

Oenococcus oeni: The Main Lactic Acid Bacteria Involved in Wine-Making



Longxiang Liu¹, Shuai Peng¹, Hongyu Zhao¹, Yun Wang¹, Hua Li^{1,2,3*} and Hua Wang^{1,2,3*}

¹College of Enology, Northwest A&F University, China

²Shaanxi Engineering Research Center for Viti-Viniculture, China

³Heyang Experimental and Demonstrational Stations for Grape, China

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*Corresponding author: Hua Li, College of Enology, Northwest A&F University, Shaanxi Engineering Research Center for Viti-Viniculture, Heyang Experimental and Demonstrational Stations for Grape, Yangling, Weinan, Shaanxi, 712100, China, Email: lihuawine@nwfufu.edu.cn

Hua Wang, College of Enology, Northwest A&F University, Shaanxi Engineering Research Center for Viti-Viniculture, Heyang Experimental and Demonstrational Stations for Grape, Yangling, Weinan, Shaanxi 712100, China, Email: wanghua@nwsuaf.edu.cn

Abstract

Based on the research advances of our team, this article examines properties of the lactic bacteria- *Oenococcus oeni*. The genetic diversity are mainly in accordance with the result of fermentation properties, random amplified polymorphic, single-enzyme amplified fragment length polymorphism, multilocus sequence typing, pulsed-field gel electrophoresis and the genome-wide comparison analysis. The adaptive stress response is based on the condition of culture, membrane lipid composition and heterologous expression of genes from *O. oeni*. Biological properties focus on the probiotic properties of *O. oeni*, such as antioxidant activity, nutritional value and sanitarian function of wine after malo-lactic fermentation. And the bacteria reconstruction and preservation are also involved. The advice on the research direction is introduced in this article.

Keywords: *Oenococcus oeni*; Wine-making; Genetic diversity; Stress response; Biological properties; Bacteria reconstruction; Bacteria preservation

Introduction

Oenococcus oeni is a wine-associated lactic acid bacterium mostly responsible for wine malo-lactic fermentation (MLF) [1], which is introduced into starter cultures, is able to grow after alcoholic fermentation [2]. Its general trait is high tolerance to harsh wine environmental conditions: high ethanol concentration, low pH, poor proportion of nutrients, etc. [3]. MLF is necessary not only to deacidify some wines and to stabilize others, but also to improve the quality of wine apparently by addition of certain products of this metabolism, which make its flavor more complex [4]. In wine, many factors can cause a delay in MLF initiation, such as low pH, low temperature, high ethanol, high SO₂, but of these, the most important factor is the property of strain. These are the things that our team has been doing over the years. This paper introduces the advances of our team to review the work can be conducted on *O. oeni*.

Genetic Diversity

Because the important position in wine-making process, the screening and identification of *O. oeni* get more and more attentions. 24 strains of *O. oeni* were isolated from different Chinese wines regions in 2000, and the isolation, culture and rapid identification system were also made [5-9]. In order to select the most active strains for MLF, the strains' abilities of resistance to wine condition (ex. low pH, high alcoholic concentration and SO₂ content) were investigated. The results indicate that different strains have different adaptability in the performance of MLF. In selected strains, *O. oeni* SD-2a was more active than commercial type strain *O. oeni* 31DH, meanwhile *O. oeni* SD-1b, *O. oeni* SD-2i and *O. oeni* SD-2h also showed strong abilities to survive in stress conditions [6]. And the wine inoculated with *O. oeni* SD-2a was stable and less volatile acid produced [10,11]. The quality comparison between

the red wine inoculated by direct vat set (DVS) *O. oeni* SD-2a starter and commercial MLF starter (Viniflora® Oenos) were conducted both at laboratory and factory, and the *O. oeni* SD-2a starter achieved good results [12,13]. All these studies have laid a solid foundation for the further application of *O. oeni* SD-2a in wine making industry. The strain *O. oeni* SD-2a have obtained patent protection (02123444.2).

Differentiation of isolates was carried out by analysis of random amplified polymorphic (RAPD) pattern. The RAPD profiles (113 different bands) are strain specific and can divide strains into two main groups. And on this basis, we established single-enzyme amplified fragment length polymorphism (SE-AFLP) reaction system to study the genetic diversity of *O. oeni* [8,14]. The SE-AFLP bands generated by four selected primers can discriminate 22 strains of *O. oeni* [14]. SE-AFLP molecular marker can be used to differentiate *O. oeni* strains. Recently, 49 strains of malolactic bacteria were isolated and purified from Xinjiang wine, Hebei wine, Gansu wine after alcohol fermentation [15,16]. Among them, five strains have higher applicability in wine producing. These strains still need a further research.

The multilocus sequence typing (MLST) and pulsed-field gel electrophoresis (PFGE) were also used to study the genetic polymorphism and evolutionary relationship of these isolates. The result showed that the MLST and PFGE method both separated the strains into two clusters which are correlated with the wine-producing regions. And the combination of results from both typing methods allowed a better discrimination of genotypes [17,18].

By now, the genome complete map of *O. oeni* SD-2a has been constructed, it is the first genome complete map of *O. oeni* isolated from Chinese wine, and the second *O. oeni* strain with a genome complete map (unpublished). From the genome-wide comparison analysis, the size difference between *O. oeni* SD-2a and *O. oeni* PSU-1 is 209,186nt (SD-2a is 1,989,703nt, and PSU-1 is 1,780,517nt), and the number of ORFs is 2071 and 1701, respectively. This indicated that substantial inter-strain genomic variation, in accordance with previous studies [19,20].

The adaptive stress response

Several studies have been made of how *O. oeni* responds under stress conditions [21]. Different mediums and initial culture pH differ in changing intracellular MLF activity, H⁺-ATPase activity [22]. Compared with FMATB and ATB medium, cells cultured in ATB medium inoculation viability and freeze-drying viability. And, ATB medium increased distinctly the relative concentration of *lactobacilli* acid (C19cyc11) and U/S ratio in cell membrane lipid composition of *O. oeni* SD-2a [22-24].

Malo-lactic enzyme gene (mleA) and malate permease gene (mleP) are two important genes of *O. oeni* involving in MLF. Malo-lactic enzyme is the function enzyme to turn L-malic acid into L-lactic acid during MLF, while malate permease performs malic acid and lactic acid transport. The expression of mleA and mleP

genes from *O. oeni* SD-2a and their co-expression have done in *Saccharomyces cerevisiae*. The co-expression of mleA and mleP gene in strain YSGA-GP results in a much higher MLF activity [25-27]. And many other stress related proteins and their genes are being discovered and reported.

Biological properties

In the process of our study, we found that *O. oeni* have antioxidant activity, which has great significance to its application in food and wine industry. The antioxidant properties and viabilities of 20 strains in simulated gastric and intestine juice and bile solution were measured and analyzed, to explore the probiotic effect of *O. oeni* strains, and assess the effects of these potentially probiotic bacteria. In addition, the possible anti-oxidative mechanism of *O. oeni* was elucidated. The results suggested that the anti-oxidative parameters were widely dispersed, irrespective of the evaluation methods used, which indicated that anti-oxidative properties depended on the strain and culture medium. The anti-oxidative mechanisms of *O. oeni* could be assigned to the 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging ability, reactive oxygen species (ROS) scavenging ability, iron ion chelation (FE), glutathione system, ferric reducing ability of plasma (FRAP), reduction activity (RA), inhibition of ascorbic acid oxidation (TAA), and linoleic acid oxidation (TLA) abilities [3,28]. Besides, the changes of amino acids were various in wine after MLF by different *O. oeni* strains: the variety and concentration of amino acids had increased, some of them have particular biochemical and physiological properties, which can improve the nutritional value and sanitarian function [10].

The *O. oeni* strains could be potentially used as a novel probiotic strain in the food and wine industry, not just the starter of MLF. Thus, the deeper studies need to be performed in order to validate their potential use as probiotics or functional food additives [29]. Furthermore, *O. oeni* possessed a unique defense system to resist ROS, and the genes involved in the defense against oxidative stress need more attention.

Bacteria reconstruction and preservation.

The research for construction of de-acidification wine yeast has been conducted by protoplast fusion with *Saccharomyces cerevisiae* 1450 and *O. oeni* SD-2a. A single clone strain named F-20-7 with strong activity was selected. This strain can degrade L-malic acid during alcoholic fermentation. The alcohol fermentation capacity of F-20-7 approximates to *S. ellisoideus* 1450, and could degrade malic acid at the same time [30,31].

Due to the feature variation of microorganism is fast, so the preservation of microbial strains is critical. The cryopreservation protocol followed by the cultures of *O. oeni* have been studied, the storage of *O. oeni* in liquid nitrogen by using the composite protective agent (yeast extraction, glycerin, sugar and monosodium glutamate) prove to be well for strains preserved in culture collections [32-33].

Conclusion

As the most important executant of MLF, efforts should be made to select strains that are more suitable for conducting of MLF and reconstruct existing strains. The biological properties of wine after MLF should be properly explored and organized. And with the widely application of molecular biology technology in various fields, more and more technologies are applied to study the stress response of microorganisms, such as omics technology, gene knock-out technology, gene knock-in technology, gene silencing technology, etc. But, in the study of *O. oeni* stress response mechanisms, these technologies are still in the start stage. This needs a further study.

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Conflict of Interest Statement

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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