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# β-glycosidase activities of *Oenococcus oeni*: Current state of research and future challenges

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*The lactic acid bacterium* Oenococcus *oeni is the most important species for the controlled malolactic fermentation (MLF) of wine and it is best known for its generally positive effect on the wine flavor. While the major impact of MLF is the reduction of acidity, the diverse metabolic side activities of* O. oeni *can exert significant influences on a wine's aroma profile. Of particular interest are glycosidase activities that catalyze the release of grape-derived aroma compounds such as terpenes. Many detailed studies conducted over the last decade revealed that* O. oeni *displays several glycosidase (glucosidase, xylosidase, arabinosidase and rhamnosidase) activities and that these activities indeed affect the complex wine aroma. Biochemical characterization of purified glycosidases from* O. oeni *led to vital insights into the mechanisms that can be made responsible, and gave further indications that could be helpful to explain the high strain-dependant variations on the molecular level. At present,* O. oeni *is probably one of the best studied organisms regarding its glycoside metabolism. Beyond its direct impact on wine making, this information is highly important to understand the ß-glycoside metabolism of LAB in general, as orthologues to the glycosidase genes from* O. oeni *can be found in several other LAB species.*
**Keywords:** *Oenococcus oeni*, wine, aroma, glycosidase, lactic acid bacteria, phenol

***β-Glycosidase-Aktivitäten von* Oenococcus oeni*: Aktueller Stand der Forschung und Ausblick.*** *Das Milchsäurebakterium* Oenococcus oeni *ist der wichtigste Organismus, um den kontrollierten biologischen Säureabbau (BSA) im Wein einzuleiten.* O. oeni *wird vor allem wegen seiner generell positiven Wirkung auf das Weinaroma geschätzt. Obwohl die Säurereduktion das eigentliche Merkmal des BSA ist, verursachen die vielfältigen Stoffwechselaktivitäten von* O. oeni *oft signifikante Änderungen des Aromaprofils. Von besonderem Interesse sind Glycosidase-Aktivitäten, welche die Freisetzung primärer Aromastoffe (z. B. Monoterpene) bewirken können. Studien der letzten Jahre ergaben, dass* O. oeni *mehrere solcher Glycosidase-Aktivitäten (Glucosidase, Xylosidase, Arabinosidase, Rhamnosidase) entfalten kann und dass diese einen bedeutenden Einfluss auf das komplexe Weinaroma ausüben können. Des Weiteren ergab die biochemische Charakterisierung von unterschiedlichen Glycosidasen aus* O. oeni *entscheidende Einblicke in die Mechanismen, die für diese Effekte verantwortlich sind. Derzeit ist* O. oeni *wahrscheinlich einer der am besten untersuchten Organismen hinsichtlich seines ß-Glycosid-Stoffwechsels. Neben seiner Bedeutung für die Weinbereitung ist diese Information auch relevant, um den Glycosid- und Kohlenhydratstoffwechsel von Milchsäurebakterien im Allgemeinen zu verstehen, da ähnliche (orthologe) Glycosidasen auch in anderen Arten gefunden werden können.*
**Schlagwörter:** *Oenococcus oeni*, Wein, Aroma, Glycosidase, Milchsäurebakterien, Phenol

***Les activités de la β-glycosidase d'*Oenococcus oeni*: l'état actuel de la recherche et les perspectives d'avenir.*** *La bactérie de l'acide lactique* Oenococcus oeni *est l'organisme le plus important pour déclencher la fermentation malolactique (BSA) dans le vin.* O. oeni *est estimé surtout pour son effet général positif sur l'arôme du vin. Bien que la réduction de l'acidité soit la caractéristique principale de la BSA, les activités métaboliques multiples d'O.* oeni *pro-*

*voquent souvent des modifications significatives du profil aromatique. Les activités de la glycosidase, qui peuvent provoquer la libération de substances aromatiques primaires (par exemple, des monoterpènes), présentent un intérêt particulier. Les études des dernières années ont révélé qu'O.* oeni *peut déployer plusieurs activités de glycosidase (glucosidase, xylosidase, arabinosidase, rhamnosidase) et que celles-ci peuvent exercer une influence considérable sur l'arôme complexe du vin. En outre, la caractérisation biochimique des glycosidases différentes d'O.* oeni *a permis de recueillir des informations décisives dur les mécanismes responsables de ces effets. À l'heure actuelle,* O. oeni *est vraisemblablement l'un des organismes les mieux étudiés quant à son métabolisme ß-glycosidique. Hormis son importance pour la vinification, cette information est également pertinente pour comprendre d'une manière générale les métabolismes glycosidique et glucidique de la bactérie de l'acide lactique, étant donné que des glycosidases similaires (orthologues) peuvent être trouvés également dans d'autres espèces.*

**Mots clés :** *Oenococcus oeni*, vin, arôme, glycosidase, bactéries de l'acide lactique, phénol

Lactic acid bacteria (LAB) have accompanied human advances in food processing and preservation throughout history. Besides the processing of dairy products, LAB play an indispensible role in the fermentation of numerous plant foods, thereby exerting a positive impact on both dietary value and sensory characteristics.*ß*-glycosidase activities are increasingly recognized as a positive side effect of the LAB metabolism since the deglycosylation of plant metabolite precursors improves the bioavailability of dietary phenols (e.g. flavonoids) with chemoprotective effects against cancer and cardiovascular diseases.

However, performing literature research in a scientific database using keywords such as "glycosidase" and "lactobacilli", one might be surprised by the abundance of records on wine aroma, wine lactic acid bacteria and especially the lactic acid bacterium *Oenococcus oeni*. For one not familiar with the field of winemaking, it may seem peculiar that so many researchers devote their attention to a seemingly rather marginal topic. Nevertheless, the complex and harsh environment of wine (low sugar content, acidity, ethanol, phenols, flavonoids, tannins etc.) presents an interesting milieu for the study of lactic acid bacteria (LAB), especially concerning the metabolic side activities that interact with these factors. The main impact of lactic acid bacteria in wine is a process called malolactic fermentation (MLF) that usually occurs after alcoholic fermentation, resulting in a decrease of acidity caused by the conversion of L-malic acid into L-lactic acid. From the biochemical point of view, MLF is a decarboxylation reaction rather than a fermentation catalyzed by the malolactic enzyme, while the exact reaction mechanism of the MLF is not understood to date. This mechanism establishes a proton motive force across the cytoplasmic membrane (Bartowsky, 2005; Salema et al., 1996; Versari et al., 1999) allowing the maintenance of the cytoplasmic pH-value. As glycolysis is effectively switched off at low pH-values, MLF is an alternative pathway to acquire metabolic energy (Bartowsky, 2005). The metabolic side activities of malolactic bacteria can exert great influence on the complex wine flavor, ranging from positive effects to severe spoilage. Due to its mostly positive effect on the wine aroma, *O. oeni* has become the preferred species for controlled MLF and is often used as commercial starter culture. Nevertheless, even *O. oeni* is not exempt from producing off-flavor (Bartowsky, 2005; Bartowsky, 2009).

Like the most plant secondary metabolites, volatile constituents of the primary (grape derived) wine aroma are often glycosylated and therefore odorless. Accordingly, the aroma profile of a wine could be substantially altered by the enzymatic release of such "dormant" aroma compounds. Most important for the characteristic varietal wine aroma are terpenoid compounds, especially monoterpenols (Maicas and Mateo, 2005; Mateo and Jiménez, 2000). The mode of precursor glycosylation involves a *ß*-D-glucopyranose moiety (monoglucosides) that can further be conjugated to *ß*-D-apiose, α-L-arabinose, α-L-rhamnose or *ß*-D-xylose residues, resulting in diglycosides (Gunata et al., 1988). Thus, the enzymes required for the sequential hydrolysis of aroma precursors are glucosidases, apiosidases, arabinosidases, rhamnosidases and xylosidases (Maicas and Mateo, 2005). It is now widely established that MLF performing LAB possess glycosidase activities and that these activities can exert a significant influence on the wine aroma profile. Especially in the last decade, numerous studies had the aim to shed light on the mechanisms and effects of the glycosidase activities of *O.oeni.* The intention of this review is to give an overview on the current state of research and to place the so far available data into a general context, with emphasis on the biochemical mechanisms that are thought to be res-

ponsible for the glycoside hydrolysis by *O. oeni*.

## Portrait of *Oenococcus oeni*

A general trend in the evolution of LAB (i.e., the order *Lactobacillales*) is one towards metabolic simplification, as it is indicated by high gene losses relative to the proposed LAB ancestor (Makarova et al., 2006). As a result, most LAB species became highly adapted to nutrient rich habitats, but are consequently restricted to narrow ecological niches. Although wine may not be a "nutrient rich habitat", it represents a "narrow ecological niche". *Oenococcus oeni* is a good example for the evolutionary adaptation of LAB, because it is highly stress-tolerant (Spano and Massa, 2006). It has often been described as the best adapted species to the harsh wine milieu, and this high adaptation might also be the reason that *O. oeni* could rarely be isolated from a different habitat (Borneman et al., 2009). Based on 16s rRNA analysis, Yang and Woese (1989) already suggested that *Leuconostocaceae* and *Oenococcus* are fast evolving (tachytelic) organisms, however, Morse et al. (1996) opposed to this observation. Based on molecular strain differentiation methods, earlier studies reported that *O. oeni* is genetically homogenous (Bon et al., 2009). Nevertheless, at present it is widely accepted that *O. oeni* and Leuconostoc are indeed fast evolving, which was also confirmed by molecular clock analysis, even showing that *O. oeni* evolves fastest among *Leuconostocaceae* (Makarova and Koonin, 2007). The absence of the mismatch repair pathway genes *mutS* and *mutL*, unique among *Lactobacillales*, is thought to be responsible for increased mutability and high rates of horizontal gene transfer (Makarova and Koonin, 2007; Marcobal et al., 2008) in the genus *Oenococcus*. Furthermore, more sensitive strain differentiation techniques recently indicated a high genetic diversity in the genus *Oenococcus* (Bon et al., 2009; Borneman et al., 2009; De Las Rivas et al., 2004). However, most probably caused by the panmictic population structure of *O. oeni*, distinct lines of clonal descent have not yet been identified (De Las Rivas et al., 2004). Ironically, although the high mutability might be the reason that *O. oeni* is so well adapted to the restrictive wine milieu, Marcobal et al. (2008) speculated that the extinction of the genus *Oenococcus* might be an eventual outcome. In any case, this genetic diversity leads to the fact that *O. Oeni* is an interesting subject to study its effect on the wine flavour.

## Classification and function of glycosidases

Glycosidases represent one of the most abundant enzyme classes in nature. While the EC nomenclature system provides a general classification that reflects the catalyzed reaction (ß-glucosidase 3.2.1.21, ß-xylosidase 3.2.1.37 etc.), the high functional and structural diversities of these enzymes can hardly be represented by a single hierarchical classification system. A widely accepted and at present probably the most important classification system for glycosidases is the Carbohydrate Active enZyme database CAZy (Cantarel et al., 2009; Henrissat and Davies, 1997). In this system, enzymes are grouped according to protein folds. As "form follows function", the assignment to a glycosyl hydrolase (GH) family provides an insight into functionality and reaction mechanism of an enzyme. Further, it has become common to use the CAZy classification (i.e., GH family) as a "brand name", which is quite helpful when it is required to compare enzymes of the same EC class. The CAZy classification system (GH family) will also be used in the present paper. This article will mainly discuss *exo*-glycosidases that are capable to hydrolyze glycosylated plant metabolites. Such glycosidases are usually termed "ß-glycosidases", reflecting their selectivity for the ß-D-glycosidic bond, which is the configuration usually encountered in structural polysaccharides and in plant glycosylation. This terming may be somewhat confusing as for example arabinose and rhamnose involved in the glycosylation of aroma precursors are bound to glucose via α-L linkage. Nevertheless, regarding the configuration on the anomeric carbon atom, α-L/ß-D are stereochemically identical. Therefore, "ß-glycosidase" is a widely accepted and sufficiently accurate term to describe enzymes involved in plant precursor glycosylation, in contrast to "α-glycosidases" like amylase or maltase. An important distinction among *exo*-glycosidases already indicated above is that between aryl-glycosidases with the ability to hydrolyze plant metabolite precursors and enzymes that preferentially hydrolyze di- or short chain oligosaccharides (Bhatia et al., 2002). In the latter case, a glucosidase should rather be classified as a cellobiase. Although this distinction is somewhat arbitrary as most enzymes will exert both activities to a certain degree, several examples for "true" aryl-glycosidases are known. The main point is that a thorough biochemical characterization using various substrates has to be performed before one can make conclusions as to the biochemical function or

metabolic role of a glycosidase. As glycosidases are usually characterized with synthetic substrates such as *p*-nitrophenyl (*p*NP) glycosides, several authors have expressed concern that the use of such substrates alone is not sufficient to determine the true function of a glycosidase. Even within aryl-glycosidases of the same enzyme class, distinct selectivities toward the aglycon can occur. An example would be the often observed selectivity towards glycosides of terpenols with primary or tertiary alcohol group.

## Glycosidase activities and aroma release by *Oenococcus oeni*

It was long suspectet, that *O. oeni* might possess glucosidase activities due to the known changes in aroma profiles and the increase of glucose during MLF (Mansfield et al., 2002). The first published report on the *ß*-glucosidase activity of an *O. oeni* strain in *vitro* can be found in Guilloux-Benatier et al. (1993). Vivas et al. (1997) observed the hydrolysis of the anthocyanic glucoside malvidin by *O. oeni.* McMahon et al. (1999) screened seven commercial strains of *O. oeni* but could not detect any glucosidase activities with synthetic substrates. The authors suspected that the composition of the growth medium might be a deciding factor to induce glucosidase activity. Grimaldi et al. (2000) screened twelve commercial *O. oeni* preparations (grown on MRS broth with 20 % apple juice) for activity towards synthetic *p*NP-glycosides and found that *ß*-glucosidase activity was present in both biomass and supernatant fractions of eleven of these preparations. Further, these activities were detectable in all growth phases. In most cases, the *ß*-glucosidase activities were increased in the presence of ethanol. Additionally, *ß*-D-xylosidase and low α-L-arabinosidase activities could be detected. Because of the variation in activity profiles found between individual strains, Grimaldi et al. (2000) already suggested the presence of multiple enzymes. In more detailed studies (22 commercial isolates), the same group confirmed that glucosidase, xylosidase, arabinosidase and even low rhamnosidase activities are present in *O. oeni,* albeit with high strain-dependant variations (Grimaldi et al., 2005b). The individual isolates responded differently to factors like pH-value, sugar concentration (glucose/fructose) and ethanol. In general, both glucose and fructose had a negative influence on the glucosidase activities. Xylosidase activity was moderately inhibited, rhamnosidase activity seemed to be increased in the presence of sugars. Arabinosidase activity was inhibited by glucose, but activated in the presence of fructose. Ethanol enhanced glucosidase and xylosidase acitivites, but had strong inhibitory effect on the arabinosidase activities. The same authors (Grimaldi et al., 2005a) also found that *ß*-glucosidase activities are widespread in *Lactobacillus* spp. and *Pediococcus* spp. mostly isolated from commercial MLF starter cultures. In a similar study, Barbagallo et al. (2004) detected *ß*-glucosidase activities in five of ten *O. oeni* isolates (wild isolates, Valpolicella) with high strain-dependant variations. In contrast to Grimaldi et al. (2000), Barbagallo et al. (2004) reported that these activities were mostly cell associated (parietal) and intracellular, no extracellular glucosidase activities were detected. In further tests, the selected strains had no detectable anthocyanase activity. The authors also described an enhancement of activity in the presence of ethanol.

These studies clearly demonstrated that *O. oeni* in fact possesses glycosidase activities and gave already interesting insights, especially regarding diversities between individual isolates. The fact that the strains were analyzed after growth on complex growth media (MRS broth) and that the enzyme assays were conducted only with synthetic substrates did not imply that these activities explain the release of aromas compounds for instance in wine. Consequently, further authors had the aim to show whether *O. oeni* is capable to hydrolyze natural glycosides of aroma compounds as well, especially in wine conditions. Mansfield et al. (2002) confirmed *ß*-glucosidase activity of *O. oeni* (7 of 9 strains positive) against synthetic substrates and found mainly parietal activity, but detected no activity on glycosides of native grapes of the 'Viognier' variety. Boido et al. (2002) found that two *O. oeni* isolates could hydrolyze glycosides of terpenols, C13-norisoprenoids and higher alcohols during MLF (variety 'Tannat'), however, the concentrations of the corresponding free aglycons remained relatively unchanged during MLF. The authors suggested that volatile compounds could be adsorbed on extracellular polysaccharides (EPS) and further concluded that the glycosidase activities might be mainly parietal, as no aglycons could be detected in the cytoplasm. Applying four commercial *O. oeni* strains in a synthetic wine supplemented with a glycoside extract from grapes of variety 'Muscat', Ugliano et al. (2003) were the first to detect the release of terpenes (linalool, terpineol, nerol, geraniol) during MLF, with a decrease of activity at lower pH-values. Strain dependant variations and differences in the release of individual com-

pounds led them to presume that the presence of distinct enzyme mechanisms may cause the selected strains to respond individually to aglycon structure and glycosylation mode. However, similar to Boido et al. (2002) the authors found that the release of free aglycons did not stoichiometrically correspond to the decrease of their glycosylated precursors. D'Incecco et al. (2004) found glucosidase and low arabinosidase and rhamonosidase acitivites in the *O. oeni* strain Lalvin EQ54. The strain could increase the concentrations of several terpenes and other volatiles (vanillin) during MLF with a model wine (supplemented with a glycoside extract from 'Chardonnay'), however to a modest extent. Interestingly, the authors also found that the rate of MLF was increased in the presence of the glycoside extract, although this did not affect the growth rate of *O. oeni*. Ugliano and Moio (2006) confirmed the hydrolysis of aroma precursors (aliphatic alcohols, benzene derivatives, terpenes, norisoprenoids) by *O. oeni* in wine, but found only low increase of the corresponding aglycons. They also determined that *O. oeni* preferentially released terpenols with a primary hydroxyl group (Ugliano et al., 2003; Ugliano and Moio, 2006). Hernandez-Orte et al. (2009) determined the release of volatile compounds (terpenes, norisoprenoids, phenols and vanillin) from a model wine during MLF with strains of *O. oeni*, *L. brevis*, and *L. casei*. Beside that they detected a low increases of volatile compounds nevertheless in tasting changes in aroma profile were noticed.
*Gagné* et al. (2011) screened a large collection (47 isolates) of *O. oeni* with synthetic glycosides and found high activities of glucosidase, xylosidase and low arabinosidase activities. In contrast to the results reported by Grimaldi et al. (2005b), the authors also detected high rhamnosidase activities. High strain-dependant variations in specific activities and notably distinct activity profiles were confirmed in this strain collection as well. Confirming the results of a previous study (Bloem et al., 2008), Gagné et al. (2011) demonstrated that *O. oeni* is capable to release glycosyated aroma compounds from oak wood extracts (e.g., whisky lactone, phenols, vanillin). The authors put emphasis on the fact that the results from assays with synthetic substrates did not correlate with those obtained with natural substrates. Strains that had only low activities toward *p*NP-glycosides were not less capable to release natural aroma compounds. Gagné et al. (2011) therefore concluded that because of the complex composition of wine, *in vitro* assays are not reliably suited to determine actual strain characteristics.

As demonstrated above *O. oeni* is capable to release glycosidically bound aroma compounds from wine during MLF and also indicated that *O. oeni* is quite versatile in these terms (Bartowsky and Borneman, 2011). From the wine makers perspective, the main impact of this information is that the choice of *O. oeni* strain for MLF has considerable impact on the aroma composition of a wine. Further, in agreement with the current opinion that *O. oeni* is genetically heterogeneous; the so far published research strongly suggests the action of distinct enzyme mechanisms, differences in gene regulation may occur as well. This is indicated by the facts that (i) enzyme assays (esp. with synthetic glycosides) demonstrated that the glycosidase activity profiles are highly strain-dependant; (ii) contradictory reports regarding the localization of enzyme activities, i.e., whether extracellular, cell-bound (parietal) or intracellular; and (iii) differences in the resulting aroma profiles, also concerning the release of primary or tertiary terpenols. Accordingly, several authors suggested that studies on the molecular level are in order to achieve a better understanding of the mechanisms in place. In the next section, we will discuss the properties of glycosidase enzymes of *O. oeni* that have so far been isolated and biochemically characterized

## Characteristics of *O. oeni* glycosidases

### Glycosidases of the phosphotransferase system

The carbohydrate:phosphotransferase system (PTS) is a key mechanism involved in bacterial sugar import and phosphorylation. The phosphate group is transferred from phosphoenolpyruvate to the sugar by a complex enzyme cascade that includes membrane associated ABC family transporters (PTS component EII) involved in substrate translocation and phosphorylation (Barabote and Saier, 2005; Postma et al., 1993). Upon phosphorylation, the activated sugar (e.g. glucose-6-phosphate) can enter glycolysis. Since its discovery in *E. coli* (Kundig et al., 1964) it has become clear that the PTS is a fundamental mechanism for carbohydrate import in both Gram positive and negative bacteria (Thompson et al., 1997). Especially since many LAB genomes are fully available now, it is obvious that the PTS is a constant in the

LAB carbohydrate catabolism as well. Phosphorylated glycosides or disaccharides are hydrolyzed by intracellular phosphoglycosidases that usually act on phosphorylated substrates only. Despite the obvious importance of PTS glycosidases, information on this topic, especially concerning ß-glycosidase activities of LAB is fairly limited. Most work so far has been done on the lactose specific PTS of LAB, as reviewed in De Vos and Vaughan (1994). Most phospho-ß-glycosidases (ß-glucosidases and ß-galactosidases) belong to GH1 and contribute to the lactose or cellobiose specific PTS. Several authors have already characterized LAB phosphoglucosidases (De Vos et al., 1990; Marasco et al., 1998; Marasco et al., 2000; Nagaoka et al., 2008; Simons et al., 1993), and such glucosidases could also be responsible for the hydrolysis of aryl-glucosides (Weber et al., 2000).

Spano et al. (2005) monitored the expression of a ß-glucosidase gene from *Lactobacillus plantarum* (lp_3629) under stress factors, and found that its expression was down-regulated in the presence of ethanol. Sequence alignment (BLASTP) implies that the gene encodes a phosphoglucosidase of GH family 1. However, as noted by Capaldo et al. (2011a, b) the difference between specificity for phosphorylated or non-phosphorylated substrates in GH1 may depend on the exchange of only a single amino acid. The genome of *O. oeni* PSU-1 contains four open reading frames encoding putative phospho-ß-glucosidase genes (OEOE_0224, OEOE_0340, OEOE_0341, OEOE_1210), "orthologues" (i.e., genes with sequential similarities) to these genes are widespread in LAB genomes. Capaldo et al. (2011a) cloned and expressed the putative phosphoglucosidase gene OEOE_0224 (bglD) and demonstrated activity against *p*NP-ß-D-glucopyranoside-6-phosphate. Olguin et al. (2011) monitored the expression of the same gene and found that the gene was expressed during MLF. Further, (Capaldo et al., 2011b) cloned OEOE_0340 (celC) and OEOE_0341 (celD) and detected that both genes are most likely organized in a single polycistronic unit (operon). While CelD could hydrolyze *p*NP-ß-Glu-6P, CelC was inactive towards this substrate and may have a distinct, yet unidentified function. BglD, CelC and CelC were inactive towards non-phosphorylated *p*NP-glycosides.

Concerning the hydrolysis of glycosylated plant metabolites by LAB, the contribution of the PTS is not sufficiently elaborated. Although phospho-ß-glucosidases are usually part of the cellobiose specific PTS, it has been shown that PTS related glucosidases can hydrolyze aryl-glucosides (arbutin, salicin) as well (Weber et al., 2000). Further, the so far publicly accessible genomes of *O. oeni* in GenBank (Benson et al., 2011) indicate the presence of genes encoding PTS transporters specific for ß-glucosides. It is therefore plausible to deduce that the PTS is responsible for the ß-glucosidase activities of *O. oeni* involved in aroma hydrolysis as well. Further, the fact that several authors reported parietal ("whole cell") glucosidase activity without detectable intra- or extracellular activities could indicate the action of the PTS in such cases. Although phosphoglucosidases are cytoplasmic, their hydrolytic activity requires the presence of functional membrane associated transporters involved in substrate phosphorylation, which may indeed result in the impression of a membrane associated activity. However, the presence of membrane bound (non-PTS) glucosidases in *O. oeni* cannot be excluded, although such enzymes have not been identified to date. Further, apart from glucosidase activities, it is not known whether PTS glycosidases can be made responsible for xylosidase, rhamnosidase or arabinosidase activities as well. Rhamnosidase activity has been recorded with *O. oeni,* but no rhamnosidase has so far been identified, neither are putative rhamnosidase genes identifiable in the published *O. oeni* genomes. The main problem is that phosphorylated substrates that would be required for a detailed analysis of kinetics and substrate specificities of phosphoglycosidases are presently not commercially available. Only few researchers have undertaken the efforts to synthesize a broad set of phosphorylated glycosides. This leads to the unfortunate situation that apart from the fact that phosphoglycosidases are ubiquitous in LAB, their role, especially regarding the release of aroma compounds by *O. oeni* remains subject to speculation. Due to the high occurrence of PTS glycosidases, diversities in functionality and regulation of gene expression can be expected as well. As noted by Capaldo et al. (2011a, b), a detailed investigation of the PTS of *O. oeni* will be most important to understand the complexity of the glycoside metabolism of this organism. It is of further interest, that genomic variations in respect to the distribution of PTS genes are indicated in the published genomes of *O. oeni*.

**Intracellular non-PTS glycosidases of** *O. oeni*

Following the purification of an intracellular GH3 ß-glucosidase from *L. brevis*, Michlmayr et al. (2010a, b) expressed and characterized a similar enzyme from *O. oeni* ATCC BAA-1163. Both glucosidases displayed similar kinetic characteristics and can be classified as aryl-glucosidases with high ß-D-xylosidase and low α-L-arabinosidase side activities. Further, activity enhancement in the presence of ethanol and moderate inhibition by glucose was determined. In general, these data are consistent with the observations made by Grimaldi et al. (2000), and indicate that this (intracellular) glucosidase may be an important factor in the glycosidase activities of *O. oeni*. Interestingly, the corresponding (GH3) glucosidase gene is widespread in LAB, but not present for example in the genomes of *L. plantarum* or *Pediococcus* sp.. In a further study, Michlmayr et al. (2011b) characterized an α-L-arabinosidase (intracellular, GH51) from *O. oeni* ATCC BAA-1163. According to a BLAST search in GenBank, the gene is rare among LAB and mainly present in the *Leuconostoc/Oenococcus/Weissella* branch. This enzyme can be classified as an aryl-glycosidase as well, as judged from the low activities toward arabinosaccharides. The enzyme's activity was inhibited by ethanol, which is in agreement with the observations of Grimaldi et al. (2005b). In laboratory tests (Michlmayr et al., 2011a), both glucosidase and arabinosidase from *O. oeni* ATCC BAA-1163 were able to release monoterpenes from natural aroma precursors. Applied in combination, these enzymes could also release high amounts of tertiary terpene alcohols. This is remarkable, since Ugliano et al. (2003) and Ugliano and Moio (2006) reported that *O. oeni* releases mainly terpenols with primary alcohol group. As the release of tertiary terpenols was mainly caused by the arabinosidase, the expression monitoring of both glycosidase genes during MLF would be interesting to determine the influence of these enzymes *in vivo.* Further, as both enzymes are intracellular, the identification and detailed study of the involved glycosidase transporters will be required as well. An interesting detail is also the genomic vicinity of these genes. Both glucosidase (OENOO_34001) and arabinosidase (OENOO_34003) genes are adjacent to a putative transporter gene (major facilitator superfamily, OENOO_34002). This topology is present in all published *O. oeni* genomes in GenBank (at the time of writing: ATCC BAA-1163 / PSU-1 / AWRIB429) but not in other LAB genomes as *L. brevis* (ATCC 367) or *L. mesenteroides* (ATCC 8293), both harboring similar glucosidase and arabinosidase genes. Therefore, it would already be interesting to investigate whether this represents a glycosidase operon unique in *O. oeni.* Further, both arabinosidase and transporter genes are interrupted (stop codons) in strains PSU-1 and AWRIB429. As some of the mutations are identical in both strains, a coincidence is unlikely. Consistent with the reported mutability of *O. oeni*, mutations in these glycosidase genes might at least be partially responsible for the often observed distinct glycosidase activity profiles of *O. oeni.*

## Conclusion

The glycosidase activities of *O. oeni* have recently been subject to numerous studies of remarkable experimental detail. Consistent with the diverse and sometimes even contradictory reports on the glycosidase profiles of *O. oeni*, the available results of biochemical and genomic analysis suggest that the glycoside metabolism of this versatile organism is a highly complex system, involving distinct mechanisms with multiple enzymes. Further, due to the reported genetic heterogeneity of *O. oeni*, strain-dependant variations regarding the genomic presence of functional glycosidase genes and their regulation can be expected as well. At present, *O. oeni* is probably one of the LAB species best studied in regard to its ß-glycoside metabolism. The information so far collected on *O. oeni* might also be interesting for the study of other LAB species, as most of the glycosidase genes so far identified in *O. oeni* are widespread in LAB (i.e., the order *Lactobacillales*) genomes.

Unfortunately, the so far available data are merely the fragments of a big puzzle that yet requires completion. Future scientists attempting to complete our understanding of the glycosidase mechanisms of LAB will not face a simple task. Several authors reported extracellular glucosidase activities (Grimaldi et al., 2000; Olguin et al., 2011; Sestelo et al., 2004) of *O. oeni.* However, no genes coding for extracellular glucosidases have so far been identified. Further, the complexity of the PTS and the regulation of its multiple components represent a challenge of its own. The fundamental question remaining, however, is that toward the metabolic roles of the diverse mechanisms/enzymes. While the PTS is mainly thought to be involved in sugar import, the role of other (intracellular) aryl-gly-

cosidases is not clear to date. These glycosidases may be an alternate sugar scavenging system specific for glycosylated plant metabolites and they could play an important role in the interaction of LAB with such compounds. In the case of *O. oeni*, most authors investigating its glycosidase activities were concerned with the release of aroma compounds. However, wine is rich in phenolic compounds (i.e. polyphenols) and most of them are glycosylated as well. Several authors have shown that phenolic compounds and their glycosides can exert both positive and negative effects on growth and metabolism of *O. oeni* (D'Incecco et al., 2004; De Revel et al., 2005; Figueiredo et al., 2008; García-Ruiz et al., 2008; Hernandez-Orte et al., 2009; Hernandez et al., 2007; Poussier et al., 2003; Vivas et al., 1997). Further, LAB also possess the ability to metabolize phenolic compounds (Rodríguez et al., 2009). Therefore, it will be most interesting to investigate how the glycosidases of *O. oeni* are involved in the degradation of phenols and glycosylated plant metabolites in general.

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