for the Brazilian sugarcane-based biorefinery. Biomass Bioenergy 119, 217–228.
Mahmoud, A.E., Fathy, S.A., Rashad, M.M., Ezz, M.K., Mohammed, A.T., 2018. Purification and characterization of a novel tannase produced by Kluyveromyces marxianus using olive pomace as solid support, and its promising role in gallic acid production. Int. J. Biol. Macromol. 107, 2342–2350.
Manera, A.P., Kuhn, G., Polloni, A., Marangoni, M., Zabot, G., Kalil, S.J., de Oliveira, D., Treichel, H., Oliveira, J.V., Mazutti, M.A., 2011. Effect of compressed fluids treatment on the activity, stability and enzymatic reaction performance of β-galactosidase. Food Chem. 125, 1235–1240.
de Mansoldo, F.R.P., Junior, A.N., da Silva Cardoso, V., Maria do Socorro, S., CARVALHO, D., VERMELHO, A.B.B., 2019. Evaluation of Kluyveromyces marxianus endo-poly-galacturonase activity through ATR-FTIR spectroscopy and chemometrics. Analyst 144, 4111–4120.
Martínez, O., Sánchez, A., Font, X., Barrena, R., 2017. Valorization of sugarcane bagasse and sugar beet molasses using Kluyveromyces marxianus for producing value-added aroma compounds via solid-state fermentation. J. Clean. Prod. 158, 8–17.
Martínez, O., Sánchez, A., Font, X., Barrena, R., 2018a. Bioproduction of 2-phenylethanol and 2-phenethyl acetate by Kluyveromyces marxianus through the solid-state fermentation of sugarcane bagasse. Appl. Microbiol. Biotechnol. 102, 4703–4716.
Martínez, O., Sánchez, A., Font, X., Barrena, R., 2018b. Enhancing the bioproduction of value-added aroma compounds via solid-state fermentation of sugarcane bagasse and sugar beet molasses: operational strategies and scaling-up of the process. Bioresour. Technol. 263, 136–144.

Martínez-Corona, R., Vázquez Marrufo, G., Cortés Penagos, C., Madrigal-Pérez, L.A., González-Hernández, J.C., 2019. Bioinformatic characterization of the extracellular lipases from Kluyveromyces marxianus. Yeast 37, 149–162.
Martins, D.B.G., de Souza Jr, C.G., Simoes, D.A., de Morais Jr, M.A., 2002. The β-galactosidase activity in Kluyveromyces marxianus CBS6556 decreases by high concentrations of galactose. Curr. Microbiol. 44, 379–382.
Mazutti, M., Bender, J.P., Treichel, H., Di Luccio, M., 2006. Optimization of inulinase production by solid-state fermentation using sugarcane bagasse as substrate. Enzym. Microb. Technol. 39, 56–59.
Mazutti, M.A., Zabot, G., Boni, G., Skovronski, A., de Oliveira, D., Di Luccio, M., Rodrigues, M.I., Treichel, H., Maugeri, F., 2010. Optimization of inulinase production by solid-state fermentation in a packed-bed bioreactor. J. Chem. Technol. Biotechnol. 85, 109–114.
McKay, A., 1988. A plate assay method for the detection of fungal polygalacturonase secretion. FEMS Microbiol. Lett. 56, 355–358.
Meehan, C., Banat, I., McMullan, G., Nigam, P., Smyth, F., Marchant, R., 2000. Decolorization of Remazol Black-B using a thermotolerant yeast, Kluyveromyces marxianus IMB3. Environ. Int. 26, 75–79.
Merico, A., Sulo, P., Piškur, J., Compagno, C., 2007. Fermentative lifestyle in yeasts belonging to the Saccharomyces complex. FEBS J. 274, 976–989.
Merico, A., Galafassi, S., Piškur, J., Compagno, C., 2009. The oxygen level determines the fermentation pattern in Kluyveromyces lactis. FEMS Yeast Res. 9, 749–756.
Mifuchi, I., HOSOI, M., YOKOKURA, T., 1969. Promotion of resistance to Ehrlich ascites carcinoma cells by injection of tumor cells mixed with yeast cell wall. GANN Japanese Journal of Cancer Research 60, 655–656.
Moré, M.I., Swidsinski, A., 2015. Saccharomyces boulardii CNCM I-745 supports regeneration of the intestinal microbiota after diarrheic dysbiosis–a review. Clin. Exp. Gastroenterol. 8, 237.
Moriyama, S., Ohta, K., 2007. Functional characterization and evolutionary implication of the internal 157-amino-acid sequence of an exoinulinase from Penicillium sp. strain TN-88. J. Biosci. Bioeng. 103, 293–297.
Morrissey, J.P., Etschmann, M.M., Schrader, J., de Billerbeck, G.M., 2015. Cell factory applications of the yeast Kluyveromyces marxianus for the biotechnological production of natural flavour and fragrance molecules. Yeast 32, 3–16.
Moyo, S., Gashe, B., Collison, E., Mpuchane, S., 2003. Optimising growth conditions for the pectinolytic activity of Kluyveromyces wickerhamii by using response surface methodology. Int. J. Food Microbiol. 85, 87–100.
Mrudula, V., Vijaya, T., Mouli, K.C., Jyothi, U.N., Aishwarya, S., Reddy, V.D., 2016. Novel method for removal of heavy metals by using low cost absorbents. American Journal of Pharmaceutical Research 6.
Murari, C.S., Machado, W.R.C., Schuina, G.L., Del Bianchi, V.L., 2019. Optimization of bioethanol production from cheese whey using Kluyveromyces marxianus URM 7404. Biocatalysis and Agricultural Biotechnology 20, 101182.
Nakamura, T., Agata, K., Mizutani, M., IINO, H., 2001. Effects of brewer's yeast cell wall on constipation and defecation in experimentally constipated rats. Biosci. Biotechnol. Biochem. 65, 774–780.
Nedeva, T., Dolashka-Angelova, P., Moshtanska, V., Voelter, W., Petrova, V., Kujumdzieva, A., 2009. Purification and partial characterization of Cu/Zn superoxide dismutase from Kluyveromyces marxianus yeast. J. Chromatogr. B 877, 3529–3536.
Negoro, H., Kito, E., 1973. Beta-fructofuranosidase from Candida kefyr. J. Ferment. Technol. 51, 96–102.
Nicolosi, R., Bell, S.J., Bistrian, B.R., Greenberg, I., Forse, R.A., Blackburn, G.L., 1999. Plasma lipid changes after supplementation with β-glucan fiber from yeast. Am. J. Clin. Nutr. 70, 208–212.
Nilsson, U., Barron, N., McHale, L., McHale, A., 1995. The effects of phosphoric acid pretreatment on conversion of cellulose to ethanol at 45 C using the thermotolerant yeast Kluyveromyces marxianus IMB3. Biotechnol. Lett. 17, 985–988.
Nitschke, M., Costa, S., 2007. Biosurfactants in food industry. Trends Food Sci. Technol. 18, 252–259.
Nonklang, S., Abdel-Banat, B.M., Cha-aim, K., Moonjai, N., Hoshida, H., Limtong, S., Yamada, M., Akada, R., 2008. High-temperature ethanol fermentation and transformation with linear DNA in the thermotolerant yeast Kluyveromyces marxianus DMKU3-1042. Appl. Environ. Microbiol. 74, 7514–7521.
Nowak, J., Hang, Y.D., 2003. The rapid degradation of sauerkraut brine by free and immobilized yeast cells. Electronic Journal of Polish Agricultural Universities. Series Food Science and Technology 6.
Nurcholis, M., Lertwattanasakul, N., Rodrussamee, N., Kosaka, T., Murata, M., Yamada, M., 2020. Integration of comprehensive data and biotechnological tools for industrial applications of Kluyveromyces marxianus. Appl. Microbiol. Biotechnol. 104, 475–488.
Ohta, K., Suetsugu, N., Nakamura, T., 2002. Purification and properties of an extracellular inulinase from Rhizopus sp. strain TN-96. J. Biosci. Bioeng. 94, 78–80.
Oliveira, C., Guimarães, P.M., Domingues, L., 2011. Recombinant microbial systems for improved β-galactosidase production and biotechnological applications. Biotechnol. Adv. 29, 600–609.
Oliveira, R.d.Q., Junior, V., Lima, G., Uetanabaro, A.P.T., Koblitz, M.G.B., Góes-Neto, A., Rosa, C.A., Assis, S.A.d., 2012. Influence of carbon source, pH, and temperature on the polygalacturonase activity of Kluyveromyces marxianus CCMB 322. Food Science and Technology 32, 499–505.
de Oliveira, S.M.M., Gomes, S.D., Sene, L., Machado, S.R., Coelho, A.C.B., Cereda, M.P., Christ, D., Piechontcoski, J., 2013. Production of 2-phenylethanol by Geotrichum fragrans, Saccharomyces cerevisiae and Kluyveromyces marxianus in cassava wastewater. J Food Agric Environ 11, 158–163.
Onken, U., Liefke, E., 1989. Effect of total and partial pressure (oxygen and carbon dioxide) on aerobic microbial processes. In: Bioprocesses and Engineering. Springer, pp. 137–169.
Ortiz-Merino, R.A., Varela, J.A., Coughlan, A.Y., Hoshida, H., da Silveira, W.B., Wilde, C., Kuijpers, N.G.A., Geertman, J.-M., Wolfe, K.H., Morrissey, J.P., 2018. Ploidy variation in Kluyveromyces marxianus separates dairy and non-dairy isolates. Front. Genet. 9, 94.
Øverland, M., Karlsson, A., Mydland, L.T., Romarheim, O.H., Skrede, A., 2013. Evaluation of Candida utilis, Kluyveromyces marxianus and Saccharomyces cerevisiae yeasts as protein sources in diets for Atlantic salmon (Salmo salar). Aquaculture 402, 1–7.
Ozmihci, S., Kargi, F., 2007. Comparison of yeast strains for batch ethanol fermentation of cheese–whey powder (CWP) solution. Lett. Appl. Microbiol. 44, 602–606.
Öztürk, S., Cerit, İ., Mutlu, S., Demirkol, O., 2017. Enrichment of cookies with glutathione by inactive yeast cells (Saccharomyces cerevisiae): physicochemical and functional properties. J. Cereal Sci. 78, 19–24.
Padilla, B., Frau, F., Ruiz-Matute, A.I., Montilla, A., Belloch, C., Manzanares, P., Corzo, N., 2015. Production of lactulose oligosaccharides by isomerisation of transgalactosylated cheese whey permeate obtained by β-galactosidases from dairy Kluyveromyces. J. Dairy Res. 82, 356–364.
Pal, R., Tewari, S., Rai, J.P., 2009. Metals sorption from aqueous solutions by Kluyveromyces marxianus: process optimization, equilibrium modeling and chemical characterization. Biotechnology Journal: Healthcare Nutrition Technology 4, 1471–1478.
Panesar, P.S., 2008. Production of ß-D-galactosidase from whey using Kluyveromyces marxianus. Res. J. Microbiol. 3, 24–29.
Parekh, S., Margaritis, A., 1986. Production of inulinase (β-fructan fructanohydrolase) by Kluyveromyces marxianus. Agric. Biol. Chem. 50, 1085–1087.
Passador-Gurgel, G., Furlan, S., Melier, J., Jonas, R., 1996. Application of a microtitre reader system to the screening of inulinase nulinase-producing yeasts. Appl. Microbiol. Biotechnol. 45, 158–161.
Pecota, D.C., Rajgarhia, V., Da Silva, N.A., 2007. Sequential gene integration for the engineering of Kluyveromyces marxianus. J. Biotechnol. 127, 408–416.
Pinheiro, R., Belo, I., Mota, M., 2003. Growth and β-galactosidase activity in cultures of Kluyveromyces marxianus under increased air pressure. Lett. Appl. Microbiol. 37, 438–442.
Pivarnik, L.F., Senecal, A.G., Rand, A.G., 1995. Hydrolytic and transgalactosylic activities of commercial β-galactosidase (lactase) in food processing. Adv. Food Nutr. Res. 1–102 Elsevier.
Plessas, S., Fisher, A., Koureta, K., Psarianos, C., Nigam, P., Koutinas, A.A., 2008. Application of Kluyveromyces marxianus, lactobacillus delbrueckii ssp. bulgaricus and L. helveticus for sourdough bread making. Food Chem. 106, 985–990.
Price, J., 2019. History of the development and application of whey protein products. In: Whey Proteins. Elsevier, pp. 51–95.
Priya, E.S., Selvan, P.S., 2017. Water hyacinth (Eichhornia crassipes)–An efficient and economic adsorbent for textile effluent treatment–a review. Arab. J. Chem. 10, S3548–S3558.
Qiu, Z., Jiang, R., 2017. Improving Saccharomyces cerevisiae ethanol production and tolerance via RNA polymerase II subunit Rpb7. Biotechnology for Biofuels 10, 125.
Radoi, F., Kishida, M., Kawasaki, H., 2005. Endo-polygalacturonase in Saccharomyces wine yeasts: effect of carbon source on enzyme production. FEMS Yeast Res. 5, 663–668.
Rajoka, M., 2007. Kinetic parameters and thermodynamic values of β-xylosidase production by Kluyveromyces marxianus. Bioresour. Technol. 98, 2212–2219.
Ramírez-Zavala, B., Mercado-Flores, Y., Hernández-Rodríguez, C., Villa-Tanaca, L., 2004. Purification and characterization of a lysine aminopeptidase from Kluyveromyces marxianus. FEMS Microbiol. Lett. 235, 369–375.
Ramírez-Zavala, B., Mercado-Flores, Y., Hernández-Rodríguez, C., Villa-Tanaca, L., 2004. Purification and characterization of a serine carboxypeptidase from Kluyveromyces marxianus. Int. J. Food Microbiol. 91, 245–252.
Rech, R., Cassini, C., Secchi, A., Ayub, M., 1999. Utilization of protein-hydrolyzed cheese whey for production of β-galactosidase by Kluyveromyces marxianus. J. Ind. Microbiol. Biotechnol. 23, 91–96.
Reed, G., 2012. Yeast Technology. Springer Science & Business Media.
Ritala, A., Häkkinen, S.T., Toivari, M., Wiebe, M.G., 2017. Single cell protein—state-of-the-art, industrial landscape and patents 2001–2016. Front. Microbiol. 8, 2009.
Roane, T.M., Pepper, I.L., Gentry, T.J., 2015. Microorganisms and metal pollutants. In: Environmental Microbiology, Third edition. Elsevier, pp. 415–439.
Rodicio, R., Heinisch, J.J., 2013. Yeast on the milky way: genetics, physiology and biotechnology of Kluyveromyces lactis. Yeast 30, 165–177.
Rodrussamee, N., Lertwattanasakul, N., Hirata, K., Limtong, S., Kosaka, T., Yamada, M., 2011. Growth and ethanol fermentation ability on hexose and pentose sugars and glucose effect under various conditions in thermotolerant yeast Kluyveromyces marxianus. Appl. Microbiol. Biotechnol. 90, 1573.
Rouwenhorst, R.J., Visser, L.E., Van Der Baan, A.A., Scheffers, W.A., Van Dijken, J.P., 1988. Production, distribution, and kinetic properties of inulinase in continuous cultures of Kluyveromyces marxianus CBS 6556. Appl. Environ. Microbiol. 54, 1131–1137.
SÁ, A.G.A., de Meneses, A.C., de Araújo, P.H.H., de Oliveira, D., 2017. A review on enzymatic synthesis of aromatic esters used as flavor ingredients for food, cosmetics and pharmaceuticals industries. Trends Food Sci. Technol. 69, 95–105.
Saber, A., Alipour, B., Faghfoori, Z., Khosroushahi, A.Y., 2017. Secretion metabolites of dairy Kluyveromyces marxianus AS41 isolated as probiotic, induces apoptosis in different human cancer cell lines and exhibit anti-pathogenic effects. J. Funct. Foods 34, 408–421.
Saini, P., Beniwal, A., Kokkiligadda, A., Vij, S., 2017. Evolutionary adaptation of Kluyveromyces marxianus strain for efficient conversion of whey lactose to bioethanol. Process Biochem. 62.
Sansonetti, S., Curcio, S., Calabrò, V., Iorio, G., 2009. Bio-ethanol production by fermentation of ricotta cheese whey as an effective alternative non-vegetable source.

Biomass Bioenergy 33, 1687–1692.
Saratale, G.D., Saratale, R.G., Ghodake, G.S., Jiang, Y.Y., Chang, J.-S., Shin, H.-S., Kumar, G., 2017. Solid state fermentative lignocellulolytic enzymes production, characterization and its application in the saccharification of rice waste biomass for ethanol production: An integrated biotechnological approach. J. Taiwan Inst. Chem. Eng. 76, 51–58.
Sarmah, N., Revathi, D., Sheelu, G., Yamuna Rani, K., Sridhar, S., Mehtab, V., Sumana, C., 2018. Recent advances on sources and industrial applications of lipases. Biotechnol. Prog. 34, 5–28.
Schultz, N., Chang, L., Hauck, A., Reuss, M., Syldatk, C., 2006. Microbial production of single-cell protein from deproteinized whey concentrates. Appl. Microbiol. Biotechnol. 69, 515–520.
Schwan, R.F., Rose, A., 1994. Polygalacturonase production by Kluyveromyces marxianus: effect of medium composition. J. Appl. Bacteriol. 76, 62–67.
Schwan, R.F., Cooper, R.M., Wheals, A.E., 1997. Endopolygalacturonase secretion by Kluyveromyces marxianus and other cocoa pulp-degrading yeasts. Enzym. Microb. Technol. 21, 234–244.
Selvakumar, P., Pandey, A., 1999. Solid state fermentation for the synthesis of inulinase from Staphylococcus sp. and Kluyveromyces marxianus. Process Biochem. 34, 851–855.
Senthilkumar, S., Perumalsamy, M., Prabhu, H.J., 2014. Decolourization potential of white-rot fungus Phanerochaete chrysosporium on synthetic dye bath effluent containing Amido black 10B. Journal of Saudi Chemical Society 18, 845–853.
Sharma, R., Soni, S., Vohra, R., Gupta, L., Gupta, J., 2002. Purification and characterisation of a thermostable alkaline lipase from a new thermophilic Bacillus sp. RSJ-1. Process Biochem. 37, 1075–1084.
Shekhar, S., Sundaramanickam, A., Balasubramanian, T., 2015. Biosurfactant producing microbes and their potential applications: a review. Crit. Rev. Environ. Sci. Technol. 45, 1522–1554.
Sheng, J., Chi, Z., Li, J., Gao, L., Gong, F., 2007. Inulinase production by the marine yeast Cryptococcus aureus G7a and inulin hydrolysis by the crude inulinase. Process Biochem. 42, 805–811.
Sheng, J., Chi, Z., Gong, F., Li, J., 2008. Purification and characterization of extracellular inulinase from a marine yeast Cryptococcus aureus G7a and inulin hydrolysis by the purified inulinase. Appl. Biochem. Biotechnol. 144, 111–121.
Shepherd, R., Rockey, J., Sutherland, I.W., Roller, S., 1995. Novel bioemulsifiers from microorganisms for use in foods. J. Biotechnol. 40, 207–217.
Sieiro, C., Villa, T.G., da Silva, A.F., García-Fraga, B., Vilanova, M., 2014. Albariño wine aroma enhancement through the use of a recombinant polygalacturonase from Kluyveromyces marxianus. Food Chem. 145, 179–185.
Šiekštelė, R., Veteikytė, A., Tvaska, B., Matijošytė, I., 2015. Yeast Kluyveromyces lactis as host for expression of the bacterial lipase: cloning and adaptation of the new lipase gene from Serratia sp. J. Ind. Microbiol. Biotechnol. 42, 1309–1317.
da Silva, E.G., Borges, M.d.F., Medina, C., Piccoli, R.H., Schwan, R.F., 2005. Pectinolytic enzymes secreted by yeasts from tropical fruits. FEMS Yeast Res. 5, 859–865.
da Silva, F.L., de Oliveira Campos, A., dos Santos, D.A., Magalhães, E.R.B., de Macedo, G.R., dos Santos, E.S., 2018. Valorization of an agroextractive residue—carnauba straw—for the production of bioethanol by simultaneous saccharification and fermentation (SSF). Renew. Energy 127, 661–669.
Silva-Santisteban, B.O.Y., Maugeri Filho, F., 2005. Agitation, aeration and shear stress as key factors in inulinase production by Kluyveromyces marxianus. Enzym. Microb. Technol. 36, 717–724.
Silva-Santisteban, B.O.Y., Converti, A., Maugeri Filho, F., 2009. Effects of carbon and nitrogen sources and oxygenation on the production of inulinase by Kluyveromyces marxianus. Appl. Biochem. Biotechnol. 152, 249–261.
Silveira, W., Passos, F., Mantovani, H., Passos, F., 2005. Ethanol production from cheese whey permeate by Kluyveromyces marxianus UFV-3: a flux analysis of oxido-reductive metabolism as a function of lactose concentration and oxygen levels. Enzym. Microb. Technol. 36, 930–936.
Singh, R., Bhermi, H., 2008a. Production of extracellular exoinulinase from Kluyveromyces marxianus YS-1 using root tubers of Asparagus officinalis. Bioresour. Technol. 99, 7418–7423.
Singh, R., Bhermi, H., 2008b. Production of extracelular exoinulinase from Kluyveromyces marxianus YS-1 using root tubers of Asparagus officinalis. Bioresour. Technol. 99, 7418–7423.
Singh, R., Sooch, B.S., Puri, M., 2007. Optimization of medium and process parameters for the production of inulinase from a newly isolated Kluyveromyces marxianus YS-1. Bioresour. Technol. 98, 2518–2525.
Singh, R.S., Dhaliwal, R., Puri, M., 2007a. Partial purification and characterization of exoinulinase from Kluyveromyces marxianus YS-1 for preparation of high-fructose syrup. J. Microbiol. Biotechnol. 17, 733–738.
Singh, R.S., Dhaliwal, R., Puri, M., 2007b. Production of high fructose syrup from Asparagus inulin using immobilized exoinulinase from Kluyveromyces marxianus YS-1. J. Ind. Microbiol. Biotechnol. 34, 649–655.
Singh, R., Kumar, M., Mittal, A., Mehta, P.K., 2016. Microbial enzymes: industrial progress in 21st century. 3 Biotech 6, 174.
Singh, R., Singh, R., Kennedy, J., 2017. Immobilization of yeast inulinase on chitosan beads for the hydrolysis of inulin in a batch system. Int. J. Biol. Macromol. 95, 87–93.
Spohner, S.C., Schaum, V., Quitmann, H., Czermak, P., 2016. Kluyveromyces lactis: an emerging tool in biotechnology. J. Biotechnol. 222, 104–116.
Stergiou, P.-Y., Foukis, A., Sklivaniti, H., Zacharaki, P., Papagianni, M., Papamichael, E.M., 2012. Experimental investigation and optimization of process variables affecting the production of extracellular lipase by Kluyveromyces marxianus IFO 0288. Appl. Biochem. Biotechnol. 168, 672–680.
Struyf, N., Laurent, J., Verspreet, J., Verstrepen, K.J., Courtin, C.M., 2017. Saccharomyces cerevisiae and Kluyveromyces marxianus cocultures allow reduction of fermentable oligo-, di-, and monosaccharides and polyols levels in whole wheat bread. J. Agric. Food Chem. 65, 8704–8713.
Struyf, N., Vandewiele, H., Herrera-Malaver, B., Verspreet, J., Verstrepen, K.J., Courtin, C.M., 2018. Kluyveromyces marxianus yeast enables the production of low FODMAP whole wheat breads. Food Microbiol. 76, 135–145.
Sudha, M., Saranya, A., Selvakumar, G., Sivakumar, N., 2014. Microbial degradation of azo dyes: a review. Int. J. Curr. Microbiol. App. Sci. 3, 670–690.
Suzuki, T., Hoshino, T., Matsushika, A., 2019. High-temperature ethanol production by a series of recombinant xylose-fermenting Kluyveromyces marxianus strains. Enzym. Microb. Technol. 129, 109359.
Tabanelli, G., Verardo, V., Pasini, F., Cavina, P., Lanciotti, R., Caboni, M., Gardini, F., Montanari, C., 2016. Survival of the functional yeast Kluyveromyces marxianus B0399 in fermented milk with added sorbic acid. J. Dairy Sci. 99, 120–129.
Tomáška, M., Stredănský, M., Gemeiner, P., Šturdík, E., 1995. Improvement of the thermostability of β-galactosidase from Kluyveromyces marxianus. Process Biochem. 30, 649–652.
Tomas-Pejo, E., Fermoso, J., Herrador, E., Hernando, H., Jiménez-Sánchez, S., Ballesteros, M., González-Fernández, C., Serrano, D., 2017. Valorization of steam-exploded wheat straw through a biorefinery approach: bioethanol and bio-oil co-production. Fuel 199, 403–412.
Ustáriz, F., Laca, A., García, L., Díaz, M., 2007. Mixed cultures of Serratia marcescens and Kluyveromyces fragilis for simultaneous protease production and COD removal of whey. J. Appl. Microbiol. 103, 864–870.
Uzunova, K., Vassileva, A., Kambourova, M., Ivanova, V., Spasova, D., Mandeva, R., Derekova, A., Tonkova, A., 2001. Production and properties of a bacterial thermostable exo-inulinase. Zeitschrift für Naturforschung C 56, 1022–1028.
Vakhlu, J., 2006. Yeast lipases: enzyme purification, biochemical properties and gene cloning. Electron. J. Biotechnol. 9 (0-0).
Vandamme, E.J., Derycke, D.G., 1983. Microbial inulinases: fermentation process, properties, and applications. Adv. Appl. Microbiol. 139–176 Elsevier.
Varela, J.A., Gethins, L., Stanton, C., Ross, P., Morrissey, J.P., 2017. Applications of Kluyveromyces marxianus in biotechnology. In: Satyanarayana, T., Kunze, G. (Eds.), Yeast Diversity in Human Welfare. Springer Singapore, Singapore, pp. 439–453.
Vigneault, F., Lachance, D., Cloutier, M., Pelletier, G., Levasseur, C., Séguin, A., 2007. Members of the plant NIMA-related kinases are involved in organ development and vascularization in poplar, Arabidopsis and rice. Plant J. 51, 575–588.
Wagner, J.M., Alper, H.S., 2016. Synthetic biology and molecular genetics in non-conventional yeasts: current tools and future advances. Fungal Genet. Biol. 89, 126–136.
Wilkowska, A., Kregiel, D., Guneser, O., Karagul Yuceer, Y., 2015. Growth and by-product profiles of Kluyveromyces marxianus cells immobilized in foamed alginate. Yeast 32, 217–225.
Willetts, A., Ugalde, U., 1987. The production of single-cell protein from whey. Biotechnol. Lett. 9, 795–800.
Williams, D.L., Pretus, H.A., McNamee, R.B., Jones, E.L., Ensley, H.E., Browder, I.W., 1992. Development of a water-soluble, sulfated (1 → 3)-β-D-glucan biological response modifier derived from Saccharomyces cerevisiae. Carbohydr. Res. 235, 247–257.
Wimborne, M.P., Rickard, P.A., 1978. Pectinolytic activity of Saccharomyces fragilis cultured in controlled environments. Biotechnol. Bioeng. 20, 231–242.
Wu, W.-H., Hung, W.-C., Lo, K.-Y., Chen, Y.-H., Wan, H.-P., Cheng, K.-C., 2016. Bioethanol production from taro waste using thermo-tolerant yeast Kluyveromyces marxianus K21. Bioresour. Technol. 201, 27–32.
Xie, Y., Zhang, H., Liu, H., Xiong, L., Gao, X., Jia, H., Lian, Z., Tong, N., Han, T., 2015. Hypocholesterolemic effects of Kluyveromyces marxianus M3 isolated from Tibetan mushrooms on diet-induced hypercholesterolemia in rat. Braz. J. Microbiol. 46, 389–395.
Xiong, C., Jinhua, W., Dongsheng, L., 2007. Optimization of solid-state medium for the production of inulinase by Kluyveromyces S120 using response surface methodology. Biochem. Eng. J. 34, 179–184.
Yadav, J.S.S., Bezawada, J., Ajila, C.M., Yan, S., Tyagi, R.D., Surampalli, R.Y., 2014a. Mixed culture of Kluyveromyces marxianus and Candida krusei for single-cell protein production and organic load removal from whey. Bioresour. Technol. 164, 119–127.
Yadav, J.S.S., Bezawada, J., Elharche, S., Yan, S., Tyagi, R.D., Surampalli, R.Y., 2014b. Simultaneous single-cell protein production and COD removal with characterization of residual protein and intermediate metabolites during whey fermentation by K. marxianus. Bioprocess Biosyst. Eng. 37, 1017–1029.
Yadav, J.S.S., Yan, S., Pilli, S., Kumar, L., Tyagi, R.D., Surampalli, R.Y., 2015. Cheese whey: a potential resource to transform into bioprotein, functional/nutritional proteins and bioactive peptides. Biotechnol. Adv. 33, 756–774.
Yadav, J.S.S., Yan, S., Ajila, C.M., Bezawada, J., Tyagi, R.D., Surampalli, R.Y., 2016. Food-grade single-cell protein production, characterization and ultrafiltration recovery of residual fermented whey proteins from whey. Food Bioprod. Process. 99, 156–165.
Yan, J., Wei, Z., Wang, Q., He, M., Li, S., Irbis, C., 2015. Bioethanol production from sodium hydroxide/hydrogen peroxide-pretreated water hyacinth via simultaneous saccharification and fermentation with a newly isolated thermotolerant Kluyveromyces marxianu strain. Bioresour. Technol. 193, 103–109.
Yarimizu, T., Nonklang, S., Nakamura, J., Tokuda, S., Nakagawa, T., Lorreungsil, S., Sutthikhumpha, S., Pukahuta, C., Kitagawa, T., Nakamura, M., Cha-aim, K., Limtong, S., Hoshida, H., Akada, R., 2013. Identification of auxotrophic mutants of the yeast Kluyveromyces marxianus by non-homologous end joining-mediated integrative transformation with genes from Saccharomyces cerevisiae. Yeast 30, 485–500.
Yazgan, A., Özcengiz, G., 1994. Subcellular distribution of accumulated heavy metals in Saccharomyces cerevisiae and Kluyveromyces marxianus. Biotechnol. Lett. 16, 871–874.
Yoshida, Y., Yokoi, W., Wada, Y., Ohishi, K., Ito, M., Sawada, H., 2004. Potent

hypocholesterolemic activity of the yeast Kluyveromyces marxianus YIT 8292 in rats fed a high cholesterol diet. Biosci. Biotechnol. Biochem. 68, 1185–1192.
Yousuf, A., Sultana, S., Monir, M.U., Karim, A., Rahmaddulla, S.R.B., 2017. Social business models for empowering the biogas technology. Energy Sources, Part B: Economics, Planning, and Policy 12, 99–109.
Yusef, H.H., 1997. Bioaccumulation of metal cations by free and immobilized cells of Kluyveromyces marxianus. Advances in food sciences 19, 120–123.
Zafar, S., Owais, M., 2006. Ethanol production from crude whey by Kluyveromyces marxianus. Biochem. Eng. J. 27, 295–298.
Zhang, S., Yang, F., Wang, Q., Hua, Y., Zhao, Z.K., 2012. High-level secretory expression and characterization of the recombinant Kluyveromyces marxianus inulinase. Process Biochem. 47, 151–155.
Zhang, G., Lu, M., Wang, J., Wang, D., Gao, X., Hong, J., 2017. Identification of hexose kinase genes in Kluyveromyces marxianus and thermo-tolerant one step producing glucose-free fructose strain construction. Sci. Rep. 7, 45104.
Zhang, B., Ren, L., Wang, Y., Xu, D., Zhang, S., Wang, H., Wang, H., Zeng, X., Xin, B., Li, F., 2020. Glycerol production through TPI1 defective Kluyveromyces marxianus at high temperature with glucose, fructose, and xylose as feedstock. Biochem. Eng. J. 161, 107689.
Zhou, H.-X., Xu, J.-L., Chi, Z., Liu, G.-L., Chi, Z.-M., 2013. β-Galactosidase over-production by a mig1 mutant of Kluyveromyces marxianus KM for efficient hydrolysis of lactose. Biochem. Eng. J. 76, 17–24.
Zhou, H.-X., Xin, F.-H., Chi, Z., Liu, G.-L., Chi, Z.-M., 2014. Inulinase production by the yeast Kluyveromyces marxianus with the disrupted MIG1 gene and the over-expressed inulinase gene. Process Biochem. 49, 1867–1874.
Zhou, J., Zhu, P., Hu, X., Lu, H., Yu, Y., 2018. Improved secretory expression of lignocellulolytic enzymes in Kluyveromyces marxianus by promoter and signal sequence engineering. Biotechnology for biofuels 11, 235.
Zoppellari, F., Bardi, L., 2013. Production of bioethanol from effluents of the dairy industry by Kluyveromyces marxianus. New Biotechnol. 30, 607–613.