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Yeast the Present and Future Cell Facture

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Abstract

Yeasts are eukaryotic microorganisms that are existing in a wide range of habitats, thanks to their ability to adapt even in extreme locations and conditions. Moreover, the unicellular nature of yeasts makes them better suited for deep liquid substrates or moist and uneven surfaces. Till now, about 1500 species of yeasts are described, and the genus *Saccharomyces* is the well-studied genus of all the yeasts in terms of physiology and genetics. In this review, we elucidate the role of yeasts as biotechnological tool and their current and potential applications. We also highlighted the features, and industrial application of some marine yeasts. Furthermore, describing rarely basidiomycetous yeast involved in different applications. Contributions of yeasts and their enzymes in food, industrial, and pharmaceuticals fields were also discussed. Finally, employment of yeasts in biotransformation process was show.

Keywords: Yeasts; Marine Yeasts; Basidiomycetous Yeasts; Biotransformation; Industrial Applications; Probiotics

Introduction

Yeasts are a multipurpose group of eukaryotic microorganisms which are heterogeneous in their nutritional abilities and are capable of surviving in a range of habitats such as in deep sea [1,2], moist and uneven surfaces including polluted waters [3], on dry substrates and in the presence of high concentrations of salt and sugar [4]. Turkiewicz M, et al. [5], suggested that yeasts may be better adapted to low temperatures than bacteria. Therefore, yeasts belonging to genera such as *Bullera, Candida, Cryptococcus, Cystofilobasidium, Debaryomyces, Kondoa, Leucosporidium, Metschnikowia, Mrakia, Pseudozyma, Rhodotorula, Sakaguchia, Sporopachydermia, Sympodiomyces* and *Trichosporon* have been identified in various habitats of Antarctica [6]. The unicellular nature of yeasts makes them better suited for deep liquid substrates or moist and uneven surfaces. Therefore, yeasts grow typically in moist environments where there is an abundant supply of simple, soluble nutrients such as sugars and amino acids. This explains why they are common on leaf and fruit surfaces, on roots and in various types of food [7,8].

Yeasts belonging to Kingdom Fungi, phylum Ascomycota, in subphylum Saccharomycotina, in the class *Saccharomycetes*, in the order Saccharomycetales, in the family Saccharomycetaceae, and in the genus *Saccharomyces* [9], with about 1500 species currently described. *Saccharomyces cerevisiae*, known as “baker’s yeast”, can reside in diverse environmental niches. *Saccharomyces cerevisiae* is probably the best studied of all the yeast species in terms of physiology and genetics, and definitely of immense industrial significance because of its involvement in fermentation of bread, beer, or wine. It is an ideal source of different enzymes and vitamins, considered as nutrient supplement and can treat antibiotic-related diarrhea [10]. Rapid growth and easy control of mass production of yeasts using simple nutrient culture medium altogether make yeast as the preferred microorganism for synthesis important natural products, as compared to other microbes [11-14].

Marine Yeasts as Unique and Promising Features and Industrial Application

Marine yeasts, defined as the yeasts that are isolated from marine environments, are able to grow better on medium prepared using seawater rather than freshwater [15]. Many marine types of yeast have been isolated from around the world from different sources, including seawater, seaweeds, marine fish and mammals as well as seabird [16]. Terrestrial yeasts have been widely used in various industries, such as baking, brewing, wine, bioethanol and pharmaceutical protein production. However, only little attention has been given to marine yeasts. Recent research showed that marine yeasts have several unique and promising features over the terrestrial yeasts, for example higher osmosis tolerance, higher special chemical productivity and production of industrial enzymes. These indicate that marine yeasts have great potential to be applied in various industries [16].

Marine yeasts provide the potential for several unique desirable properties to be used in various industries. The latest development in the methodology of marine yeast isolation and cultivation offers the opportunity of discovering novel marine yeasts. Various media have been proposed by different research groups to suit for the different requirement of marine yeasts. These media are rich in nutrients, and they are common to contain antibiotics to reduce the bacterial and mould contamination. Using marine yeasts in bioethanol production shows distinctive advantage on the osmosis tolerance, the possibility of utilization of seawater instead of fresh water and the potential of advantage in using marine biomass as a substrate. Marine yeasts have already been investigated for the production of pharmaceutical and enzymatic products, such as astaxanthin, siderophore, riboflavin, inulinase and amylases. Yet, the commercial application of marine yeasts is still limited [16]. More direct evaluation studies should be carried out to give further evidence on the advantages of marine yeasts compared with terrestrial yeasts in bioethanol industry, especially in bioethanol fermentations. More marine yeasts should be isolated to explore their potential. The isolates should be selected based on their capability of utilizing and fermenting a wide range of sugars that presented in marine biomass hydrolysate [16].

Applications of the Rarely Isolated Basidiomycetous Yeasts

A dominant feature of basidiomycetous yeasts is a growth phase consisting of round, oval, and elongate cells that reproduce by enteroblastic budding (blastoconidia), fission (arthroconidia), and/or forceful ejection (ballistoconidia). These yeasts are not restricted to one group of basidiomycetes, i.e., class or order; rather, they are polyphyletic; there are approximately 220 recognized species in 34 genera distributed among the Urediniomycetes, Ustilaginomycetes, and Hymenomycetes. Consequently, life cycles and ultrastructure among these yeasts are not uniform; rather, they reflect phylogenetic diversity. These yeasts have considerable economic, agricultural and medical importance and estimates suggest that the number of known yeasts represents only about 1 to 5 % of the species that exist in nature. There is an increased interest in exploration of these species for economic exploitation and there is a need to understand their biodiversity and ecological roles [17].

Yeasts and Pharmaceutical Applications

Since the early 1980s, yeasts have been utilized for heterologous production of a variety of proteins. The production of heterologous proteins in yeasts holds enormous potential for biotechnological processes. A major breakthrough in heterologous protein expression in yeast was the cloning, expression, processing, and secretion of human proinsulin in *S. cerevisiae* in the 1980s [18]. Yeasts are intensively being developed as protein expression systems and in comparison, to mammalian cell lines have higher productivity, higher cell yields, shorter fermentation cycles, can be cultured in defined media under relatively inexpensive conditions, can efficiently secrete proteins, possess posttranslational modification pathways, non-pathogenic and non-pyrogenic [19]. Several yeast species have been investigated for heterologous protein production, namely *Saccharomyces cerevisiae, Pichia pastoris, Hansenula polymorpha, Kluyveromyces lactis, Schizosaccharomyces pombe, Yarrowia lipolytica, Arxula adeninivorans* [20,21].

Yeast has become a prominent model for human diseases and path ways. At least 31% of the proteins encoded in the yeast genome have a human orthologue and nearly 50%of human disease genes exhibit yeast orthologues [22]. Furthermore, yeast has been the testing ground of new gene expression profiling of drug action [23], synthetic lethal screens [24], drug-induced haploinsufficiency [25], and drug-induced phenotypic responses have been implemented and validated in yeast [26]. Yeast cells as preformed microcapsules can be used to improve the bioavailability of poorly soluble drugs in the gastro-intestinal tract. Microorganisms have been recognised as potential preformed natural microcapsules since the early 1070s, when Swift and Co., USA, patented a technique using specifically prepared yeast containing high concentrations of lipid, greater than 40% by weight. Using commercially available yeast strains, such as *Saccharomyces cerevisiae*, from the baking and brewing industry [27]. Yeast cells can be utilized as microcapsules for the encapsulation of lipophilic drug molecules. The drug remains stable within the capsule until release is initiated by addition of a surfactant or by contact with a mucous membrane. When administered directly into the duodenum, the lipophilic drug is released from the cell and enters the blood stream with a reduced burst effect and prolonged release profile [27].

Yeast cultures do not require elaborate sterile techniques or complex media and can be stored in standard refrigerator stocks. Furthermore, there is an arsenal of strains, vectors and genetic tools that allow researchers to quickly develop yeast-based bioassays [27]. Proteins from any origin can be expressed in yeast, RNA levels can be easily manipulated, and gene expression can also be made inducible [27]. Yeast has been traditionally overlooked in cancer drug discovery, because of the general belief that drugs are difficult to deliver in yeast cells. This was reported to occur by activities of multidrug transporters which extrude drugs out of the cell. However, this problem can be easily overcome by deleting genes involved in mem-brane permeability and/or drug efflux. Accordingly, the National Cancer Institute (NCI), as part of its large-scale drug screen, has generated a panel of yeast strains with deletions in the ERG6 [28], (ergosterol biosynthesis-sis), PDR1 and PDR3 (drug efflux) genes [29,30].

Yeasts and Industrial Enzymes

Terrestrial yeasts and marine yeasts (*Aureobasidium* sp. and *Pichia* sp.) both have been investigated for the production of enzymes, such as inulinase [15], amylase [31,32], superoxide dismutase [33], and lipase [15]. Several yeasts are exploited for their enzymes and enzymatic activities. *Cryptococcus albidus* produces xylanases [34], *Cryptococcus cellulolyticus* produces cellulases [35], and amylolytic activity has been demonstrated in *Filobasidium capsuligenum, Cryptococcus curvatus, Pseudozyma tsukubaensis*, and *Trichosporon pullulans* [36,37]. Lipid accumulation occurs in *Cryptococcus laurentii, Cryptococcus curvatus, Rhodotorula glutinis, R. gracilis, R. graminis, R. mucilaginosa, Trichosporon cutaneum, T. pullulans* [38,39]. *Trichosporon pullulans* accumulates more than 65% of its biomass as lipid [40]. Mutants of *Cryptococcus curvatus* are able to produce cocoa butter equivalents [41,42]. Species of *Pseudozyma* produce mannosylerythritol lipids [43,44] and beta-lipase for the synthesis of glucoside esters exhibiting surfactant properties [45].

Yeast Biotechnology Potential and Applications

The yeasts have been exploited by mankind for thousands of years for food and fermentation processes. Traditionally the yeast has been used for the production of alcoholic beverages, biomass and glycerol [46]. *Saccharomyces cerevisiae* has been described as mankind's most domesticated organism and still widely exploited yeast species in industry today. The number of yeast species described so far is about 1500 and only a little is used at industrial scale. Some 70-80 species have been shown to possess potential value for biotechnology. According to modest estimate, known yeast species represent roughly 5% of the total number that may inhabitant Earth surface. Modern applications of yeasts have been greatly expanded beyond classical applications [47]. Yeasts, especially *Saccharomyces cerevisiae* and other non-saccharomyces yeasts today are increasingly used for the heterologous production of enzymes and pharmaceutical proteins. Yeasts have important roles in environmental applications such as bioremediation and removal of heavy metals from wastewaters. Yeasts are also used in agriculture as bio-control agents. Several chemicals can be produced using yeast as a biocatalyst. New developments in engineering yeast have introduced novel capabilities to extend substrate range and produce new products so far yeast cannot produce. *Saccharomyces cerevisiae* is largest cultivated organism so far. Having in mind diversity and potential of all yeast species, the cultivation and utilization of *Saccharomyces* yeasts are still the tip of the iceberg and there is a vast potential yet to be discovered for the production of valuable products using *Saccharomyces* and non-*Saccharomyces* yeasts [47].

Role of Yeasts in Food Process

Fermented foods and beverages have been an important part of our lives in all over the world. Their production is one of the oldest manufacturing and preservation methods, dating back to ancient times. Yeasts, mainly *Saccharomyces cerevisiae*, and lactic acid bacteria have long been used for the production of many fermented products. In food industry, yeasts have an important role in the production of alcoholic beverages, bioethanol, baker's yeast and yeast-derived products. Lactic acid bacteria also have a fundamental effect on the production of some food products such as yoghurt, fermented vegetables, sour-dough bread and others [48]. Yeast plays a vital role in the production of all alcoholic beverages. Yeast plays a vital role in the production of all alcoholic beverages and the selection of suitable yeast strains is essential not only to maximize alcohol yield, but also to maintain beverage sensory quality [49]. In wine fermentation, strains with specific characteristics are needed, for instance, highly producers of ethanol to reach values of 11-13%v/v, typically found in this beverage. Yeasts are largely responsible for the complexity and sensory quality of fermented beverages. During fermentation, yeast cells convert cereal-derived sugars into ethanol and CO2. At the same time, hundreds of secondary metabolites that influence the aroma and taste of beer are produced. Variation in these metabolites across different yeast strains is what allows yeast to so uniquely influence beer flavour [27]. Although most breweries use pure yeast cultures for fermentation, spontaneous or mixed fermentation is nowadays used for some specialty beers. Traditional ciders are produced from spontaneous fermentation of juice carried out by autochthonous yeasts, cerevisiae strains are also commonly used to carry out alcoholic fermentation. This ensures consistent quality of the finished products [29]. Some other non-*Saccharomyces* yeast species are involved in spontaneous fermentation of apple juice for cider production. However, these yeasts contribute at a lesser extent than *Saccharomyces* and can be producers of off-flavours [30].

Yeast as Biotechnological Tool in Food Industry

In addition to these three worldwide-famous fermented beverages, the fermentative yield of yeast cells during this fermentation is crucial and determines the final quality of the bread. Yeasts not only produce CO_2 and other metabolites that influence the final appearance of the dough, volume, and texture, and of course, the taste of the bread. Commercial bread producers currently produce various types of dough such as lean, sweet or frozen dough. Depending on the type of dough, and to obtain optimal fermentation rates, it is recommended to use suitable yeast strains with specific phenotypic traits [50].

Yeasts play an important role in coffee production, in the post-harvest phase. Its performance can be done in two phases. On the one hand, aerobically, in which the berries just collected are deposited in a tank and the yeasts are allowed to act. This process is carried out under control of basic parameters, such as time and temperature. This second process is more homogeneous and easy to control than the aerobic. Sometimes, coffee beans are even fermented in a mixed process, first in an aerobic and finally anaerobic manner [50]. Raw cacao beans have a bitter and astringent taste, because of high phenolic content. Anthocyanins are one group of these polyphenols, and it both contributes to astringency and provides the reddish-purple color. Fermentation allows the enzymatic breakdown of proteins and carbohydrates inside the bean, creating flavour development [50].

Yeasts as Probiotics

Probiotics have been defined as viable microorganisms that (when ingested) have a beneficial effect in the prevention and treatment of specific pathological conditions [51]. In fact, probiotics have been used for as long as people have eaten fermented foods. In the early 20th century, the Russian immunologist *Elie Metchnikoff* suggested that *lactobacilli* ingested in yogurt could have a positive influence on the normal microbial flora of the intestinal tract [52]. He hypothesized that *lactobacilli* were important for human health and longevity. In recent years, the definition of a probiotic has changed, primarily because of the recognition that probiotic bacteria can influence the physiological outcomes, distant from the gut lumen. Most probiotic microorganisms are bacteria. Strains of *Lactobacillus acidophilus* and *Lactobacillus rhamnosus* strain GG (formerly *Lactobacillus casei*) probably have the longest history of application as probiotics because of their health benefits. Currently used commercial probiotic products include *Lactobacillus* Sp., Bifidobacterium and even a few non-lactic acid bacteria [52].

Although most probiotics are bacteria, one strain of yeast, *Saccharomyces boulardii*, which isolated about a hundred years ago, has been found to be an effective probiotic in double-blind clinical studies. *Saccharomyces boulardii*, a patented yeast preparation, is the only yeast probiotic that has been proven effective in double-blind studies [53]. This yeast is used in many countries as both a preventive and therapeutic agent for diarrhoea and other the gastrointestinal (GI) disorders caused by the administration of antimicrobial agents. *Saccharomyces boulardii* possesses many properties that make it a potential probiotic agent, i.e., it survives transit through GI tract, its temperature optimum is 37ºC, both in vitro and in vivo, it inhibits the growth of a number of microbial pathogens. However, *Saccharomyces boulardii* belongs to the group of simple eukaryotic cells (such as fungi and algae) and, it thus differs from bacterial probiotics that are prokaryotes [53].

Some Contributions of Yeasts in Biotransformation Processes

Yeasts have been studied as biotechnological tools for the biosynthesis of targeted secondary metabolites that can be introduced in different applications [54,55]. In the field of biotransformation, many types of yeast such as *Candida tropicalis*, *Hortaea werneckii*, *Saccharomyces cerevisiae*, and *Trimatostroma salinum* were known for producing 17 β-hydroxysteroid dehydrogenases which are critically important for the control of steroidal hormones biological potency through catalysing oxidation or reduction at C 17. 17 β-HSDs may also work on other substrates including alcohols, fatty acids, bile acids, and retinols [56]. *Saccharomyces cerevisiae* was employed to express a mammalian hydroxylase for stereospecific hydroxylation of dehydroepiandrosterone [57]. *Saccharomyces cerevisiae* has extensive contributions in biotransformation of various compounds. For example, production of testosterone from androstenedione [58]; and from androsten-4-en-3,17-dione [59]. Also, for Ring F opening, and 15 α-hydroxylation of timosaponin A-III [60]. In biotransformation of cellulosic sugars [61]. Conversion of furfural to furfuryl alcohol [62]. Additionally, *Saccharomyces cerevisiae* is used during biotransformation of spent coffee grounds [63].

Applications of Yeasts in Various other Fields

Besides bioenergy, pharmaceutical and enzyme production, marine yeasts have also showed potential to be utilized in various other fields, such as synthesis of metal nanoparticles [5], degradation of pollutants [64-66]. Bioethanol and biodiesel are two important liquid biofuels. Bioethanol production has been increased worldwide [67]. In the last few decades, halo-tolerant yeasts have been investigated as promising alternative candidates for bioethanol production. Urano N, et al. [68], isolated several marine yeasts from various aquatic environments. Most of these isolates belonged to two genera, namely Candida and Debaryomyces. Kathiresan K, et al. [69], isolated 10 marine yeast strains from mangrove sediments on the south-east coast of India. These isolated strains were *Candida albicans*, *Candida tropicals*, *Debaryomyces hansenii*, *Geotrichum* sp., *Pichia capsulata, Pichia fermentans, Pichia salicaria, Rhodotorula minuta*, *Cryptococcus dimennae* and *Yarrowia lipolylica*. Kathiresan K, et al. [69], reported that *Pichia salicaria* was the best strain for ethanol production. Obara N, et al. [70], studied the bioethanol production from the hydrolysate of paper shredder scrap using marine yeast isolated from Tokyo Bay. It was found that the marine yeast *Saccharomyces cerevisiae* (C-19) showed high osmotic tolerance and high ethanol production [71,78].

Conclusion

Yeasts are important eukaryotic microorganisms that are employed in various food, pharmaceuticals, and industrial applications. Their potency and variable capabilities encourage for screening for new, promising, and potent species. Understanding the importance of these unicellular microbes, and their current as well as prospective applications can contribute in putting them in their right position as potent eco-friendly, and low cost biotechnological tools.

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