Chapter 12
Polysaccharide Production by Grapes Must and Wine Microorganisms

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List of Abbreviations

EPS Exopolysaccharide
MLF Malolactic fermentation
MP Mannoproteins
PS Polysaccharide

12.1 Introduction

In this chapter, we describe the formation of polysaccharides (PS) by some of the microorganisms most frequently encountered in grapes, must and wine: *Botrytis cinerea*, *Saccharomyces cerevisiae*, non-*Saccharomyces*, *Oenococcus oeni* and other wine lactic acid bacteria. The structure of the polymer produced, the metabolic pathways identified, the putative or demonstrated benefits linked to capsular PS formation for the microorganism and the impact of the PS released on wine quality are described.

Several species of fungi, yeasts and bacteria develop on the grape berry during ripening and, afterwards, throughout the winemaking process. All contribute, via their own metabolic pathways, to the final chemical composition of the wine. Polysaccharides (PS) form part of the molecules produced by microbial metabolism which affect wine quality. They constitute the highest molecular weight component of wine and consist of repeating sugar units. These repeat units can be made of several different monosaccharides (heteropolysaccharides) or of the repetition of a

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H. König et al. (eds.), *Biology of Microorganisms on Grapes, in Must and in Wine*,
DOI 10.1007/978-3-319-60021-5_12

single one (homopolysaccharides). The chain length, degree of branching and type of osidic bounds are also important characteristics of the molecule structure.

The PS content in must and wine varies throughout the winemaking process due to synthesis and degradation reactions. Only the more soluble grape PS are extracted in must (pectins and arabinogalactan). From picking until the end of alcoholic fermentation, pectins are gradually degraded into smaller PS, due to the action of grapes and microbial pectolytic enzymes (Pellerin and Cabanis 1998). The first microbial event that significantly modifies the wine's final PS composition is when the grapes are infected by *Botrytis* (Sect. 12.2): the pectins are hydrolysed and specific neutral polymers are formed (Dubourdieu 1982). In the next stage, during alcoholic fermentation and ageing on the lees, yeasts (*Saccharomyces* and non-*Saccharomyces*) release mannoproteins. These molecules constitute the second group of wine PS in quantitative terms, after those originating from grapes (Sect 12.3) (Ribéreau-Gayon et al. 2000). Pectolytic yeast species may also hydrolyse certain grape PS, thus providing substrates for the subsequent growth of other microbial species (Louw et al. 2006). Afterwards, as a result of the natural selection among bacteria occurring during alcoholic fermentation, *Oenococcus oeni* generally becomes dominant for the subsequent malolactic fermentation (MLF). During this stage, many changes occur in wine PS composition, indicating that, like *Botrytis* and yeast, *O. oeni* has the ability to produce and degrade PS (Dols-Lafargue et al. 2007). Though, most of the time, *O. oeni* PS have no evident impact on wine quality, some of them, which are also produced by other wine bacteria, have long been associated with the spoilage named "ropiness". Indeed, the bacterial PS structures and biosynthetic pathways are diverse and strain specific, and some associated genes are shared by several species (Sect. 12.4).

This chapter focuses on PS synthesis by microorganisms in grapes and wine, describing the structures of the polymers produced and, when identified, the biosynthetic pathways, with molecular aspects and regulation. Microbial PS are usually, at least partially, linked to the cells, thus forming a capsule, while the remainder is released into the surrounding medium (Sutherland 1993). The putative or demonstrated physiological benefits linked to capsular PS formation are discussed, and, finally, the impact of the released PS on wine quality is examined.

12.2 PS Produced by *Botrytis cinerea*

Botrytis cinerea is a deuteromycete (Hyphomycete) fungus. It is an important plant pathogen with an exceptionally broad host range. Its development on grapes may be dreaded (grey rot) or desired ("noble rot") (Ribéreau-Gayon et al. 2000).

In terms of PS, must extracted from rotten grapes no longer contains pectic PS, and its galactose and mannose concentrations are modified. Moreover, these musts contain exopolysaccharides (EPS), specifically produced by *B. cinerea*. When the fungus is cultivated on liquid medium, it is possible to separate two groups of soluble PS by alcoholic precipitation (Dubourdieu 1982):

- The more alcohol-soluble fraction consists of heteropolysaccharides.
- The less alcohol-soluble polymer is a glucan (glucose homopolysaccharide), known as cinerean. This is also the only polymer observed with certain strains of *B. cinerea* (Leal et al. 1976; Stahmann et al. 1992). Most of this extracellular polymer is attached to the hyphal cell wall, forming capsules (60%), while the rest (40%) is released as slime (Pielken et al. 1990).

12.2.1 Structure of the PS Produced

The heteropolysaccharide fraction has been less studied than the β-glucan. It consists of mannose, galactose, glucose and rhamnose (60/30/5/5), with molecular weights between 10 and 50 kDa (Dubourdieu 1982).

Cinerean has a linear backbone of β-1,3 linked glucosidic residues, with branched chains, consisting of a single β-1,6 linked glucosidic residue, attached to every second or third glucose molecule (Fig. 12.1) (Dubourdieu et al. 1981). This structure is common in cell wall polymer of yeast and filamentous fungi. The chains can be linked by low energy bonds. This increases the apparent molecular weight and leads to the trapping of a black pigment, melanin, by the glucan. The molecular weight of the glucan was estimated at 10^5–10^6 Da by size exclusion chromatography and 10^9–10^{10} by low-angle laser light scattering. Ultrasound treatment was used to separate the polymer from the melanin, resulting in glucan fibrils of 50–250 kDa (Dubourdieu et al. 1981; Dubourdieu 1982; Stahmann et al. 1995; Doss et al. 2003).

12.2.2 PS Production Kinetics

The two families of PS are produced during active growth on glucose in model medium: 300 mg l^{-1} cinerean and about 50 mg l^{-1} heteropolysaccharides (Dubourdieu 1982). In batch fermentation, a decrease in cinerean is observed after glucose exhaustion, leading to a striking decrease in viscosity. Indeed,

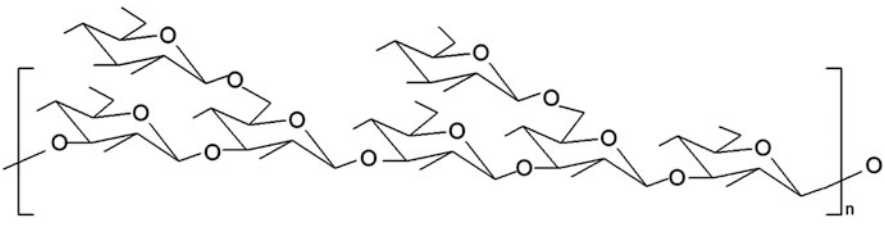

Fig. 12.1 Schematic representation of the repeating unit of *Botrytis cinerea* β-glucan (Dubourdieu et al. 1981)

B. cinerea produces several β-1,3 glucanases. Cinerean may be considered an external carbon reserve (Leal et al. 1976; Dubourdieu and Ribereau-Gayon 1980; Martinez et al. 1983; Stahmann et al. 1995). The PS content of wines produced from botrytised musts is up to 750 mg l^{-1} higher than in wines obtained from uncontaminated musts (Dubourdieu et al. 1978).

The genes and enzymes responsible for PS synthesis in *B. cinerea* have not been studied. Only Monschau et al. (1997) evidence the β-1,3 glucan synthase activity of membrane fraction of *B. cinerea* and suggest that the branching enzyme for the β-1,6 glycosidic bonds does not have the same location. Most studies have been done with other filamentous fungi but the biosynthetic pathway may be similar in *B. cinerea*. They suggest that the membrane-bound glucan synthase complex releases the polymer in the periplasmic space, where a remodelling occurs. In *Epicococcum niger*, Schmid et al. (2006) show that the synthesis of epiglucan (β-1,3 β-1,6 branched fungal glucan) occurs via the transfer of glucosyl residues (probably from UDP-glucose) to the non-reducing end of the growing chain. The side β-1,6 linked residues are incorporated gradually, as β-1,3 backbone glucan elongates. Furthermore, they suggest two PS formation mechanisms involving either (1) a single transmembrane glycosyltransferase, as proposed for *Streptococcus pneumoniae* and *Pediococcus parvulus* β-glucans (Sect. 12.4), or (2) a complex set of glycosyltransferases, as described for lactic acid bacteria EPS synthesis (Sect 12.4). Identification of single or multiple genes associated with β-glucan formation would clarify which mechanism is actually responsible.

12.2.3 Benefit for the Fungus

Like for other filamentous fungi, the *B. cinerea* glucan is essential for the cell wall rigidity. Most of the exocellular part of the β-glucan produced sticks to the cells, thus forming a thick capsule (Pielken et al. 1990). This capsule protects them from drought and assists in cell attachment on grapes (Dubourdieu 1982; Doss et al. 1995). Gil-ad et al. (2001) show that the presence of the glucan sheath strongly modifies the fungus morphology, protecting it from host responses, by slowing the diffusion of host secretions. In addition, the glucan sheath traps enzymes (peroxidase, laccase and catalase), which thus constitute an "arsenal" outside the cells (Doss 1999). Eventually, *Botrytis* PS undoubtedly play a key role in the biofilm established on the grape berry, containing yeasts, bacteria and other fungi.

12.2.4 Impact on Wine Quality

Cinerean is responsible for the high viscosity of musts produced from rotten grapes. After alcoholic fermentation, in the presence of ethanol, this glucan tends to form aggregates which block filters, making more difficult spontaneous clarification by

sedimentation and impairing wine filterability. Commercial glucanases are thus applied to such wines. They mainly display exo-β-1,3 glucanase and β-1,6 glucosidase activities, which finally hydrolyse the glucan to glucose (Villetaz et al. 1984; Dubourdieu et al. 1985; Humbert-Goffard et al. 2004). The direct pressing of rotten grapes without crushing them can also reduce the amount of glucan released into the must.

B. cinerea glucan affects yeast physiology and metabolism. Its addition to a fermenting medium slows down the alcoholic fermentation and stimulates the glyceropyruvic pathway, leading to increased excretion of glycerol and acetate (Ribéreau-Gayon et al. 1979; Dubourdieu 1982).

12.3 Yeast Mannoproteins

Mannoproteins (MP) constitute the outer part of the yeast cell wall polysaccharide layer. Some MP with enzyme activity (such as the external invertase) are immobilised in the structure of the MP matrix (Ballou 1976). During alcoholic fermentation and ageing on lees, some of these MP are released into the wine, where they interact with many other wine components.

12.3.1 Yeast Cell Wall Organisation and MP Structure

The most studied cell wall of *Saccharomyces cerevisiae* makes up 15–30% of the cell's dry weight, depending on growth conditions. It consists of separate, interconnected PS layers (Fig. 12.2). The outer layer is made of MP, connected to a matrix of amorphous β-1,3 glucan, while the inner layer consists of fibrous β-1,3 glucan, over a small quantity of chitin; β-1,3 glucan is the main component (85%) responsible for the mechanical properties of the cell wall. The β-1,6 glucan (15%) probably links the components of the inner and outer walls (Kollár et al. 1997; Klis et al. 2002).

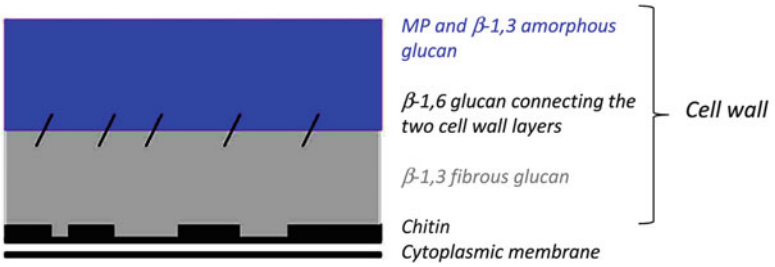

Fig. 12.2 *Saccharomyces cerevisiae* cell wall organisation

In the genus *Saccharomyces*, MP are made of mannose (about 90%), *N*-acetyl-glucosamine and mannosylphosphate (0.1–1%), in varying proportions, depending on the strain and growth phase (Ballou 1976, 1990; Jigami and Odani 1999; Klis et al. 2002). Their molecular weights vary from 20 to 450 kDa. A glycosylphosphatidylinositol anchor attaches the carboxylic group of the peptide chain of certain MP, which cross the cell wall, to the plasma membrane. Then, three forms of glycosylation have been described for *S. cerevisiae* MP, but they do not necessarily coexist in all of the MP (Fig. 12.3). The first form of glycosylation consists of mainly α-1,6-linked glucomannan chains, but their peptide point of attachment has not been clearly identified yet. The second form of glycosylation consists of small α-1,2- and α-1,3-linked mannooligosaccharide chains, which are sometimes phosphorylated. These small chains are attached to the peptide chain, via *O*-glycosidic bonds on serine or threonine residues. The last form of glycosylation is a *N*-linked PS attached to the peptide chain, via an asparagine residue. The core of this PS consists of a double unit of β-1,4-linked N-acetyl-glucosamine, to which a α-1,2-, α-1,3- and α-1,6-linked phosphorylated mannooligosaccharide is attached. A highly ramified outer chain (150–250 mannose units) is then attached to the core. This consists of a skeleton of α-1,6-linked mannosyl units, supporting short side chains of α-1,2- and α-1,3-linked mannosyl residues and phosphodiester-branched mannosyl residues (Ballou 1990; Jigami and Odani 1999).

The core of the PS fraction occurs in several yeast species, while the external PS chain is strain specific (Ballou 1976). The structure of the MP released into wine

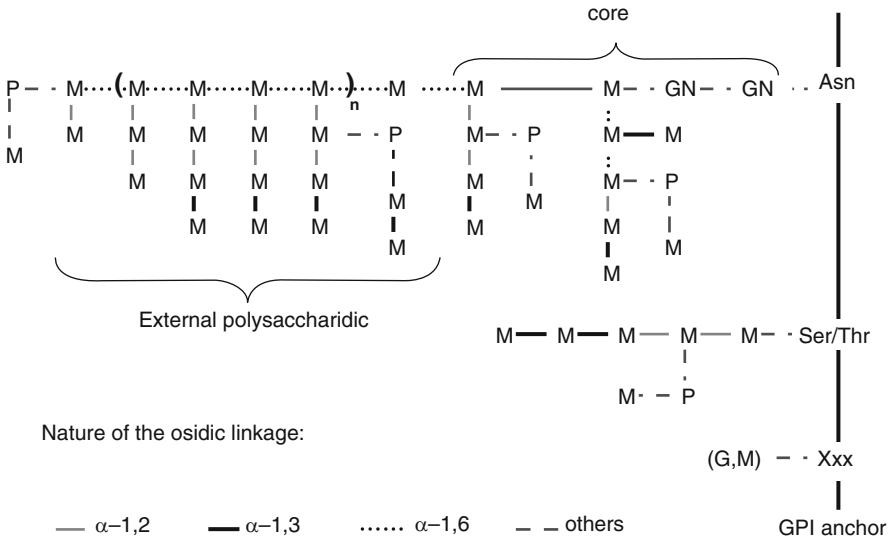

Fig. 12.3 Schematic representation of the O-linked oligosaccharide fraction and N-linked polysaccharide fraction of *S. cerevisiae* mannoproteins, MP ($n = 0$–10) (Adapted from Ballou 1990; Jigami and Odani 1999). *GNAc* N acetyl glucosamine, *M* mannose, *P* phosphate, *Asn* asparagine, *Ser* serine, *Thr* threonine

depends on the yeast strain, but is always similar to that of the yeast cell wall, with a molecular mass between 50 and 500 kDa (Villetaz et al. 1980; Llaubères 1987).

So far, non-*Saccharomyces* species MP have been less studied. However their structure is presumably similar to the one of *Saccharomyces*. These molecules are mainly mannoproteins with close composition to that of *S. cerevisiae*, excepted in *Schizosaccharomyces pombe*, whose MP contains also galactomannans (Giovani et al. 2012).

12.3.2 Physiology of MP Release

The cell wall construction is a dynamic, tightly regulated process, involving a large number of genes (Lussier et al. 1997; Smits et al. 1999; de Groot et al. 2001). The growing cells produce β-glucanases and other enzymes that partially degrade the β-1,3/β-1,6 glucan network, weakening the cell wall and facilitating cell division, budding or mating. No mannosidase or N-Ac-glucosaminidase is detected (Llaubères 1987; Klis et al. 2002; Gonzales-Ramos and Gonzales 2006). As a result, yeasts release PS, and especially MP, from the cell wall during active growth. In model medium, 100–250 mg l^{-1} MP are released, depending on the yeast strain, contact time, temperature and agitation of the yeast biomass. This phenomenon slows down when cells enter the stationary phase, as the walls become thicker and more resistant to β-glucanases, while the level of MP phosphorylation increases (Llaubères 1987; de Nobel et al. 1989, 1990; Shimoi et al. 1998; Jigami and Odani 1999).

The same phenomena occur during alcoholic fermentation in wine. *S. cerevisiae* MP are mainly released by active yeasts during the early stages of alcoholic fermentation but also by dying or dead cells (Giovani et al. 2010). According to Domizio et al. (2014), non-*Saccharomyces* PS are mainly released during growth. However, β-glucanases present in the cell wall maintain some residual activity a few months after cell death. As a result, ageing on the lees further raises the MP level by 150–200 mg l^{-1}, depending on the yeast strain, especially when lees are stirred and consist of fermented yeasts rather than additional dry yeasts (Llaubères 1987; Ribéreau-Gayon et al. 2000; Guilloux-Benatier and Chassagne 2003; Juega et al. 2015).

Given the positive effect of MP on wine (see Sect. 12.3.4), yeasts richer in MP are sought. Besides strain selection, genetic approaches such as recombinant genetics or random mutagenesis have been tried (Gonzalez-Ramos et al. 2008, 2010). Pérez-Través et al. (2015) obtained a *S. cerevisiae* strain with high producing MP ability and high fermentation performance. However another way to take advantage of yeast MP release is to use non-*Saccharomyces* yeasts. Namely, *Torulaspora delbrueckii* has been generally recognised as a high MP producing species (Giovani et al. 2012; Belda et al. 2014).

12.3.3 Benefit for the Wine Yeasts

MP in the outer cell wall layer play an important role in controlling the exchange of macromolecules (proteins, etc.) between the periplasmic space and the environment (de Nobel et al. 1989, 1990; Kapteyn et al. 1996). Several enzymes are thereby retained in the periplasmic space (Klis et al. 2002). Moreover, the external PS fraction of MP, which emanates from the cell surface, is involved in cell–cell recognition events.

MP are also involved in cell protection and survival in hostile environments, e.g. water retention and drought protection (Klis et al. 2002). Furthermore, various studies have shown that mannosylphosphorylation or modified MP patterns help the cells to overcome stress and contribute to yeast flotation during velum formation (Jigami and Odani 1999; Parascandola et al. 1997; Martinez et al. 1997; Alexandre et al. 1998, 2000). In an evolutionary engineered *S. cerevisiae* wine strain, genes linked to cell wall MP synthesis proved to be upregulated in response to low temperature, suggesting a direct involvement of MP in cold stress (López-Malo et al. 2015).

12.3.4 Impact on Wine Quality

Today, the use of yeast and yeast cell wall derivatives is accepted in winemaking, during or after fermentations, for fining or in replacement of lees for ageing. Most studies report that the presence of MP is beneficial to wine quality (Caridi 2006), although in specific cases, they may be responsible for a decrease in wine colour intensity or lower filterability (Vernhet et al. 1999; Morata et al. 2003; Rizzo et al. 2006).

In the pH range of wine, MP are negatively charged and establish interactions with other components, especially phenolic compounds (anthocyanins and tannins) and aromas, thus increasing colour stability, decreasing astringency and modulating aroma intensity and volatility (Lubbers et al. 1994; Vernhet et al. 1996; Escot et al. 2001; Riou et al. 2002; Caridi et al. 2004; Chalier et al. 2007; Juega et al. 2012; Mekoue Nguela et al. 2016; Gonzales-Royo et al. 2016). This property is used to stabilise wine via the legally authorised addition of purified MP (mannostab^{TM}) (Dubourdieu and Moine 1996). MP also inhibit the crystallisation of tartrate salts (Lubbers et al. 1993; Gerbaud et al. 1996) and prevent protein haze or adsorb molecules that would otherwise be implicated in oxidation reactions. This explains the stabilisation of white wines aged on lees (Waters et al. 1994; Escot et al. 2001; Charpentier et al. 2004; Dufrechou et al. 2015). Some MP have been shown to significantly adsorb ochratoxin A, a mycotoxin sometimes reported in grapes, must and wine (Caridi 2006). In addition, MP contribute to yeast flocculation as well as to yeasts and bacteria co-flocculation, during sparkling wine production (Suzzi et al. 1984; Peng et al. 2001; Fleet 2003; Pérez-Magariño et al. 2015). Some have

been reported to stimulate the growth of malolactic bacteria (Guilloux-Benatier et al. 1995; Guilloux-Benatier and Chassagne 2003). And last but not least, a keen interest for MP was recently observed for improving mouthfeel perception, aroma persistence and body or sweetness. This MP enrichment could be achieved through addition of purified molecules (Moine 2009; Pérez-Magariño et al. 2015) or by using selected MP producing strains or species. For example, the great impact on sensorial mouthfeel by *T. delbrueckii* is clear in all reports (Giovani et al. 2012; Belda et al. 2016; Domizio et al. 2014). This positive impact is also achievable by using *T. delbrueckii* lees in wine ageing (Belda et al. 2016).

12.4 Production of PS by Wine Lactic Bacteria

Many lactic bacterial species can be found in wines especially after alcoholic fermentation, when they drive malolactic fermentation, MLF (Chap. 1). Soluble PS concentrations increase or decrease during MLF, depending on the wine considered, suggesting that *Oenococcus oeni*, the bacterial species most often responsible for MLF, can both produce and degrade PS without altering the wine (Dols-Lafargue et al. 2007). However, in some cases, lactic acid bacteria cause "ropiness" or "oiliness", one of the four major types of bacterial spoilage in wine (Pasteur 1866). Spoiled wines display an oily, ropy texture, due to the liberation of a specific bacterial PS (Llaubères 1987).

However, recent studies show that it is not so easy to distinguish on one side harmless or beneficial bacterial EPS and, on the other side, those causing wine spoilage.

12.4.1 Structure and Location of Wine Bacterial PS

The first wine bacteria studied for their ability to produce EPS were chosen because they displayed visible and singular thickening or sticking properties (see examples Fig. 12.4a, b). They had been isolated from spoiled ropy wines, beer and cider. Such singular ropy strains belong to genera *Streptococcus*, *Leuconostoc*, *Pediococcus*, *Lactobacillus* and *Oenococcus* (Luthi 1957; Van Oevelen and Verachtert 1979; Lonvaud-Funel and Joyeux 1988; Manca de Nadra and Strasser de Saad 1995; Duenas et al. 1995; Fernandez et al. 1995; Walling et al. 2005b; Werning et al. 2006; Ibarburu et al. 2007; Garai-Ibabe et al. 2010; Dimopoulou et al. 2014, 2016; Caggianiello et al. 2016). All ropy strains produce significant EPS amounts in model media, when compared to other strains of the same species.

The first and most studied ropy EPS is the high molecular weight (500–2000 kDa) β-glucan produced by *P. parvulus* 2.6 and IOEB_8801. These strains were first considered as *Pediococcus cerevisiae* (Lonvaud-Funel and Joyeux 1988), later classified as *Pediococcus damnosus* by DNA/DNA hybridisation

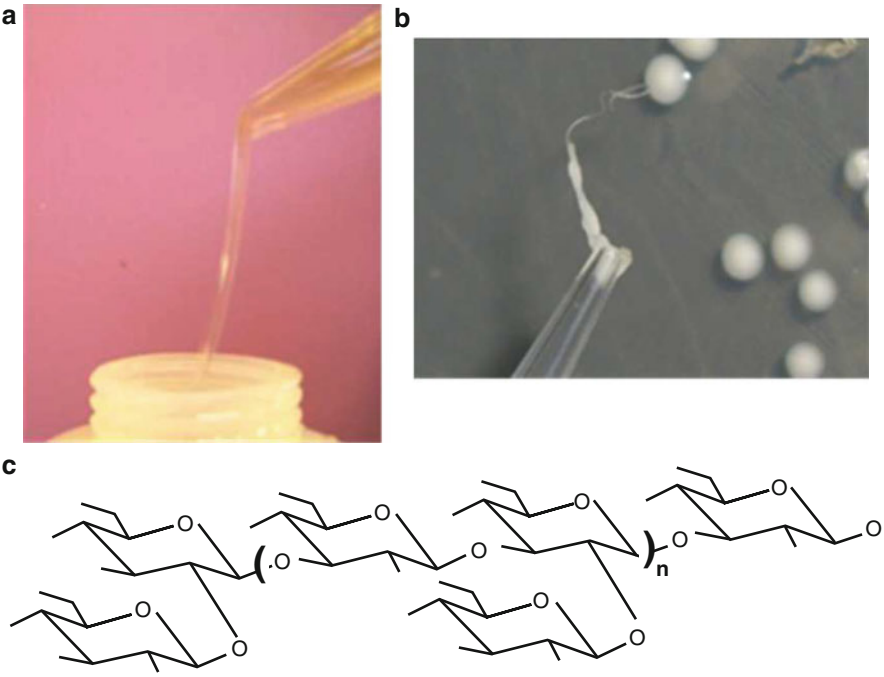

Fig. 12.4 (**a**) Ropiness induced in liquid model medium (MRS) by *P. parvulus* IOEB_8801. (**b**) Ropiness detection by picking colonies of *O. oeni* IOEB_0205 after growth on solid model medium (MRS) (**c**) Schematic representation of the chemical structure of *Pediococcus parvulus* β-glucan

(Lonvaud-Funel et al. 1993; Duenas et al. 1995, Walling et al. 2005a, b) and then, finally, as *Pediococcus parvulus* based on 16S RNA sequencing (Werning et al. 2006). The ropy β-glucan consists of a trisaccharide repeating unit with a β-1,3-linked glucosyl backbone branched with a single β-1,2-linked D-glucopyranosyl residue (Fig. 12.4c). Its structure is close to the one of capsular PS of *S. pneumoniae* type 37 (Adeyeye et al. 1988; Llaubères et al. 1990; Duenas-Chasco et al. 1997; Walling et al. 2005b). Transmission electron microscopy analyses show that the β-glucan forms a large but loosely attached layer around the cells (Fig. 12.5a). However, the β-glucan is probably not the only PS produced by *P. parvulus*, if one believes the dense halos still visible around the cells after β-glucan removal (Fernandez de Palencia et al. 2009; Coulon et al. 2012). Other less studied species, such as *Pediococcus damnosus*, *Lactobacillus diolivorans* and *Lactobacillus suebicus*, are described to produce this specific β-glucan (Walling et al. 2005b; Duenas-Chasco et al. 1998; Garai-Ibabe et al. 2010). However, PS other than this β-glucan may be responsible for the ropy character of *Lactobacillus collinoides* and *Lactobacillus hilgardii* strains (Walling et al. 2005b).

More recently, *O. oeni* was shown to produce EPS, independently of the ropy phenotype of the strain studied (Ibarburu et al. 2007; Dols-Lafargue et al. 2007,

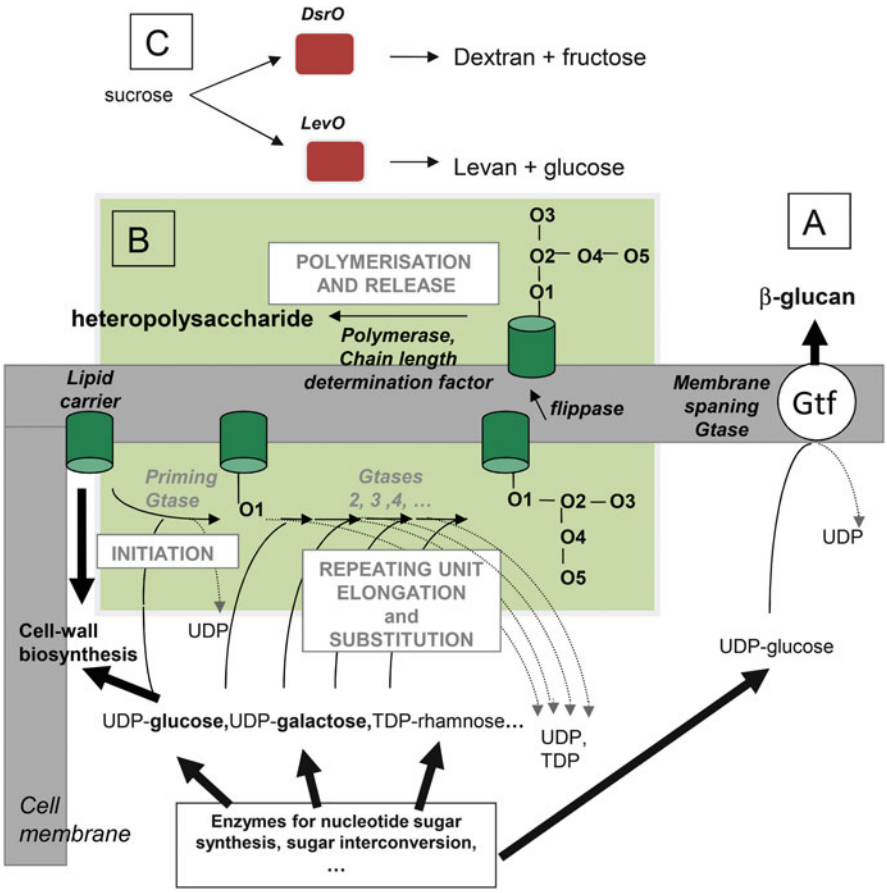

Fig. 12.5 Schematic representation of heteropolysaccharide biosynthesis by lactic acid bacteria. *O* osyl (e.g. glucosyl, rhamnosyl, galactosyl, etc.), *Gtase* glycosyltransferase [Adapted from Dimopoulou et al. (2012)]

2008; Ciezack et al. 2010; Dimopoulou et al. 2012, 2014). The EPS molecular weight distribution and chemical structure show that most strains produce a mixture of PS. With glucose as sole carbon source in the growth medium, the amounts of soluble PS recovered are low but significant. More than 75% of the studied strains produce heteropolysaccharides, made of glucose galactose and rhamnose, in varying proportions depending on the strain. These polymers are found in either a free or capsular form, but do not induce ropiness (Ibarburu et al. 2007; Dimopoulou et al. 2012, 2014). The capsule is dense but very thin, as shown in Fig. 12.5b. Some strains also produce the same β-1,3-β-1,2 glucan as *P. parvulus*, in either a free or a capsular form, and clearly display the ropy phenotype (Ibarburu et al. 2007; Dols-Lafargue et al. 2008; Dimopoulou et al. 2014). Moreover, with glucose and sucrose in growth medium, most *O. oeni* strains produce high amounts of soluble dextran

(>500 mg l^{-1}) and some strains also produce soluble levan (>1000 mg l^{-1}). Dextran is a glucose homopolymer with α-1,6-linked residues (95%) and some α-1,3-linked branched residues (5%), while levan is a β-2,6 fructan. None of these two polymers induce any obvious viscosity change in *O. oeni* growth media (Dimopoulou et al. 2012, 2014).

Furthermore, several *Leuconostoc mesenteroides* strains isolated from wine produce both dextrans and fructans in model media (Montersino et al. 2008).

12.4.2 Biosynthetic Pathways and Associated Genes

In *P. parvulus*, a single glucosyltransferase gene *(gtf)* is associated with β-glucan synthesis (Walling et al. 2005b; Werning et al. 2008). It codes a 567 amino-acid, 65 kDa protein (Gtf). The cloned *gtf* gene of *P. parvulus* expressed in *S. pneumoniae or L. lactis* produces a functional transmembrane Gtf which subsequently synthesises β-glucan (Werning et al. 2008; Dols-Lafargue et al. 2008). The role of Gtf in ropiness is thus clearly demonstrated. Gtf, like the glucosyltransferase of *S. pneumoniae* type 37, is a bifunctional transmembrane protein belonging to GT-2 family (www.cazy.org). It catalyses the synthesis of two distinct osidic bonds, as well as the export of the polymer (Fig. 12.6a) (Llull et al. 2001; Walling 2003; Werning et al. 2008).

In *O. oeni*, several complementary EPS biosynthetic pathways are active. Two have been characterised:

1. The glucan synthase pathway (Gtf), involved in ropy β-glucan synthesis from UDP-glucose (Fig. 12.6a) (Dols-Lafargue et al. 2008; Dimopoulou et al. 2014).
2. A Wzy-dependent synthetic pathway, resulting in production of heteropolysaccharides made of glucose, galactose and rhamnose from sugar nucleotides which originate in the central metabolic pathways (Fig. 12.6b). The repeating unit is assembled on a lipid carrier molecule, anchored in the cytoplasmic membrane. The first monomer is linked to the lipid carrier by the priming glycosyltransferase. Then, the following monomers are linked by other specific glycosyltransferases. Each glycosyltransferase uses the energy of the UDP-osyl bond to transfer the osyl to the growing repeating unit, forming in turn a specific osidic bond. After completion, the resulting repeating unit is assumed to be exported and polymerised on the outer face of the cell membrane. The lipid carrier is externalised by a flippase, and the repeating unit is added to the non-reducing end of the growing PS chain by a polymerase. A chain length determination factor may limit the extension of the molecule. This pathway is similar to that described in *Pneumococci* or in milk lactic bacteria (Dimopoulou et al. 2012, 2014).
3. The last pathway consists of homopolysaccharide synthesis from sucrose (α-glucan or β-fructan) thanks to glycoside hydrolases of the GH-70 (dextransucrase, DsrO) and GH-68 (levansucrase, LevO) families (Fig. 12.6c) (Dimopoulou et al. 2014).

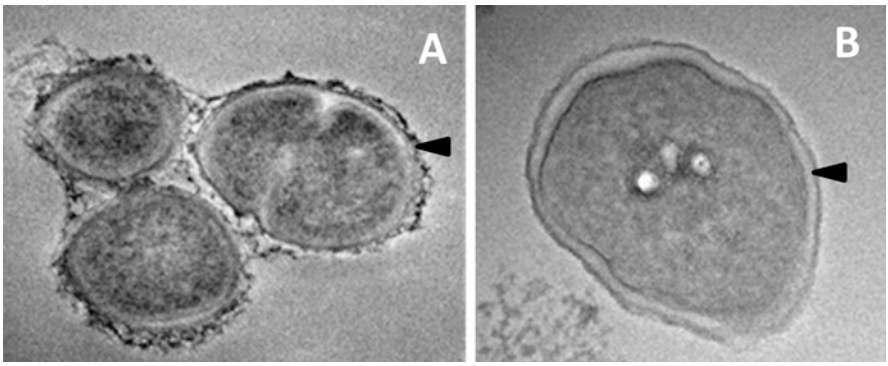

Fig. 12.6 Visualisation of PS capsules by transmission electron microscopy. (**a**) The β-glucan network around *P. parvulus* IOEB_8801 cells. (**b**) The thin PS layer around *O. oeni* IOEB_0607 cells (Adapted from Coulon et al. (2012) and Dimopoulou et al. (2014))

In *O. oeni*, all the genes dedicated to PS synthesis are located on the chromosome, and all strains studied display several *eps* genes. Most of them are inserted into two complex gene clusters named *eps1* and *eps2*. The composition of the *eps* gene clusters diverges from one strain to another and *eps2* is highly truncated or absent in specific strains. Other *eps* genes are spread over the chromosome: three glycoside hydrolase genes named *dsrO*, *dsrV* and *levO* and three glycosyltransferase genes named *gtf*, *it3* and *it4*. These last six genes are present or absent depending on the strain. Truncated genes or clusters are also found in some strains (Dimopoulou et al. 2014).

Analysis of sequences surrounding the *eps* genes and the *eps* gene distribution among distinct wine bacterial species and among distant strains in a same species (see Chap. 19 for *O. oeni*) brings some information on the mode of acquisition and mobility of the genes. More than 20% of the *Pediococcus* analysed by Garai-Ibabe et al. (2010), 20% of the *O. oeni* analysed by Dols-Lafargue et al. (2008) and 43% of *O. oeni* strains isolated from Champagne region (France) by Dimopoulou et al. (2016) display the *gtf* gene. In *O. oeni* strains originating from Champagne, *gtf* is located in a phage remnant (Dimopoulou et al. 2014, 2016). However, in a red wine *O. oeni* strain, the gene is inserted in a prophage of distinct origin, in another region of the chromosome. In red wine *Pediococcus*, the *gtf* gene is located on a 5.5 kb plasmid, on another 5.5 kb plasmid in *Lb. diolivorans* strains and on a 35 kb plasmid in cider *Pediococcus* (Gindreau et al. 2001; Werning et al. 2006). It displays over 98% identity from one bacterial species to another (Dols-Lafargue et al. 2008). The gene *gtf* is thus a mobile gene, via either phages or plasmids.

On the other hand, the *eps* gene clusters *eps1* and *eps2* of *O. oeni* display a mosaic structure. They are quite conserved in their 5′ end and more divergent in their 3′ end. Gene by gene, they have similarities with *eps* gene clusters found in bacteria isolated in very different ecological niches, and their mode of acquisition remains unclear (Dimopoulou et al. 2014).

12.4.3 Physiology of PS Release and Benefits for the Bacteria

All *O. oeni* strains studied so far have several genes dedicated to EPS metabolism. This suggests that these polymers are significant for the adaptation of *O. oeni* to its ecological niche and possibly contribute to the technological performance of malolactic starters. The same may apply to other wine lactic acid bacteria species.

In *O. oeni*, the exopolysaccharide production can be stimulated by changing the growth medium composition (Ciezack et al. 2010). Moreover, as previously stated, addition of sucrose to the growth medium may modify the biosynthetic pathway and final polymer structure (Dimopoulou et al. 2012). All the *Pediococcus* strains studied produce larger amounts of β-glucan when grown on glucose rather than other carbon sources, up to 140–200 mg l^{-1} β-glucan. Depending on the strain, β-glucan is also produced with fructose, maltose, galactose, xylose and arabinose as carbon source. It can be stimulated by adding malic acid or ethanol to the growth medium. β-glucan production is not directly linked to cell growth. However, an efficient preliminary growth phase is essential for subsequent "large-scale" EPS production. Agitation and aeration are detrimental (Llaubères 1987; Lonvaud-Funel and Joyeux 1988; Duenas et al. 2003; Walling et al. 2005a; Velasco et al. 2006, 2007).

Most of the EPS are not consumed by the bacteria that produce them and do not constitute external carbon sources (Walling et al. 2005a; Dols-Lafargue et al. 2008; Dimopoulou et al. 2012). Their biological role is probably to overcome stress commonly encountered in wine (Spano and Massa 2006; Dols-Lafargue et al. 2008; Dimopoulou et al. 2016; Caggianiello et al. 2016). Actually, the β-glucan capsule ensures resistance of ropy strains to SO_2, ethanol and low pH (Lonvaud-Funel and Joyeux 1988; Lonvaud-Funel et al. 1993; Walling et al. 2005a, b; Dols-Lafargue et al. 2008; Caggianiello et al. 2016) but also to lysozyme (Coulon et al. 2012). The β-glucan is supposed to enhance bacteria survival in the gut of insects or animals and hence contributes to dissemination of the bacteria present on fruits (Fernandez de Palencia et al. 2009; Stack et al. 2010; Deutsch et al. 2012). This polymer also modulates cell adhesion to biotic and abiotic surfaces (Dols-Lafargue et al. 2008; Fernandez de Palencia et al. 2009; Stack et al. 2010; Blättel et al. 2011). The heteropolysaccharidic capsule and the dextran released increase *O. oeni* resistance to cold shock, low pH or freeze-drying (Dimopoulou et al. 2016). Furthermore, *O. oeni* EPS may contribute to biofilm formation on grapes and winemaking equipment (Bastard et al. 2016). These biofilms are known to favour cell survival under extreme conditions, as well as genetic exchanges between species (Mah and O'Toole 2001). As a result, EPS production should contribute to the diversification of PS structures.

12.4.4 Impact on Wine Quality and Winemaking Practices

Ropiness due to beta-glucan production occurs all over the world in red and white wines, as well as beer and cider. The most frequently incriminated species is *P. parvulus*. Ropiness due to *O. oeni* in wine is not clearly reported, though it occurs in model growth media. High viscosity is sometimes reported during winemaking, in tanks or in barrels. At these stages, β-glucan is often produced by *Pediococcus* and 20 mg l^{-1} may be sufficient to spoil the wine (Lonvaud-Funel and Joyeux 1988). But afterwards, ropiness is easily decreased during the following winemaking steps like racking without any damage for the wine. The problem is when spoilage occurs later in bottles. Even if glucan has no impact on human health and has no specific taste, the wine's viscosity makes it impossible to market. Wine can be reconditioned after being agitated to reduce the viscosity and properly treated for its microbial stabilisation, especially for elimination of ropy bacteria (Ribéreau-Gayon et al. 2000). However, these are highly resistant to sulphur dioxide (Dols-Lafargue et al. 2008). It is the reason why bacterial detection and preventive treatment prior to the development of high population levels and the formation of ropiness are more appropriate. PCR-based methods were therefore developed to detect the presence of the *gtf* gene in wine microflora, as early as possible in the winemaking process (Gindreau et al. 2001; Delaherche et al. 2004; Walling et al. 2005b; Werning et al. 2006; Ibarburu et al. 2010). Then a well management of wine fermentations or ageing is generally sufficient to avoid the product alteration. Methods complementary to sulphuring like lysozyme treatment or beta-glucanases (Blättel et al. 2011; Coulon et al. 2012) have been proposed.

Conversely, the protective role of either cell-linked heteropolysaccharides or dextrans produced by *O. oeni* is demonstrated during freeze-drying or inoculation in wine and may be exploited in the future to produce even more resistant malolactic starters (Dimopoulou et al. 2016).

Furthermore, the soluble PS released by *gtf* negative *O. oeni* strains may interact with many wine molecules and contribute to the positive impact of MLF on wine quality. In addition, thanks to the EPS produced, *O. oeni* biofilm can develop on oak. This biofilm was shown to drive MLF more efficiently than free cells and it modulated the wood–wine transfer of volatile aromatic compounds during MLF and ageing by decreasing furfural, guaiacol and eugenol (Bastard et al. 2016).

12.5 Conclusion

All the microorganisms on grapes, in must and in wine produce exocellular PS. Acetic bacteria were not considered in this chapter, as their EPS-producing abilities have never been studied for wine strains. However, studies of strains of other origin suggest that the situation is as complex as that of lactic acid bacteria (Jansson et al. 1993; Geremia et al. 1999; Ua-Arak et al. 2016).

Whatever the species, some of the microbial PS remain attached to the cell, forming a capsule, which constitutes a protection to environmental constraints, especially in the final stages in winemaking. The remainder of the PS is released into the surrounding medium. Depending on the PS structure, on the species involved and on the winemaking stage, this may be neutral, beneficial or detrimental to wine quality and/or subsequent growth of other species (Lonvaud Funel 1999). Genetic exchanges between species are probably responsible for the present high diversity of microbial PS structure, particularly in bacteria. As a result, microbial PS remain important research topics in wine microbial ecology.

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