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Review

Bio-products produced by marine yeasts and their potential applications

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HIGHLIGHTS

- The yeasts are widely distributed in different marine environments.
- The marine yeasts can produce different kinds of biomolecules with a high yield.
- Many biosynthetic pathways and relevant genes for biomolecules are still unknown.
- All the functional biomolecules from marine yeasts have highly potential applications.

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ABSTRACT

It has been well documented that the yeasts isolated from different marine environments are so versatile that they can produce various fine chemicals, enzymes, bioactive substances, single cell protein and nanoparticles. Many genes related to the biosynthesis and regulation of these functional biomolecules have been cloned, expressed and characterized. All these functional biomolecules have a variety of applications in industries of food, chemical, agricultural, biofuel, cosmetics and pharmacy. In this review, a summary will be given about these functional biomolecules and their producers of the marine yeasts as well as some related genes in order to draw an outline about necessity for further exploitation of marine yeasts and their bio-products for industrial applications.

1. Introduction

Marine yeasts are the ones that are derived from marine environments and that can grow better in a medium prepared with seawater than in that prepared with fresh water. Actually, all the marine yeasts can grow well in the medium without marine elements.

Marine yeasts are widely distributed in native marine environments like seawater, marine sediment, estuaries, weeds and algae, marine invertebrates and animals and mangrove ecosystems (Kutty and Philip, 2008; Chi et al., 2012a). Even they were also found in some extremely adverse sources such as deep sea extreme environments (Nagano and Nagahama, 2012), benthic animals and seafloor sediment at the depths ranging from 6400 to 11,000 m (Gadanho and Paulo Sampaio, 2005). Surely such an environment is dark, anoxic, and cold down there, unless near a hydrothermal vent. Maybe this type of environment provides some of the potential advantages of bioproducts derived from the marine yeasts – particularly their presumed psychrophilic properties. The genera of marine yeasts identified include *Rhodotorula*, *Rhodosporidium*, *Candida*, *Debaryomyces*, *Cryptococcus*, *Yarrowia*, *Aureobasidium*, *Metschnikowia*, *Torulopsis*, *Pichia*, *Kluyveromyces*, *Saccharomyces*, *Williopsis*, *Pseudozyma*, *Hansenula*, *Trichosporon*, *Filobasidium*, *Leucosporidium* and so on. Some psychrophilic yeasts such as *Mrakia frigida* and *Guehomyces pullulans* are also widely distributed at the sea sediment of Antarctica (Zhang et al., 2012).

Because marine yeasts have a strong ablitily to live in the extreme marine environments, they must have versatile potentials for synthesis of functional biomolecules. The functional biomolecules discovered have included fine chemicals, biofuels, enzymes, bioactive substances, single cell protein and nanoparticles. All the functional biomolecules have valuable potential applications in food, chemical, agricultural, biofuel and pharmaceutical industries. Therefore, it is of importance to exploit marine yeasts, their bio-products and corresponding fuctional genes for industrial applications. In this review article, the functional biomolecules, their producers and potential applications will be summarized.

2. Fine chemicals

In recent years, the research on marine yeasts has revealed that they also could produce some fine chemicals in bulk. In this

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section, the fine chemicals such as pullulan, polymalic acid (PMLA), biosurfactants, xylitol and citric aicd produced by marine yeasts together with some critical genes for biosynthesis of these substances have been discussed.

2.1. Pullulan

Pullulan which consists of maltotriose units is an edible tastless biopolymer that can be used in medical and food industries such as gene delivery, targeted drug therapy, tissue engineering, and medical imaging. It has also been thought that the pullulan may have many physiological functions (Li et al., 2015).

So far, most of the pullulan producers have been obtained from terrestrial *Aureobasidium pullulans* (Chen et al., 2012). New discoveries have been made where different strains of *Aureobasidium* spp. isolated from the marine environments, were reported as good producers of pullulan. For example, a novel strain of *A. pullulans* CJ001 isolated from sea mud at Eastern China Sea could produce pullulan of 26.13 g/l in an optimized medium (Chen et al., 2012). Another better producer was *A. pullulans* var. *melanogenium* P16 strain isolated from a mangrove system, which produced and 67.4 g/l of pullulan and 23.1 g/l of cell dry weight in a 10-liter batch fermentation (Ma et al., 2015). The genetically engineered mutant carrying an inulinase gene could produce 70.57 ± 1.3 g/l of pullulan in the fermented medium containing 138.0 g/l inulin within 108 h (Ma et al., 2015). This pullulan production from inulin instead of traditional glucose which is mainly produced from starch in food grain has reached the industrial level just in the batch fermentations.

Although pullulan has been used in industries for a long time, the detailed pathway for pullulan biosynthesis in *Aureobasidium* spp. is still unclear (Li et al., 2015). In our previous work, we have proposed that α-phosphoglucose mutase, UDPG – pyrophosphorylase, several glucosyltransferases, and pullulan synthetase may be related to pullulan biosynthesis in *Aureobasidium* spp. (Li et al., 2015). Indeed, the genes responsible for α-phosphoglucose mutase and UDPG – pyrophosphorylase have been identified. Disruption of the pullulan synthetase gene in *A. pullulans* HN6.2 led to decreased production of pullulan by the mutants (Ma et al., 2012). However, it is still unknown if the *PUL1* gene is identical to the pullulan synthetase gene in *Aureobasidium* spp. (Li et al., 2015). Furthermore, over-expression of the *PUL1* gene promoted pullulan production by the genetically modified strain of *A. pullulans* var. *melanogenium* P16 strain (unpublished data). However, the genes encoding glucosyltransferases are still completely unknown. So further research is needed to fully interpret the detailed biosynthesis pathway of pullulan in order to genetically modify the pullulan producers and further enhance pullulan production by the recombinant producers.

2.2. Polymalic acid (PMLA)

PMLA is another safe and valuable bio-polymer composed of repetitive sole unit of L-malate. PMLA and its derivatives have a great potential application as promising candidates for a new prototype of biomedical materials in the specific drug delivery systems (Li et al., 2015). Till now, almost all the PMLA-producing yeasts *A. pullulans* have been isolated from the terrestrial sources (Li et al., 2015). Surprisingly, two marine yeasts exhibit a much higher production of PMLA than any other producers ever discovered. Firsly, *Aureobasidium* sp. P6 strain isolated from a mangrove system in South of China could produce over 118.3 g/l of Ca^{2+}-PMLA during the 10-liter batch fermentation (Ma et al., 2013). Then, *A. pullulans* var. *pullulans* MCW which was also isolated from the same mangrove ecosystem in South of China could produce 152.52 g/l of Ca^{2+}-PMLA in a 10-liter batch fermentation with a fine product property of 2.054×10^5 (g/mol) of molecular mass and 1.263 of polydispersity (Wang et al., 2015). This indicated that the marine-derived *Aureobasidium* spp. may be the better producers of PMLA than any other strains of *A. pullulans* isolated from the terrestrial sources. The reason for these is still completely unclear.

However, the concrete pathway and the relevant genes involved in PMLA biosynthesis have not yet been specified. In our previous work, we also have proposed an assumption for this unique product biosynthesis for which pyruvate carboxylase, malate dehydrogenase, thiokinase and PMLA synthetase are mainly responsible (Li et al., 2015). So far, the genes responsible for pyruvate carboxylase and malate dehydrogenase have been found to be closely related to malate biosynthesis in *Aspergillus oryzae* (Li et al., 2015). However, the genes encoding thiokinase and PMLA synthetase are still completely unclear. Therefore, further comparative whole-genome sequencing and transcriptome sequencing are required for the specification of the PMLA biosynthesis in *Aureobasdium* spp. and the genes coding for thiokinase and PMLA synthetase must be elucidated in order to further promote PMLA biosynthesis.

2.3. Biosurfactants

Biosurfactants are prominent in several industrial and environmental uses (Valappil Sajna et al., 2015). In recent years, many yeasts isolated from marine environments also have been found to be able to produce biosurfactants. For example, the emulsifier, named Yansan was produced by the wild strain IMUFRJ50682 of *Yarrowia lipolytica* isolated from Guanabara Bay in Rio de Janeiro. It has an 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). The biosurfactant produced by *Pseudozyma hubeiensis* SY62 isolated from the deep-sea cold-seep clam at 1156 m in Sagami bay was MEL-C (4-O-[4′-O-acetyl-2′,3′-di-O-alka (e) noil-β-D-mannopyranosyl]-D-erythritol) with saturated C_6, C_{10}, and C_{12} fatty acids and its concentration in the fermented medium was estimated to be around 30 g/l. This novel MEL-C showed larger critical micelle concentration (1.1×10^{-5} M) than a conventional MEL-C with C_{10} and C_{12} fatty acids (Konishi et al., 2010). However, the genes and the enzymes related to the biosurfactants synthesis in the marine yeasts are yet to be identified.

2.4. Citric acid

Right now, citric acid is being obtained industrially by a large-scale fermentation of *Aspergillus niger*. Lately, the yeast *Y. lipolytica* has been found as an alternative citric acid producer and has many advantages over *A. niger* (Liu et al., 2013). For example, *A. niger* has narrower substrate spectrum (only glucose from food grain or sucrose from sugar cane), a complex process control (the yeast can be easily cultivated in the fermentor compared with the filamentous fungus), and is not easily genetically modified compared to *Y. lipolytica.* In our recent work, the *INU1* gene encoding an exo-inulinase cloned from *Kluyveromyces marxianus* CBS 6556 has been expressed in the cells of *Y. lipolytica* SWJ-1b, a high citric acid producer isolated from the gut of a marine fish at Bohai Sea. The recombinant yeast displaying the inulinase (22.6 U/mg of cell dry weight) could produce 68.9 g/l of citric acid in the fermented medium within 312 h of a 2-l fermentation (Liu et al., 2013). Then, we did further genetic manipulation to *Y. lipolytica* SWJ-1b to enhance its citric acid production. For example, after some of the ATP-citrate lyase genes were removed and the copy number of the iso-citrate lyase gene was increased in *Y. lipolytica* SWJ-1b displaying the recombinant inulinase, the newly engineered yeast strain 30 could yield 84.0 g/l of citric acid from 10.0% of inulin during a 2-l fermentation (Liu et al., 2013). The engineered yeast strain 30