Oenococcus oeni: Queen of the cellar, nightmare of geneticists

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Graphical abstract

The slow but steady evolution of O. oeni genetics. Timeline of the history of genetic research on O. oeni from the first description of the species by Gravies in 1967 to sequencing of the genome by Mills et al. (FEMS Microbiol Rev 2005;29:465–475) and the last major advance that has allowed the application of anti-sense technology to modulate gene expression in this highly xenophobic acid lactic bacterium (Darsonval M, Msadek T, Alexandre H, Grandvalet C. Appl Environ Microbiol 2016;82:18–26). Scanning electron microscopy of a chain of O. oeni cells by M. Maitre. Bar, 1 µm.

Abstract

Oenococcus oeni is a wine-associated lactic acid bacterium (LAB) responsible mostly for wine malolactic fermentation (MLF). This fastidious bacterium (auxotrophic for many amino acids and slow growing) possesses remarkable adaptability to harsh physicochemical conditions and can reprogramme its metabolic pathways to enhance its survival in wine. Thus, O. oeni is an instructive bacterial model for investigating stress response mechanisms in LAB. However, the lack of appropriate techniques to modify the O. oeni genome has hampered molecular studies of this species. The application of recent advances in molecular genetics promises to provide a better understanding of the regulation of stress responses in this species in the future.
TAXONOMY

Domain Bacteria, phylum Firmicutes, class Bacilli, order Lactobacillales, family Leuconostocaceae, genus Oenococcus, species Oenococcus oeni.

PROPERTIES

Oenococcus oeni is a Gram-positive, non-motile, facultative anaerobic and chemo-organotrophic bacterium that organizes in chains or pairs of circular to ellipsoidal cells. It belongs to the lactic acid bacteria (LAB) group and can use oxygen for respiration or obtain energy by fermentation. This heterofermentative bacterium occurs naturally in fruit mashes and related environments and proliferates in wine during or after yeast-driven alcoholic fermentation. From grape must to wine, autochthonous O. oeni species are part of the microbial fauna that can perform malolactic fermentation (MLF), the crucial secondary fermentation step in the production of wine that converts malic acid to lactic acid. This LAB gradually becomes the dominant bacterial species during the winemaking process and carries out malolactic conversion with a concomitant rise in pH, microbiological stabilization and sensory impacts on the wine, resulting in increased complexity of both aroma and flavour. O. oeni can survive in acidic conditions below pH 3.0 and tolerate ethanol levels above 10 % (v/v). It is the most tolerant LAB to acidity and ethanol, as well as to sulfite used during wine processing or produced by yeast during alcoholic fermentation. O. oeni not only survives, but actively proliferates, in wine, impacting positive sensory properties, whereas most other wine-associated bacterial species are associated with spoilage. It releases multiple end products from fermenting sugar: ethanol, CO2 and acetate, as well as aromatic molecules such as diacetyl, a buttery flavour sought after in some wine varieties. MLF has been the subject of much research because of its economic importance. O. oeni is often added as a starter culture in wine for more rapid and reliable MLF, allowing better control and increased robustness of the process.

As for most LAB, O. oeni is auxotrophic for many amino acids (18–21 amino acids). Furthermore, other amino acids are also needed for optimal growth. O. oeni metabolizes hexoses and pentoses via the phosphoketolase pathway. Citrate cannot be used as the sole carbon source, but co-metabolism with sugars stimulates growth. Glucose and fructose, both principal sugar sources of must and wine, heighten biomass yield when they are used in the culture medium. Optimal growth occurs between 17 to 30 °C on sugar and protein-rich media adjusted to an initial pH between 4.8 to 5.5. The slow specific growth rate of O. oeni (0.066 h−1) implies an incubation period from 48 h up to 10 days. Growth on plates is greatly enhanced by semi-anaerobic or micro-aerophilic incubation. White, opaque colonies, less than 1 to 1.5 mm in diameter, usually develop only after 5 days.

GENOME

Mills et al. were the first to deposit the complete genome sequence of the O. oeni PSU-1 strain, isolated at Penn State University [1]. O. oeni PSU-1 has a compact genome of 1.78052 Mb, encoding 1662 proteins, with a GC content of 37.9 %. This genome contains no temperate bacteriophages, or larger tracts of obvious bacteriophage origin, often found in other LAB. Nevertheless, putative phage-related genes identified in the PSU-1 genome may be a sign of imprecise prophage excision from this genome. Currently, 202 additional genome sequences of commercial and environmental strains are available from the National Center for Biotechnology Information in various stages of completeness. Comparative studies show genetic variation among strains with differences in sugar utilization, amino acid and exopolysaccharide biosynthesis, and bacteriophage and plasmid content. Plasmids, ranging from 2.5 to 60 kb, have been found in some strains isolated from wines. Six small cryptic plasmids (2544 to 4410 bp), encoding replication and mobilization proteins, have a GC content of 31 to 36 %, and do not carry any genes potentially involved in winemaking. More recently, two large plasmids, named pOENI-1 (18.3 kb) and pOENI-1v2 (21.9 kb), were described and are presumed to contribute to the fitness of bacteria performing MLF. Finally, the existence of four distinct groups of temperate bacteriophages in O. oeni strains makes prophages a major contributor to the genetic diversity of the O. oeni species [2]. The presence of bacteriophages may play a significant role in shaping microbial communities during the oenological process.

PHYLOGENY

In 1995, Dicks et al. proposed a reclassification of Leuconostoc oenos into the new genus, Oenococcus oeni (Oeno- suffix, Greek for wine) on the basis of the 16S rRNA sequence. O. oeni is distinguished from Leuconostoc species by its ability to use lactose, saccharose and maltose as a carbonate substrate and its growth at an initial pH of 4.8 and in media containing 10 % (v/v) ethanol. The two genera share little DNA homology. Yang and Woese [3] first proposed accelerated evolution of the Leuconostoc group in general, and of O. oeni in particular. This was confirmed by a phylogenetic comparison of concatenated ribosomal and RNA polymerase subunits from sequenced LAB. The adaptation of O. oeni to growth in wine and related habitats is one explanation for the divergence of this species away from the other Leuconostocs. The Oenococcus genus is composed of three species: Oenococcus oeni, Oenococcus alcoholitolerans and Oenococcus kitaharae. O. alcoholitolerans was isolated during bioethanol production in Brazil. O. kitaharae, isolated from a composting distilled shochu residue in Japan, is a non-malolactic-fermenting bacterium, less acidophilic and resistant to ethanol than O. oeni.

KEY FEATURES AND DISCOVERIES

The four principal wine parameters that induce stress and affect growth and cell integrity are ethanol (12–16 % v/v), low pH (3.5 to 2.5), SO2 (10–70 mg l−1) and low temperature (down to 12 °C). O. oeni can reprogramme its metabolic pathways to enhance its resistance. The O. oeni ATCC BAA-1163 strain has been widely used for the characterization of wine-related stress responses. Three primary mechanisms have been reported: (i) the establishment of a...

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The involvement of Lo18 in stress tolerance. Moreover, this sense attenuation was successfully carried out to confirm technology to modulate gene expression [5]. A new stable has been achieved by the application of anti-sense RNA for electroporation of does not allow gene replacement, as the transfer frequency transfer by conjugative means. However, conjugal transfer the plasmid pGK13 into Dicks first used electroporation to successfully transform protein protection [4]. The expression of many other stress genes has been investigated under wine-related stressful conditions: trxA, clpX, ftsH, omrA, clpP-clpL1 and cfa. In addition to these stress genes, ubiquitous operons groES-groEL and grpE-dnak-cbpA, as well as clpATPase genes, have been identified in oenococcal genomes. All stress-response genes in O. oeni, characterized so far, are under the control of a single stress response regulator, CtsR, which is unusual for a Gram-positive bacterium.

The absence of appropriate techniques to modify the O. oeni genome has obstructed molecular studies of this species. The timeline of genetic research on O. oeni of the last half century highlights the slow but steady evolution of the genetics of this recalcitrant LAB (graphical abstract figure). Unlike other LAB, transformation of O. oeni is laborious. Dicks first used electroporation to successfully transform the plasmid pGK13 into O. oeni. However, this transformation protocol has not been confirmed in any other laboratory. The development of molecular tools has allowed transfer by conjugative means. However, conjugal transfer does not allow gene replacement, as the transfer frequency is lower than that for recombinant. An improved method for electroporation of O. oeni, using ethanol as a membrane fluidifier, was developed in 2008, allowing effective and reproducible DNA transfer. More recently, a major advance has been achieved by the application of anti-sense RNA technology to modulate gene expression [5]. A new stable and effective expression vector was developed and anti-sense attenuation was successfully carried out to confirm the involvement of Lo18 in stress tolerance. Moreover, this efficient expression vector allows the expression of genes of oenological interest, such as esterase genes which can modulate the ester profile of wine during MLF. These latest developments constitute a revolutionary genetic application for the functional exploration of the O. oeni genome, providing access to the in vivo study of gene function.

**KEY QUESTIONS**

- No expression of exogenous genes has been reported so far in O. oeni. Is O.oeni xenophobic?
- What are the molecular mechanisms implicated in the transduction of cellular signals to CtsR, the master transcriptional regulator gene expression in the O. oeni stress response?
- Do bacteriophages influence the shaping of microbial communities during the oenological process, resulting in the *inter alia* selection of O. oeni?
- As a member of the Leuconostocaceae family, what have been the genetic evolutionary pathways underlying the colonization of wine by O. oeni?

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**References**


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