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MINI-REVIEW

Marine yeasts as biocontrol agents and producers of bio-products

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Abstract As some species of marine yeasts can colonize intestine of marine animals, they can be used as probiotics. It has been reported that β-glucans from marine yeast cells can be utilized as immuno-stimulants in marine animals. Some siderophores or killer toxins produced by marine yeasts have ability to inhibit growth of pathogenic bacteria or kill pathogenic yeasts in marine animals. The virulent factors from marine pathogens can be genetically displayed on marine yeast cells, and the yeast cells displaying the virulent factors can stimulate marine animals to produce specific antibody against the pathogens. Some marine yeast cells are rich in proteins and essential amino acids and can be used in nutrition for marine animals. The marine yeast cells rich in lipid can be used for biodiesel production. Recently, it has been reported that some strains of *Yarrowia lipolytica* isolated from marine environments can produce nanoparticles. Because many marine yeasts can remove organic pollutants and heavy metals, they can be applied to remediation of marine environments. It has been shown that the enzymes produced by some marine yeasts have many unique properties and many potential applications.

Keywords Probiotics · Marine yeasts · Industrial enzymes · Vaccine · Siderophore · Bio-products

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Introduction

Yeasts are regarded as fungi with vegetative states that predominantly reproduce by budding or fission, in vegetative phase growing mainly as single cells. They include ascomycetous and basidiomycetous yeasts. It has been well-documented that yeasts have many applications in fermentation, food, feed, agricultural, biofuel, medical, and chemical industries as well as environmental protection (Kurtzman and Fell 2000).

Marine yeasts are the yeasts that are isolated from marine environments and that can grow better in the medium prepared with seawater than in the medium prepared with fresh water. Recent research has shown that marine yeasts also have highly potential uses in food, feed, and medical industries as well as marine biotechnology (Chi et al. 2009a).

Although the properties of some enzymes such as amylases, cellulase, lipase, phytase, protease, inulinase, and killer toxin produced by marine yeasts, and their genes have been summarized by us (Chi et al. 2009a, b), the present review article is mainly focused on the marine yeast applications in disease control, bioremediation of marine environments, and new bio-product production. We hope that this review article will arouse more researchers to be interested in marine yeasts and their bio-products.

Marine yeasts as biocontrol agents

Probiotics

Probiotics is defined by Verschuere et al. (2000) as "a live microbial adjunct which has a beneficial effect on the host by modifying the host-associated or ambient microbial community by ensuring improved use of the feed or enhancing its

nutritional value, by enhancing the host immunity response towards diseases, or by improving the quality of its environment". In order to minimize and even avoid application of antibiotics and chemicals in rearing environment and feeds in maricultural industries, it is necessary to develop the new methods for inhibition of the pathogenic bacteria in rearing animals by using probiotics and vaccine. It has been reported that after the probiotics is applied to rearing environment, the ecological balance of the habitats of the marine animals and the microbial ecological balance of rearing objections are regulated and improved, the immunity of rearing animals is enhanced and the pathogenic microorganisms are inhibited so that the outbreaks of diseases are controlled effectively (Verschuere et al. 2000; Wang et al. 2002). In addition to bacteria, cyanobacteria, actinomycetes, and microalgae that can be used as probiotics, different species of yeasts are also found to be probiotics in marine animals. The yeast genera *Yarrowia*, *Metschnikowia*, *Candida*, *Debaryomyces*, *Kluyveromyces*, *Pichia*, *Saccharomyces*, *Hanseniaspora*, *Kloeckera*, *Exophiala*, *Leucosporidium*, *Cryptococcus*, *Sporobolomyces*, *Rhodotorula*, and *Trichosporon* have been reported to occur in fish microbiota (Gatesoupe 2007). The ability of yeasts such as *Rhodotorula rubra*, *Rhodotorula glutinis*, *Candida zeylanoides*, *Saccharomyces cerevisiae*, *Debaryomyces hansenii*, and *S. cerevisiae* var. *boulardii* to colonize the intestine of rainbow trout and turbot has been confirmed because they can adhere and grow on the intestinal mucus of rainbow trout as the sole food source. This was particularly evident after single administration either in the rearing water or by force feeding. Because the yeasts in the intestine can grow rapidly and produce extracellular proteases, siderophores and killer toxins as discussed below, they play a role for antagonism to some pathogens, such as pathogenic fungi and enteropathogenic bacteria. It has been reported that *D. hansenii* HF1 can produce spermidine which can induce intestinal maturation in European sea bass larvae and improve growth, survival, and vertebral conformation of the larvae of European sea bass, possibly due to the acceleration of the maturation of the digestive system (Tovar-Ramírez et al. 2002). In addition, the polyamines play a fundamental role in proliferating, fast growing, and regenerating tissues of marine animals. However, it is necessary to find more yeast strains that can actively adhere and grow on the intestine of the cultivating marine animals and play an important role for strong antagonism to most of pathogens in the animals by producing antibacterial and antifungal substances.

Cell wall (immuno-stimulants)

As many of marine animals such as sea cucumber and crustaceans have no specific immune system, and their immune system is mainly non-specific; they must rely on phagocytosis, encapsulation, agglutination, and antioxidation in fighting the pathogens in marine environments. The β-1,3-glucans of certain fungi and yeasts have been successfully used as immuno-stimulants to enhance the defense potential of fish and shellfish against bacterial and viral infection. Much research has shown that β-glucan, mannoprotein, and chitin in yeast cell wall, even the whole cell of *S. cerevisiae* can produce a general immune response and increase immunoglobulin serum titers so that good protection against infection of pathogenic bacteria can be obtained in marine fish fed either *S. cerevisiae*, β-glucan, or deacylated chitin (Gatesoupe 2007). As a glucan receptor is involved in phagocytosis by gilthead sea bream leukocytes during the immune response, β-glucan may be the most important components to account for immuno-stimulation of fish by yeast (Gatesoupe 2007). Sung et al. (1994) reported an enhanced resistance to vibriosis in *Penaeus monodon* postlarvae administered with β-glucan isolated from *S. cerevisiae* cells. Increased post-challenge survival was observed in glucan-fed *P. monodon* to white spot syndrome virus (WSSV) (Song et al. 1997). Chang et al. (2003) showed that oral administration of β-glucan at an optimal level of 10 g/kg diet for 20 days effectively enhanced the immune system, resulting in improved survival against WSSV infection in *P. monodon*. The efficacy of a marine yeast *Candida sake* as source of immuno-stimulant to Indian white shrimp *Fenneropenaeus indicus* was estimated. The results show that marine yeast *C. sake* at 10% in diet (*w*/*w*) may be used as an effective source of immuno-stimulant in *F. indicus* (Sajeevan et al. 2006).

Killer toxin

It has been well known that most of the diseases in marine animals are caused by marine bacteria and marine viruses (Zhu et al. 2006). However, in recent years, the studies have shown that some marine yeasts are also pathogenic to some marine animals. It has been found that some *Candida* spp., *Metschnikowia bicuspidate*, *Cryptococcus* spp., *Sporobolomyces salmonicolor*, and *Trichosporon* sp. are the pathogens of amago (*Oncorhynchus rhodurus*), chinook salmon (*Oncorhynchus tshawytscha*), the githead seabream (*Sparus aurata*), crab (*Portunus trituberculatus*), and teach (*Tinca tinca*), respectively (Gatesoupe 2007; Wang et al. 2007a). It should be emphasized that some yeast and other fungi may be potentially harmful in an immunocompromised host or in stress environmental conditions. However, most of yeast strains are likely harmless to healthy fish reared in good conditions. The pathogenic agent for the milky disease in crab is the yeast *Metschnikowia bicuspidata* WCY (Wang et al. 2007a), and the milky disease has caused large economic losses in maricultural industry in some regions of China. The yeast *Torulopsis*

mogii is a pathogen to some shrimp (Sun and Sun 1998). *M. bicuspidata* var. *bicuspidate* was capable of infecting aquaculture-reared and disease-free *Artemia* (Moore and Strom 2003). Therefore, it is very important to develop some effective and sustainable methods to control the pathogenic yeasts in marine environments. It has been confirmed that nystatin, benzalkonium bromide, and extracts of goldthread root and garlic are active against *M. bicuspidata* WCY (collection number 2E00088 at MCCC). However, the compounds with minimum inhibitory concentration were toxic to the crab. Furthermore, it would be impossible to apply antibiotics in the open sea (Xu et al. 2003).

Much research has shown that killer yeasts can be applied to control the growth of pathogenic yeasts in humans, animals, and plants (Marquina et al. 2002). Killer toxins produced by some yeast strains are low molecular mass proteins or glycoprotein toxins which kill sensitive cells of the same or related yeast genera without direct cell–cell contact (Magliani et al. 1997). It is generally regarded that the mechanisms of killer toxin system are binding of killer toxin to cell wall, the formation of trans-membrane channels, ion leakage, arrest of cell division, interference with the synthesis of glucan in the cell wall and cell death, induction of DNA damage and apoptosis and a strong β-1,3-glucanase activity (Magliani et al. 1997, 2008; Klassen and Meinhardt 2005). However, it is very important to find the killer toxins that only interfere with the biosynthesis and hydrolysis of glucan in the cell wall of the pathogenic yeasts because such killer toxins are not toxic to animal and human cells that have no cell wall. Multiple yeast strains from seawater, sediments, mud of salterns, guts of marine fish, and marine algae for killer activity against the yeast *M. bicuspidata* WCY (pathogenic to crab *P. trituberculatus*; collection number 2E00088 at MCCC) were screened. It was found *Williopsis saturnus* WC91-2 (collection number 2E00219 at MCCC), *Pichia guilliermondii* GZ1 (collection number 2E00008 at MCCC), *Pichia anomala* YF07b (collection number 2E00058 at MCCC), *D. hansenii* hcx-1(collection number 2E00089 at MCCC), and *Aureobasidium pullulans* HN2.3 (collection number 2E00078 at MCCC) could secrete toxin into the medium and kill the pathogenic yeast (Wang et al. 2008a). Finally, it was observed that the marine-derived *W. saturnus* WC91-2 has much higher killing activity and wider killing activity spectra than the marine-derived *P. anomala* YF07b (Wang et al. 2008a). The molecular mass of the purified killer toxin from the marine killer yeast YF07b is 47.0 kDa and the toxin also actively hydrolyzes laminarin and kills the whole cells of the pathogenic yeast in crab (Wang et al. 2007b). The molecular mass of the purified killer toxin produced by the marine-derived *W. saturnus* WC91-2 is 11.0 kDa and the toxin has no β-1,3-glucanase activity that catalyzes hydrolysis of laminarin (Peng et al. 2009b). After the amino acids of the 11.0 kDa killer toxin are aligned with those of yeast killer toxin superfamily (pfam 09207), the results in Fig. 1 show that the amino acids of the 11.0 kDa killer toxin from 2 to 88 and those of yeast killer toxin superfamily (pfam 09207) from 1 to 87 are very highly conserved, suggesting that the 11.0 kDa killer toxin belongs to yeast killer toxin superfamily. It has been shown that all the killer toxins of the yeast killer toxin superfamily can inhibit biosynthesis of β-glucan of fungal cell wall (Magliani et al. 1997). Therefore, the 11.0 kDa killer toxin produced by the marine-derived *W. saturnus* WC91-2 can be used to inhibit biosynthesis of β-glucan of the pathogenic yeast *M. bicuspidata* WCY. After the amino acids deduced from the cloned gene encoding the killer toxin in the marine killer yeast YF07b is aligned with those of exo-β-1,3-glucanases from other yeasts (Wang et al. 2007c), the results in Fig. 2 indicate that all of them contain the conserved amino acid sequences NLCGEWSAA (in box), where the Glu (E) has been shown convincingly to be the catalytic nucleophile for β-1,3-glucan hydrolysis (Martin et al. 2007). It has been shown that exo-β-1,3-glucanases catalyze hydrolysis of β-1,3-glucan, the main component of the fungal cell wall. This means that the 47.0 kDa killer toxin produced by the marine killer yeast YF07b can catalyze hydrolysis of β-1,3-glucan in the pathogenic yeast *M. bicuspidata* WCY. Indeed, the purified 47. 0 kDa killer toxin produced by the marine killer yeast YF07b can hydrolyze laminarin into monosaccharide and disaccharides, and its strong β-1,3-glucanase activity is the primary killing mechanism of the killer toxin (Wang et al. 2007b). All the results mentioned above demonstrate that these two kinds of killer toxins are suitable for killing the pathogenic yeasts in marine animals. However, it is still unknown if the killer toxins can be used to control the milky disease in crab in marine environments.

Siderophore

Siderophores are low molecular weight, iron-chelating ligands produced by nearly all the microorganisms during extreme iron-depleted conditions for the solubilization of extracellular ferric iron (Wang et al. 2009b). The most significant feature of siderophores is their extremely high affinity for ferric ion. Siderophores are also found to have many applications in medical industry, agricultural industry, and environmental sciences (Renshaw et al. 2002). For example, they can be used to control the growth of the pathogenic bacteria isolated from diseased marine fish, and the high iron-chelating ability of siderophores can be used to develop processes for metal recovery or remediation of waste sites, including radioactive waste as they are

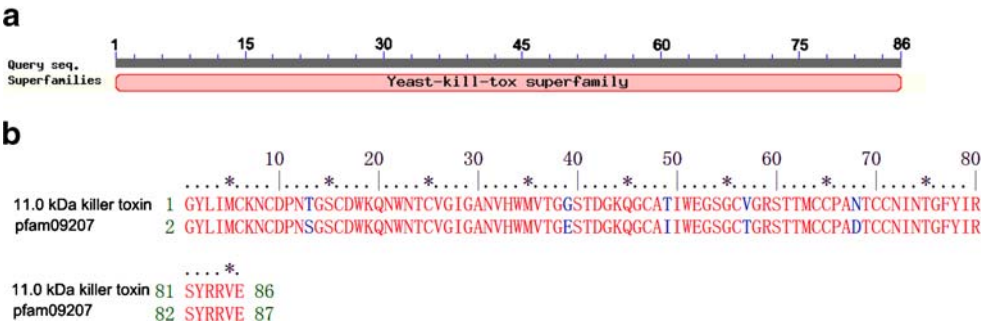


Fig. 1 Conserved domains of killer toxins (**a**) and alignment of amino acids of 11.0 kDa killer toxin and pfam09207 (**b**). The 11.0 kDa killer toxin is produced by the marine-derived *W. saturnus* WC91-2; Pfam09207 is the known yeast killer toxin superfamily

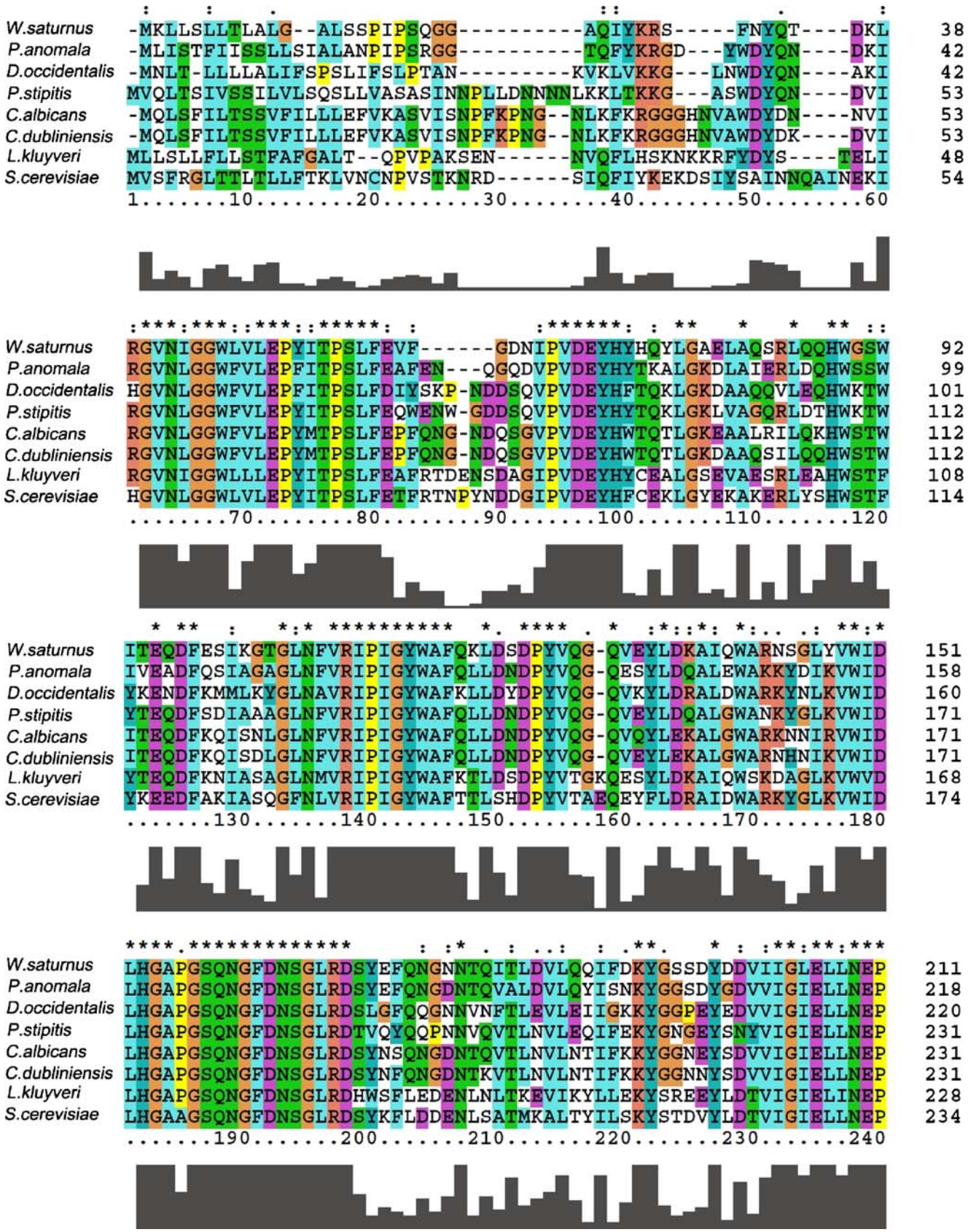
extremely effective at solubilizing actinides and other metals from polluted environments (Renshaw et al. 2002).

It has been confirmed that yeasts produce only hydroxamate-type compound (Riquelme 1996). Over 300 yeast strains isolated from different marine environments were screened for their ability to produce siderophore. Among them, only the yeast strain HN6.2 (collection number 2E00322 at MCCC) which was identified to be *A. pullulans* was found to produce the highest level of the siderophore. Under the optimal conditions, this yeast strain can produce 1.1 mg/ml of the siderophore. The crude siderophore produced by the yeast strain HN6.2 is able to inhibit cell growth of *Vibrio anguillarum* and *Vibrio parahaemolyticus*, isolated from the diseased marine animals (Wang et al. 2009b). After analysis using HPLC and electron-spray ionization mass spectroscopy, the purified siderophore produced by the marine-derived *A. pullulans* HN6.2 was found to be fusigen (Fig. 3a). The purified desferric fusigen still has strong inhibition activity against growth of the pathogenic *V. anguillarum* while the fusigen chelated by Fe^{3+} looses the ability to inhibit the growth of the pathogenic bacterium (Wang et al. 2009c). It has been reported that *V. anguillarum* also can produce its own siderophore, anguibactin (Fig. 3b; Hossain et al. 1998). However, the siderophore produced by the yeast strain HN6.2 has much higher affinity for iron than that produced by the pathogenic bacterium, leading to its strong inhibition activity against growth of the bacterium by depriving the bacterium of iron. The structures (Fig. 3) show a monomeric complex in which the anguibactin is a tetradentate ligand while fusigen is a hexadentate ligand. Therefore, it can be expected for the fusigen to have a higher complexation constant than anguibactin, and thus, the fusigen can prevent the ferric anguibactin to be formed and thus preventing growth of *V. anguillarum.* The marine bacterium *V. anguillarum* is responsible for vibriosis, a systemic disease of fish characterized by hemorrhagic septicemia. Outbreaks of the vibriosis worldwide result in high mortality rates of infected fish (Zhang et al. 2006). Thus, it is economically important how to control the growth of this pathogenic bacterium in marine environments. As the siderophore produced by the marine-derived *A. pullulans* HN6.2 is very stable in the environments (Wang et al. 2009b), it may have many advantages over the antimicrobial peptide (Li et al. 2007) which is temperature-sensitive and easily attacked by proteinase in the environments when the siderophore is applied to biocontrol of the pathogenic bacterium in marine environments. In addition, the yield of siderophore produced by the marine yeast is usually much higher than that of the antimicrobial peptide produced by some marine animals. However, it has been well-evidenced that Fe^{2+} available in the medium can repress the siderophore biosynthesis in the marine yeast. In contrast, ornithine available in the medium can derepress the siderophore biosynthesis. The repression and derepression happen at the transcriptional level (Wang et al. 2009c). So, the siderophore production can be further enhanced by over-expressing the genes encoding the transcriptional activator and deleting the genes encoding the transcriptional repressor in the yeast (Johnson 2008).

Vaccine

It is well-documented that most bacterial infections in marine animals are caused by vibrios (Li and Woo 2003). So far, conventional approach, such as the use of antimicrobial substances, has limited the success in the prevention or cure of the bacterial disease in marine animals. We think that the best alternative strategies to the use of antimicrobial chemicals are to use vaccines. Vaccines can be classified into live attenuated vaccine, killed vaccine, DNA vaccine, and live vaccine. In recent years, live vaccines have received increasing attention as they have many advantages over any other vaccines (Zhu et al. 2006). One of the best approaches for production of live

Fig. 2 CLUSTAL W program alignment of the deduced amino acid sequences of exo-β-1,3-glucanases from *W. saturnus* WC91-2 (accession number: ACP74152.2), *P. anomala* YF07b (accession number: EF029071), *D. occidentalis* (accession number: Q12700.1), *Pichia stipitis* (accession number: XP_001385760.2), *C. albicans* (accession number: CAA21969.1), *Candida dubliniensis* (accession number: XP_002416951.1), *Lachancea kluyveri* (accession number: Q875R9.1), *S. cerevisiae* (accession number: EDN63719.1); the conserved domains were *boxed*


W.saturnus
P.anomala
D.occidentalis
P.stipitis
C.albicans
C.dubliniensis
L.kluyveri
S.cerevisiae

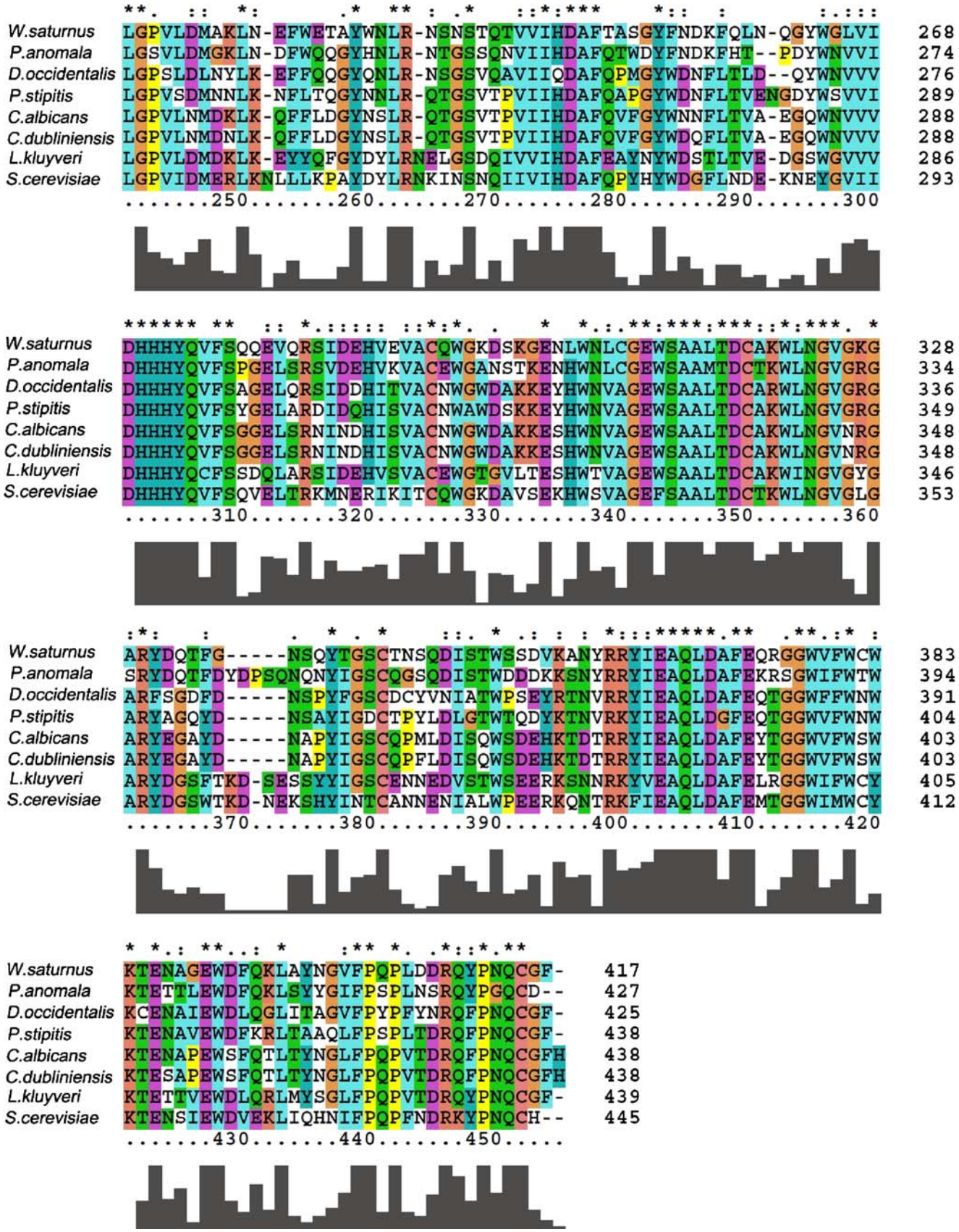


Fig. 2 (continued)

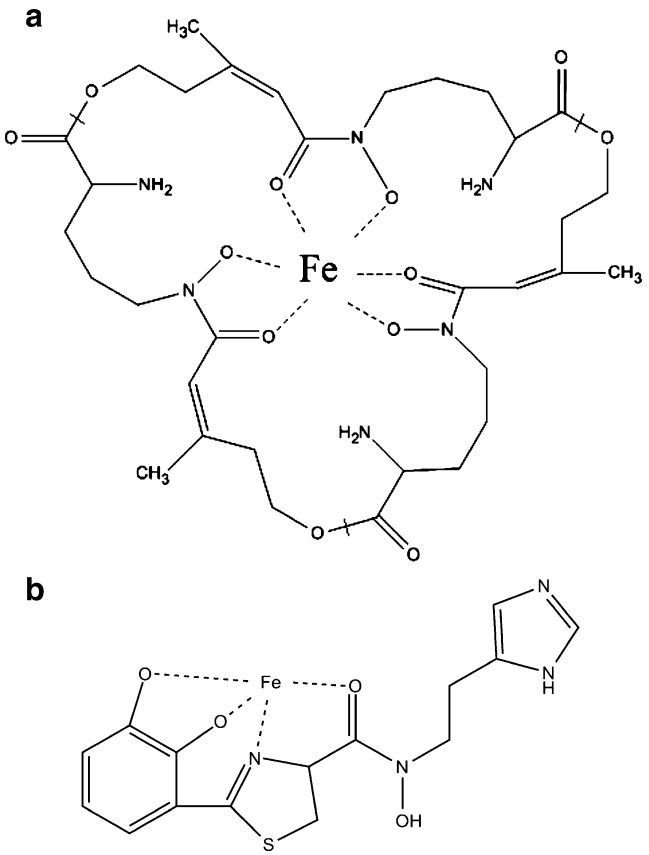


Fig. 3 The chemical structures of fusigen **a** produced by *A. pullulans* HN6.2 and anguibactin **b** produced by *V. anguillarum*

vaccine is to display virulence factors from the pathogens on cell surface of *S. cerevisiae* due to its generally regarded as safe (GRAS) status, easy cultivation, cheap production and distribution, clear genetic background, available surface displaying system, regeneration, and adjuvant function (Ueda and Tanaka 2000). In recent years, it has been found that *Vibrio harveyi* emerges as a serious pathogen of marine animals and hemolysins produced by it might be involved in pathogenesis in Atlantic salmon and rainbow trout (Li and Woo 2003). In general, bacterial hemolysin has been suggested to be an important factor of the pathogenic vibrios by causing hemorrhagic septicemia and diarrhea in the host. Many of these hemolysins are well-characterized, and the genes encoding them have been cloned from *V. parahaemolyticus*, *Vibrio cholerae*, *Vibrio hollisae*, *Vibrio mimicus*, *Vibrio vulnificus*, *Vibrio anguillarum*, and *V. harveyi* (Li and Woo 2003). Hemolysin-encoding gene *HL1* from *V. harveyi* SF-1 was expressed on the yeast cell surface. After induction for 36 h in the galactose-containing medium, one-third of the cells contained the displaying protein and the displayed cells had hemolytic activity on erythrocytes from flounder. The double diffusion agar analysis showed that the sera from the flounder immunized with the displayed yeast cells having the hemolytic activity could form precipitate with the purified hemolysin. ELISA analysis indicated that immunization times had great influence on increased production of the specific antibody against hemolysin in turbot immunized with the displayed yeast cells having the hemolytic activity. After the challenge with *V. harveyi* SF-1, it was found that earlier protection in flounder and significant protection in turbot, both of which were immunized with the displayed yeast cells having the hemolytic activity, were achieved. These results suggested that the yeast cells displaying the hemolysin could be used as potential live vaccine in marine fish (Zhu et al. 2006). In another study (Wang et al. 2009a), it has been observed that the same hemolysin displayed on cells of the laboratory strain *Yarrowia lipolytica* Po1h and the marine-derived yeast *Y. lipolytica* with high protein content also has hemolytic activity towards erythrocytes from flounder. The flounder and turbot immunized with the yeast cells displaying the hemolysin also produce the specific antibody against the hemolysin.

As many species of *Vibrio* sp. can exist in the same marine environments where the marine animals grow and each species of *Vibrio* sp. can produce different kinds of virulent factors which can cause disease in marine animals, usually it is impossible for one specific antibody produced by marine animals to neutralize the different pathogens and virulent factors. The results in Fig. 4 show that the amino acids of outer membrane protein K (OmpK) from different species of *Vibrio* sp. are very conserved, suggesting that OmpK can be a better candidate for developing new vaccine (Mao et al. 2007). Hence, it is possible that one specific antibody produced by the marine animal immunized with one kind of OmpK displayed on marine yeast cells can react with the different pathogens. This technique can be used to develop multivalent vaccine against infection of different pathogens in marine environments.

Marine yeasts as producers of bio-products

Industrial enzymes

It has been shown that amylase, cellulase, inulinase, lipase, alkaline protease, acid protease, and phytase can be produced by different species of the marine-derived yeasts (Chi et al. 2009a). All the enzymes have been purified and characterized. The genes encoding the inulinase, lipase, alkaline protease, acid protease, and phytase have been cloned and characterized. Some of them have been heterologously expressed in other hosts. It has been shown that the enzymes and their encoding genes have many potential applications. All these have been thoroughly reviewed by Chi et al. (2009a). For example, the inulinase gene cloned from the marine-derived yeast *P. guilliermondii*

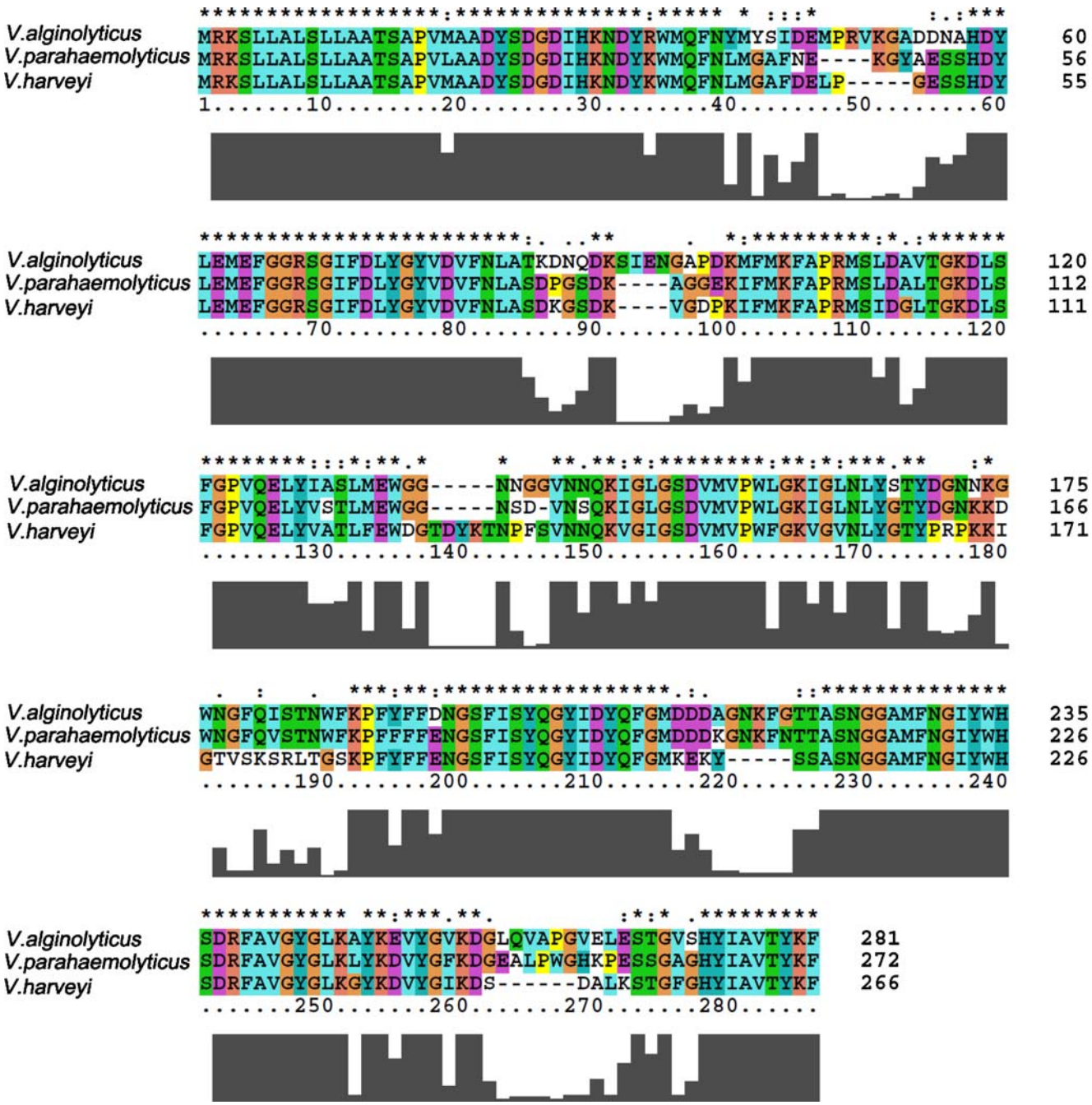


Fig. 4 Alignment of OmpKs from *V. alginolyticus* (accession number: DQ063588), *V. parahaemolyticus* (accession number: DQ016304), *V. harveyi* (accession number: DQ279075). The conserved amino acids are *marked in different colors* in the amino acid sequences and the conservation degrees are *marked in black* below the amino acid sequences. *Asterisk*: shows the range of the conserved amino acids

strain 1 (collection number 2E00005 at MCCC) was expressed in *Pichia pastoris* X-33. After the optimization of the conditions for production of the recombinant inulinase, 286.8±5.4 U/ml of the recombinant inulinase activity in the supernatant of the culture of 2-l fermentor was attained at 120 h of the fermentation (Zhang et al. 2009c). The purified recombinant inulinase also had high exoinulinase activity (Zhang et al. 2009a). After the inulin (over 20% *w*/*v*) was completely hydrolyzed by the recombinant inulinase, the hydrolysate produced could be converted into ethanol by a high ethanol producing yeast, *Saccharomyces* sp. W0 and ethanol concentration in fermented medium could reach over 15% (*v*/*v*). Recently, the inulinase gene has been cloned into the high ethanol producing yeast and expressed in it. The recombinant yeast can produce over 15% (*v*/*v*) ethanol from inulin and 12%

(*v*/*v*) ethanol from powder of tubers of Jerusalem artichoke in one-step fermentation. This indicates that the recombinant inulinase and the gene cloned from the marine yeast may have highly potential applications in biofuel industry. Inulin is present as a reserve carbohydrate in the roots and tubers of plants such as Jerusalem artichoke, chicory, dahlia, and yacon and one of the most important raw materials in biotechnology (Chi et al. 2009c).

The polysaccharides produced by marine algae and marine animals include chitin, alginate, agar, and carrageenan. They have many applications in food, pharmaceutical, and agricultural industries as well as biological research. It also has been found that oligosaccharides produced from the polysaccharides have more applications and functions than the polysaccharides. Although many marine bacteria can produce chitinase, alginate lyase, agarase, and carrageenase, it has not been evidenced that marine-derived yeasts can produce such enzymes (Liu et al. 2009; Chi et al. 2009a). Many genes encoding chitinase, agarase, alginate lyase, and carrageenase in marine bacteria have been cloned and characterized (Stefanidi and Vorgias 2008; Barbeyron et al. 1998; Lee et al. 2000) and hydrolysates (oligosaccharides) from chitin, agar, and carrageenan also have a variety of bioactivities and many applications in medical and food industries. Thus, the chitinase, agarase, and carrageenase from marine bacteria or other sources could also be expressed in the marine yeasts in order to overproduce the recombinant chitinase, agarase, and carrageenase for hydrolysis of chitin, agar, alginate, and carrageenan and production of their bioactive oligosaccharides. Because some of the enzyme-producing marine bacteria are the pathogens to marine algae and marine animals, it is necessary to overexpress the genes in the marine yeasts that have GRAS status. For example, the alginate lyase structural gene (*Aly*VI gene) was amplified from plasmid pET24-ALYVI carrying the alginate lyase gene from the marine bacterium *Vibrio* sp. QY101 which is a pathogen of *Laminaria* sp (Han et al. 2004) and expressed in cells of *Y. lipolytica.* The recombinant alginate lyase can be used to hydrolyze poly-β-D-mannuronate (M) and poly-α-L-guluronate (G) and sodium alginate to produce different lengths of oligosaccharides (more than pentasaccharides; Liu et al. 2009). As shown below, *Y. lipolytica* is also widely distributed in marine environments. The expression vector pINA1317 also can be used for cloning and expression of genes from different sources in the marine-derived *Y. lipolytica* (Wang et al. 2009a).

Many marine fungi and *Laminaria* sp also synthesize β-glucans. The non-cellulosic β-glucans are important biopolymers because of their peculiar biological activities that include anti-tumor, anti-inflammatory, and immunomodulation. One way of increasing the water solubility of β-glucans is to fragment the polysaccharide into shorter chain lengths using microbial β-glucanases. These enzymes are having potential application in biotechnology such as cell fusion, transformation, and protoplast preparation, in food, feed, agricultural, pharmaceutical, and fermentation industries and during the clarification of slimy must (Martin et al. 2007). Recently, it has been found that the crude enzymes from many marine-derived yeasts can hydrolyze laminarin, β-1,3-glucan isolated from *Laminaria* sp (Wang et al. 2008a). The purified β-1,3-glucanase from the marine yeast *W. saturnus* WC91-2 with molecular mass 47.5 kDa could convert laminarin into monosaccharides and disaccharides (Peng et al. 2009a), suggesting that the enzyme is an exo-β-1,3-glucanase. As mentioned above, the purified killer toxin from the marine killer yeast YF07b also actively hydrolyzes laminarin into monosaccharides and disaccharides (Wang et al. 2007b). This reveals that β-1,3-glucanases produced by the marine-derived yeasts can be used to actively hydrolyze β-1,3-glucan from *Laminaria* sp. The gene (*WsEXG1*) in *W. saturnus* WC91-2 has been cloned and characterized (Peng et al. 2009a). The amino acids deduced from the *WsEXG1* gene contain the conserved amino acid sequences NLCGEWSAA (in box in Fig. 3), where the Glu (E) has been shown convincingly to be the catalytic nucleophile for β-1,3-glucan hydrolysis (Martin et al. 2007). The gene can be over-expressed in *Y. lipolytica*, and the secreted β-1,3-glucanase can actively hydrolyze laminarin into sole monosaccharides and minor disaccharides (Peng et al. 2009a). This may suggest that the recombinant β-1,3-glucanase produced by the engineered *Y. lipolytica* can be used to hydrolyze β-1,3-glucan.

Single-cell protein

Single-cell protein has many applications in food and feed industries. The microorganisms which can be used as single-cell protein include a variety of marine microalgae, bacteria, yeasts, and molds (Ravindra 2000; Gao et al. 2007). However, as single-cell protein, yeast cells are better than any other microorganisms (Ravindra 2000). Rhishipal and Philip (1998) isolated 33 strains of marine yeasts from the coastal and offshore waters off Cochin in India. The isolates were inoculated into the prawn-shell wastes, and the protein content of the final products was increased from 38.5% to 70.4% after the transformation of the prawn-shell waste by strain M15 of *Candida*. Brown et al. (1996) found that the marine yeasts *D. hansenii* ACM 4784, *Dipodascus capitatus* ACM 4779, and *Dipodascus* sp. ACM 4780 contained 23%, 32%, and 36% of crude protein, respectively, while terrestrial *Candida utilis* ACM 4774 contained 42% of crude protein. The authors concluded that high protein content, high levels of carbohydrate, and good amino acid composition characterized all the marine yeasts they used, and high levels of saturated fats characterized

some of the marine yeasts (Brown et al. 1996). However, all the marine yeast strains lacked the 20:5n-3 and 22:6n-3 fatty acids, making them unsuitable as a complete diet for larval raising (Brown et al. 1996). A total of 327 yeast strains from seawater, sediments, mud of salterns, guts of the marine fish, and marine algae were obtained. After crude protein of the yeasts was estimated by the method of Kjehldahl, it was found that eight strains of the marine yeasts grown in the medium with 20 g/l glucose contained more than 30.4 g protein per 100 g of cell dry weight. They belong to *Metschnikowa reukaui* (collection number 2E00007 at MCCC), *Cryptococcus aureus* (collection number 2E00005 at MCCC), *Aureobasidium pullulan* (collection number 2E00009 at MCCC), *Y. lipolytica* (collection number 2E00018 at MCCC), and *Hanseniaspora uvarum* (collection number 2E00028 at MCCC), respectively. Analysis of amino acids indicates that the yeast strains have a large amount of essential amino acids, especially lysine and leucine which are very important nutritive components for marine animals (Chi et al. 2008a). It has been observed that the marine yeast *C. aureus* G7a, which is able to grow on a wide range of carbon sources and secrete a large amount of inulinase into the medium, can be used as the candidate of single-cell protein because 10.1 g of cell dry weight per liter of medium and 53.0 g of crude protein per 100 g of cell dry weight (5.4 g/l of medium) were achieved (Gao et al. 2007; Sheng et al. 2007). It was also found that the yeast strain only contained 2.1 g of nucleic acid per 100 g of cell dry weight, but its cells contained a large amount of $C_{16:0}$ (19.0%), $C_{18:0}$ (46.3%), and $C_{18:1}$ (33.3%) fatty acids and had a large amount of essential amino acids, especially lysine (12.6%) and leucine (9.1%), and vitamin C (2.2 mg per 100 g of cell dry weight; Gao et al. 2007).

It has been well-known that yeast cells have a rigid thick cell wall of about 200 nm in thickness outside of the plasma membrane (Ueda and Tanaka 2000). However, some animals cannot synthesize the enzymes which hydrolyze mannoproteins and β-linked glucans in the yeast cell wall. Therefore, it is difficult for the yeast cell wall to be attacked in the guts of some marine animals. A highly thermosensitive and permeable mutant is the mutant from which intracellular contents including proteins can be released when it is incubated both in the low osmolarity water and at the non-permissive temperature (usually 37°C). After mutagenesis using nitrosoguanidine, such mutant named Z114 was obtained from the marine yeast *C. aureus* G7a. Of the total protein, 65.3% was released from the mutant cells suspended in distilled water after they were treated at 37°C overnight. It was found that no big changes in cell growth, protein content, vitamin C content, nucleic acid content, fatty acids, and amino acid compositions of both the mutant, and its wild-type were detected. Therefore, the highly thermosensitive and permeable mutant still can be a good candidate as single-cell protein (Zhang et al. 2009b). After the mutant was grown in the yacon extract for 45 h, the crude protein content in the highly thermosensitive and permeable mutant Z114 was 59.1% and over 61% of the total protein can be released from the cells treated at 37°C. The mutant cells grown in the yacon extract still contain high level of essential amino acids and other nutrients (Zhao et al. 2009). This suggests that the yacon extract can be used as the medium for production of the single-cell protein.

So far, little has been known about the physiological role of the accumulated proteins, and the enzymes and genes responsible for improvement of single-cell protein production. It has been reported that when the glutamate dehydrogenase system isolated from *Escherichia coli* was transferred to a glutamine–ketoacid–transaminase mutant of *Pseudomonas methylotrophus* by genetic engineering, improved nitrogen assimilation was observed (Ravindra 2000). This may imply that it is crucial how to effectively transform inorganic nitrogen source, such as NH_4Cl and NH_4SO_4, into amino acids for enhancement of single-cell production. We also think that disruption of the genes encoding the intracellular proteases in the yeast cells with high protein content may further enhance protein production in yeast cells.

Single-cell oil

In the past years, biodiesel, which is a renewable, biodegradable, and nontoxic fuel has received increasing attention because of the environmental pollution and energy crisis worldwide. Biodiesel can be produced by transesterification of triacylglycerols from renewable biomass (single-cell oils), yielding monoalkyl esters of long-chain fatty acids with short-chain alcohols. It has been well-documented that single-cell oils are produced by some oleaginous microorganisms, such as yeast, fungi, bacteria, and microalgae. In general, yeast and molds can accumulate much more lipids than bacteria and microalgae (Meng et al. 2009). It was also found that the single-cell oil production by oleaginous yeasts has many advantages due to their fast growth rate, high oil content, and the resemblance of their triacylglycerol fraction to plant oil. So far, *Cryptococcus albidus*, *Cryptococcus curvatus*, *Lipomyces lipofera*, *Lipomyces starkeyi*, *Rhodosporidium toruloides*, *R. glutinis*, *Trichosporon pullulan*, and *Y. lipolytica*, have been intensively used to accumulate oils from glucose, xylose, arabinose, mannose, glycerol, and other agricultural and industrial residues and different yeast species can accumulate different amount of oils in their cells (Meng et al. 2009; Li et al. 2008; Hassan et al. 1996).

At present the high production cost of biodiesel is a major barrier to its commercialization. The high cost of biodiesel production is partially associated with the cost of raw material, making it a less competitive fuel. Therefore, using a low-cost raw material is crucial in reducing the cost of biodiesel production. In order to reduce the cost of the single-cell oil production by yeasts, other carbon sources instead of glucose should be used. Cassava (*Manihot esculenta*) is a root crop of tropical American origin and is the fourth most important staple crop in the tropics. The cassava plant is extremely robust, is resistant to disease and drought, and can grow in relatively low-quality soils (Li et al. 2010).

Rhodotorula mucilaginosa TJY15a (collection number 2E00267 at MCCC) which was isolated from surface of marine fish could accumulate a large amount of lipid from hydrolysate of cassava starch. The cells contained 47.9% (*w*/*w*) oil during batch cultivation, whereas 52.9% (*w*/*w*) of lipid was obtained during the fed-batch cultivation. Therefore, the marine-derived *R. mucilaginosa* TJY15a was another candidate for single-cell oil production. The fatty acids from *R. mucilaginosa* TJY15a were mainly composed of palmitic acid ($C_{16:0}$), palmitoleic acid ($C_{16:1}$), stearic acid ($C_{18:0}$), oleic acid ($C_{18:1}$), and linolenic acid ($C_{18:2}$), suggesting that the fatty acids could be used as feedstock for biodiesel production (Li et al. 2010).

Like in single-cell protein, little has been known about the physiological role of the accumulated lipids and the genes coding for the key enzymes that contribute to fatty acid synthesis and accumulation. It has been well-documented that when the oleaginous microorganisms grow in a medium with an excess of carbon substrate and a limiting amount of nitrogen, lipid content in their cells can reach very high. It is thought that ATP/citrate lyase and AMP deaminase involved in formation of acetyl-CoA in oleaginous microorganisms are of importance for improvement of fatty acid biosynthesis (Ratledge 2004).

Nanoparticles

Nanoparticles have many applications in chemical, food, medical, and agricultural industries and great influence on chemical, energy, electronic, and biological sciences. Recently, it has been found that many microorganisms, such as *Pichia jadinii*, *Verticillium luteoalbum*, *Rhodopseudomonas capsulata*, *Actinobacter* sp., *Bacillus megatherium*, and *Cladosporium cladosporioides* can biosynthesize nanoparticles. It was found that gold nanoparticles could be synthesized from $HAuCl_4$ by the tropical marine yeast *Y. lipolytica* NCIM 3589 in seawater and in fresh water. Both the yeast and filamentous forms can synthesize nanoparticles under a variety of conditions, and the nanoparticles produced are associated with the cell wall. Acidic pH favored nucleation on the cell surfaces and the subsequent formation of gold crystals. At pH 7.0 and 9.0, there was nanoparticle synthesis with a size of 15 nm. The reductases or proteases in the marine yeast cells may play a role in the reduction of the gold salt into nanoparticles (Pimprikara et al. 2009; Agnihotri et al. 2009). However, it is still unknown if other marine yeasts also can synthesize such nanoparticle and how to enhance the yield of the nanoparticles in their cells. Also, it is very important to know the molecular and physiological mechanisms involved in the process of nanoparticle synthesis.

Riboflavin

Recently, it has been found that the marine yeast strain W14-3 isolated from seawater of China Eastern Sea could produce riboflavin. It is interesting to observe that the marine yeast strain produces a large amount of riboflavin in the medium containing xylose, sucrose, galactose, and maltose under the conditions of vigorous shaking. The yeast strain was found to belong to *Candida membranifaciens* subsp. *flavinogenie* (collection number 2E00025 at MCCC). The amino acid sequences deduced from the cloned genes encoding riboflavin biosynthesis in the yeast exhibit high identity with those of the corresponding enzymes for riboflavin biosynthesis in other yeasts. Fe^{3+} available in the medium represses riboflavin production and expression of the genes responsible for riboflavin biosynthesis in the yeast (Wang et al. 2008b). Under the optimized conditions, 22 μg/ml of riboflavin was reached in the culture of strain W14-3 within 54 h of fermentation (Chi et al. 2008b). It has been well-known that riboflavin has many applications in food and pharmaceutical industries (Stahmann et al. 2000). It is thought that Fe^{3+} can bind the repressor, then the repressor bind to the promoters of the genes responsible for biosynthesis of riboflavin. As Fe^{3+} widely exists in natural medium and it is not easy to remove it from the natural medium, it is also important to clone the repressor gene from the riboflavin-producing yeasts and then delete it in order to further promote riboflavin biosynthesis.

Removal of pollutants from marine environments

As you know, the marine environments are also heavily contaminated by different kinds of pollutants, including organic substances and heavy metals, especially hydrocarbons. However, marine microorganisms including bacteria, filamentous fungi, and yeasts can play an important role in degradation of organic pollutants and transformation of heavy metals. Transport, pumping of ballast waters, effluent from oil processing factories, servicing of oil tankers, and ships are the major factors contributing

towards oil pollution which also affect beaches contiguous to the ports. Ahrean et al. (1971) have reported the natural occurrence of marine yeasts in oil-polluted coastal regions. Analysis of 20 samples of marine mud and water around Mumbai led to the isolation of 17 bacterial strains and yeast strains all of which were able to degrade more than 10% of the supplied crude oil (Zinjarde and Pant 2002). The authors found that the yeasts strains were important degraders of the aliphatic fraction of crude oil. They include *Candida parapsilosis*, *Candida albicans*, *Candida guilliermondii*, *Y. lipolytica*, *Candida tropicalis*, and *Candida intermedia*. Among them, *Y. lipolytica* was the best degrader utilizing 78% of the aliphatic fraction of Bombay high crude oil. None of these isolates degraded the aromatic or ashphaltene fractions. All the isolates required aeration, nitrogen, and phosphate supplementation for optimal

Table 1 The various applications of marine yeasts and their products

Products	The marine yeasts	Mechanisms	Applications	References
Probiotics	*R. rubra*, *R. glutinis*, *C. zeylanoides*, *S. cerevisiae*, *D. hansenii*, *S. cerevisiae* var. *boulardii*	Strong antagonism, induction of intestinal maturation, and improvement of growth and survival	Inhibition of pathogens	Gatesoupe 2007
Immuno-stimulants	*S. cerevisiae*, *C. sake*	Immuno-stimulation of fish	Stop of bacterial and viral infection	Sajeevan et al. 2006
Siderophore	*A. pullulans* HN6.2	High iron-chelating ability	Inhibition of pathogen	Wang et al. 2009b
Killer toxins	*W. saturnus* WC91-2, *P. anomala* YF07b	Inhibition and hydrolysis of cell wall of the sensitive cells	Killing of pathogens	Peng et al. 2009b
Vaccine	*S. cerevisiae*, *Y. lipolytica*	Antibody	Enhanced immunity	Zhu et al. 2006
Amylase	*A. pullulans*	Hydrolysis of starch	Fermentation	Chi et al. 2009a
Alkaline protease	*A. pullulans*	Hydrolysis of protein	Food and pharmaceutical industries	Chi et al. 2009a
Acid protease	*Metschnikowia reukaufii*	Hydrolysis of protein	Food and pharmaceutical industries	Chi et al. 2009a
Lipase	*A. pullulans*	Hydrolysis of lipid	Chemical industry	Chi et al. 2009a
Cellulase	*A. pullulans*	Hydrolysis of cellulose	Chemical industry	Chi et al. 2009a
Inulinase	*P. guilliermondii*	Hydrolysis of inulin	Food and fuel industry	Zhang et al. 2009a
Phytase	*K. ohmeri*	Hydrolysis of phytate	Feed industry	Chi et al. 2009a
Alginate lyase	*Y. lipolytica*	Hydrolysis of alginate	Pharmaceutical industries	Liu et al. 2009
Glucanase	*W. saturnus*	Hydrolysis of β-glucan	Pharmaceutical industry	Peng et al. 2009a
Riboflavin	*C. membranifaciens* subsp. *flavinogenie*	Nutrition	Food and pharmaceutical industry	Wang et al. 2008b
Single-cell protein	*C. aureus*, *Y. lipolytica*	Nutrition	Food and feed industry	Zhang et al. 2009b
Single-cell oil	*R. mucilaginosa*	Bioenergy	Biodiesel industry	Li et al. 2010
Nanoparticles	*Y. lipolytica*	Materials	Biomaterial industry	Agnihotri et al. 2009
Degrader of pollutants	*Y. lipolytica*	Degradation of pollutants	Bioremediation	Bankar et al. 2009a

degradation. Treatment of palm oil mill effluent from a factory site in India using *Y. lipolytica* NCIM 3589, a marine hydrocarbon-degrading yeast isolated from Mumbai, India, gave a carbon oxygen demand (COD) reduction of about 95% with a retention time of 2 days (Oswal et al. 2002). Jain et al. (2004) have reported that a tropical marine yeast *Y. lipolytica* has the ability to transform trinitrotoluene (TNT) into products such as 2,4-DNT (dinitrotoluene) which in turn could be metabolized by other microbes, and this yeast strain can be used in the bioremediation of TNT-polluted marine environments. It was also found that *Y. lipolytica* has the genetic make-up to degrade other environmental pollutants, such as triglycerides and aliphatic hydrocarbons. The results indicate that the marine-derived yeast *Y. lipolytica* has high ability to degrade different organic pollutants, especially hydrocarbons. Our results also show that the yeast is indeed widely distributed in different marine environments (Wang et al. 2009a). It is regarded that the efficient cytochrome P450-dependent hydroxylation in the hydrophobic substrate degrading yeast, *Y. lipolytica* can play an important role in degradation of the aromatic or ashphaltene fractions (Bankar et al. 2009a). It has been reported that the *Y. lipolytica* cells can bind large amounts of heavy metals and produce specific protein metallothionein as a protective agent against the heavy metals. This approach offers the possibility to apply the yeast in bioremediation of soil and water contaminated by petroleum products in connection with heavy metals (Strouhal et al. 2003). Indeed, the biomass of two marine strains of *Y. lipolytica* (NCIM 3589 and 3590) can remove chromium (VI) ions from aqueous solutions and maximum biosorption was observed at pH 1.0 and at a temperature of 35°C (Bankar et al. 2009b). These results indicate that the marine yeasts can be used to remove pollutants from marine environments.

In addition, it was found that *Y. lipolytica* IMUFRJ50682 isolated from Guanabara Bay in Rio de Janeiro can produce Yansan, a bioemulsifier. The emulsifier presents high emulsification activity and stability in the pH range of 3.0–9.0 and is capable of stabilizing oil-in-water emulsions with several aliphatic and aromatic hydrocarbons (Amaral et al. 2006). This may be related to its high ability to degrade aliphatic and aromatic hydrocarbons.

Perspectives

The various applications of marine yeasts and their products are summarized in Table 1. It can be clearly seen from the table that marine yeasts and their metabolites have many potential applications. However, in order to apply the probiotic, immuno-stimulants from marine yeasts and yeast cell wall, killer toxin, siderophore and vaccine to the practical exercises, much further work on field tests needs to be done. In order to produce higher yields of the enzymes and metabolites, we should focus more attention on the detailed regulation of gene expression in marine yeasts. We also think that more bio-products from marine yeasts need to be discovered.

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