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ORIGINAL PAPER

Evaluation of the antimicrobial activity of bacteriocin-like inhibitory substances of enological importance produced by *Oenococcus oeni* isolated from wine

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Abstract
We experimentally determined that bacteriocin-like inhibitory substances synthetized by *Oenococcus oeni* may act as a natural preservative and may thus be of importance for the wine industry. An *O. oeni* strain isolated from wine was tested for antimicrobial activity against ten indicator lactic acid wine spoilage bacteria of enological origin from the genera *Lactobacillus, Leuconostoc*, and *Pediococcus*. A strong competition effect of *O. oeni* against all the tested indicator bacteria was noted. The antimicrobial extracellular metabolites were produced at the end of *O. oeni's* exponential growth phase and the maximum activity occurred between hours 15 and 20 of cultivation. The bioactive compounds synthetized by *O. oeni* were found to be strain-specific. Their bactericidal activity was stable at high temperatures (100 °C, 20 min) and in low pH environments (with the greatest activity at pH 2–8), but was totally degraded by proteolytic enzymes (pronase E, proteinase K, trypsin, pepsin, and α-chymotrypsin). They can thus be classified as heat-resistant proteins active at low pH. The dynamics of the biosynthesis and their activity were temperature-dependent, being best at 20 °C. Our results indicate that the use of an *O. oeni* starter culture with the appropriate antimicrobial potential may be a useful tool in winemaking procedures to control microbial stability and inhibit wine spoilage.

Keywords Malolactic bacteria · Wine microflora · *Oenococcus oeni* · Bacteriocin-like inhibitory substances

Introduction

Oenococcus oeni is Gram-positive, heterofermentative bacteria commonly used in winemaking. The strains perform malolactic fermentation (MLF) and are currently used in commercial starter cultures for controlled malolactic fermentation. This specific process of secondary fermentation takes place after the alcoholic fermentation and involves the bioconversion of malic acid into lactic acid. Malic acid is a dicarboxylic acid, responsible for the enhanced total acidity of grape musts which contributes to the sourness of wine. Lactic acid is a monocarboxylic acid, that is weaker than malic acid, and which possesses a smoother and lighter acidity. Malolactic bioconversion thus reduces the wine's total acidity (by about 1–3 g/L calculated as tartaric acid) and increases its pH (by about 0.1–0.2). Malolactic fermentation also modifies wine's aroma, reducing vegetal and herbaceous aromas while enhancing fruity aromas. Furthermore, the body and mouthfeel of the wine is also improved giving a longer aftertaste [1–5].

MLF also contributes to the microbial stability of wines. Malolactic bacteria (MLB) use up residual sugars left by yeast after alcoholic fermentation, and they also metabolize malic acid, which could also act as a carbon source for spoilage microflora. Additionally, malolactic bacteria, as lactic acid bacteria (LAB), are able to biosynthesize bacteriocins. These are low-molecular peptide compounds with antimicrobial properties secreted by some bacteria to inhibit the growth and activity of competing microorganisms [6, 7]. At present, the bacteriocins produced by LAB are perceived as

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very desirable natural food preservatives. Most LABs have been granted a qualified presumption of safety (QPS) and the bacteriocins produced by these bacteria may serve as beneficial alternatives to chemical food preservatives.

The indigenous microflora of malolactic fermentation includes bacteria of the genera: *Lactobacillus, Pediococcus, Leuconostoc* and *Oenococcus*. Some of these have already been described as synthetizing compounds with bacteriocinic properties. *Lactobacillus plantarum* is the best-known strain of enological origin that can produce bacteriocin—in this case, various types of plantaricin: plantaricin C [8], plantaricin W [9], plantaricin C19 [10], plantaricin 423 [11, 12], plantaricin EF and JK [13, 14], plantaricin S [15], plantaricin NC8 [16], plantaricin F [17], and plantaricin 1.25α [18]. *Pediococcus pentosaceus*, which was isolated from wine [19], as well as *Leuconostoc mesenteroides* subsp. *cremoris* [20] have also been found to produce some inhibitory compounds. However, all these are strains of malolactic bacteria involved in spontaneous processes, which entail a significant risk of wine spoilage through the overproduction of undesirable metabolites, such as acids, flavour compounds, acetaldehyde, biogenic amines, ethyl carbamate [21–24]. For a controlled, dynamic and safe malolactic process, *Oenococcus oeni* strains are therefore often advised in the wine industry. It is thus of significant importance to know whether this microorganism can also produce antimicrobial compounds. The selection of such strains for industrial use could help in controlling microbial activity and differentiation during the vinification process.

To our knowledge, only one research group [25] testing for the presence of bacteriocin-encoding genes in lactic acid bacteria examined strains of *Oenococcus oeni*. We have not found any research directly related to the biosynthesis of antimicrobial compounds by either *Oenococcus oeni* isolated from wine or by commercial starter cultures that include *O. oeni*. The objective of our study was thus to find and characterize the bacteriocin-like inhibitory substances (BLIS) synthetized by an *O. oeni* wine isolate. The activity of the bioactive metabolites synthetized by *O. oeni* was evaluated against ten indicator bacteria of enological origin belonging to wine spoilage MLB of the genera *Lactobacillus, Leuconostoc*, and *Pediococcus*.

Materials and methods

Microorganisms

One isolate of *Oenococcus oeni* was tested for synthesis of bacteriocin-like inhibitory substances (BLIS). Ten other strains of malolactic bacteria isolated from wine were used in the experiments as indicator microorganisms. All the strains, listed in the Table 1, are of enological origin and belong to the Spanish Culture Type Collection (CECT). The strains were stored in a Cryobank (Bacteria storage system, MAST Diagnostica) at − 20 °C. Prior to the tests, the strains were defrosted and passaged twice in MRS broth medium under anaerobic conditions (Anaxomat, temp. 30 °C, 24 h).

Supernatants

A 2% v/v inoculum of *Oenococcus oeni* CECT 217T was used and cultures were carried out in MRS broth medium at pH 6.2 under anaerobic conditions (Anaxomat), without pH regulation at 30 °C for 20 h. The supernatants we obtained after centrifugation (5500 g, 10 min) were analyzed for their antibacterial activity against ten indicator microorganisms. Two types of supernatants were tested: (1) native supernatant (directly after centrifugation), and (2) treated supernatant (after pH neutralisation and catalase treatment). The native supernatant was applied as indicator of the activity of the total extracellular metabolites synthetized by the tested strain. The treated supernatant was neutralized to pH 6.5 using 1 N NaOH, to eliminate the antimicrobial activity of the organic acids, and was treated with 68,000 IU/ml catalase to degrade hydrogen peroxide. Both types of supernatants were evaluated separately.

Table 1 Bacteria wine isolates used in this study

Oenococcus oeni CECT 217T	Indicator microorganisms: *Lactobacillus hilgardii* CECT 4786T *Lactobacillus hilgardii* CECT 4681 *Lactobacillus brevis* CECT 4121T *Lactobacillus brevis* CECT 216 *Lactobacillus buchneri* CECT 4111T *Lactobacillus buchneri* CECT 4674 *Leuconostoc mesenteroides* CECT 219T *Leuconostoc mesenteroides* CECT 394 *Pediococcus pentosaceus* CECT 4695T *Pediococcus pentosaceus* CECT 923

Evaluation of antimicrobial activity

Inhibition zones evaluation Twenty microliter samples of liquid cultures of *Oenococcus oeni CECT* 217T (including live cells) or their supernatants (with no live cells) were plotted on Petri dishes with MRS agar medium (1% agar) inoculated with 10^6 cfu/ml of indicator malolactic bacteria isolates. The plates were incubated under anaerobic conditions at 30 °C for 14 h. After incubation, the inhibition zones were evaluated by a computer scanning system (MultiScan-Base v14.02).

Critical dilutions method The supernatants were diluted twice using 100 mM phosphate buffer with pH 6.5. The diluted samples were then plotted on Petri dishes with MRS agar medium (1% agar) inoculated with 10^6 cfu/ml of indicator malolactic bacteria isolates. The plates were incubated under anaerobic conditions at 37 °C for 18–20 h. Antimicrobial activity was expressed in AU/ml (the reciprocal of the highest dilution of the sample) which give clear inhibition zones (those zones with diameters over 2 mm).

Physiochemical characteristics of the synthetized BLIS

The supernatants with the highest bacteriostatic activity were treated with enzymes, temperature, and pH to evaluate their physiochemical nature.

For enzymes' treatment The supernatants were incubated for 2 h with the selected enzymes (each of the enzyme separately) such as pronase E, proteinase K, pepsin, trypsin, α-chymotrypsin, α-amylase, lysozyme, catalase and lipase (Sigma). The applied concentration of the tested enzymes was 1 mg/ml. After incubation, the antibacterial activity of the supernatants was evaluated.

For temperature treatment The supernatants were heated at 80 and 100 °C for 10, 20 and 30 min and at 121 °C for 15 min. After incubation, the antibacterial activity of the supernatants was evaluated.

For pH treatment The supernatants' pH was adjusted from 2.0 to 10.0 ($\Delta = 0.5$) using 1 N NaOH and 1 N HCl. This was incubated at room temperature for 2 h. After incubation, the pH of the samples was regulated back to 6.5 and the antibacterial activity of the supernatants was evaluated.

After these treatments, the antibacterial activities of the supernatants were evaluated using the critical dilution method and expressed as AU/ml.

Dynamics of BLIS biosynthesis

The MRS broth medium was inoculated with 2% (v/v) of overnight *O. oeni* culture. The incubation was conducted without pH regulation at temperatures of 10, 20, 30, and 37 °C for 60 h. During incubation, at 5, 10, 15, 20, 25, 30, 35, and 60 h, samples were taken to evaluate antimicrobial activity using the critical dilution method. The results were expressed as AU/ml.

Influence of BLIS on *Pediococcus pentosaceus* growth

Having undergone *O. oeni* incubation under anaerobic conditions (30 °C, 24 h), the supernatants were centrifuged (5500 g, 10 min), and incubated at 80 °C for 10 min and sterilized by membrane filters (Millex GV, 0.22 µm, Millipore) to eliminate the activity of proteolytic enzymes and remove the remaining cells. We refer to these supernatants as active extracts (AE). MRS broth medium was inoculated with 10^3 cfu/ml of *Pediococcus pentosaceus* CECT 4695T and incubated at 30 °C for 4 h. Then, 200, 400, and 800 AU/ml of AE were introduced into the growing culture, which was incubated for a further 20 h. Every 4 h, samples were evaluated for the number of *P. pentosaceus* bacteria (as cfu/mL) during growth in presence of the BLIS. A culture without BLIS (0 AU/ml) was monitored as a control.

Human/animal rights

This article does not contain any studies with human or animal subjects.

Results and discussion

Antimicrobial activity of *Oenococcus oeni* isolate

To date, a few studies have been carried out on the antimicrobial compounds synthetized by bacteria of enological origin and have presented their spectrum of inhibition activity, like diverse bacteriostatic potential against different indicator strains from the group of wine or general food spoilage bacteria [2, 11, 17, 19, 20, 26, 27]. However, *O. oeni* strains have been used in these studies only as indicator microorganisms showing differential sensitivity to these bactericidal metabolites, and not as a producer. In our study, *O. oeni*, being the most important microorganism in the malolactic process, was evaluated for its production of antimicrobial substances including BLIS productions.

Evaluation of the antimicrobial activity of extracellular metabolites was performed using liquid medium that had undergone cultivation with *Oenococcus oeni* CECT 217T (the medium containing live *O. oeni* cells). This was done to estimate the antagonistic potential of the *O. oeni* strain and its metabolites against ten indicator malolactic bacteria also isolated from wine. The indicator microorganisms (bacteria of the genera *Lactobacillus, Leuconostoc*, and *Pediococcus)* were chosen as elements of the native wine flora, that take part in spontaneous malolactic fermentation and which

potentially present the dangers of wine spoilage. In all cases, intense inhibition zones (≥ 10 mm) were observed, with the highest bactericidal activity (a 14 mm inhibition zone) noted against *Leuconostoc mesenteroides* and *Pediococcus pentosaceus* strains (Table 2).

Table 2 Antimicrobial activity of *Oenococcus oeni* CECT 217T and their exogenous metabolites against ten indicator malolactic bacteria

Indicator microorganisms	Liquid *O. oeni* culture	Native supernatants	Treated supernatants (pH 6.5, catalase treatment)
Lactobacillus hilgardii	+	−	−
Lactobacillus hilgardii	+	−	−
CECT 4786T	+	±	−
Lactobacillus hilgardii	+	±	−
Lactobacillus hilgardii	+	−	−
CECT 4681	+	−	−
Lactobacillus brevis CECT	++	+	±
Lactobacillus brevis CECT	++	++	+
4121T	++	++	+
Lactobacillus brevis CECT	++	+	±
216			
Lactobacillus buchneri			
CECT 4111T			
Lactobacillus buchneri			
CECT 4674			
Leuconostoc mesenteroides			
CECT 219T			
Leuconostoc mesenteroides			
CECT 394			
Pediococcus pentosaceus			
CECT 4695T			
Pediococcus pentosaceus			
CECT 923			

– no antagonistic activity, ± weak bacteriostatic activity (inhibition zone with dia < 10 mm), + strong bacteriostatic activity (inhibition zone with dia 10–14 mm), ++ very strong bacteriostatic activity (inhibition zone with dia > 14 mm)

To identify the antimicrobial substances as exogenous metabolites released by the *O. oeni* isolate, the bacterial biomass was separated and native, cell-free supernatants were analyzed. Generally, after removing the alive *O. oeni* cells, the inhibition activity registered was significantly lower. *Leuconostoc mesenteroides* and *Pediococcus pentosaceus* remained notably sensitive to the exogenous metabolites active in the supernatants (with inhibition zones of ≥ 10 mm). Weak growth inhibition was observed for *Lactobacillus brevis*. No antagonistic properties of extracellular metabolites were observed against *Lactobacillus hilgardii* and *Lactobacillus buchneri* (Table 2).

Next, the native supernatants were neutralized (to eliminate the antimicrobial effect of organic acids) and treated with catalase (to degrade hydrogen peroxide). This additional treatment was necessary to ensure that no lactic acid or H_2O_2 (LAB metabolites with inhibitory properties) affected the growth of the indicator microorganism [28]. Evaluation of these supernatants showed that there were some BLIS active against *Leuconostoc mesenteroides* and *Pediococcus pentosaceus* among the bioactive metabolites synthetized by *O. oeni* isolate (Table 3).

The presence of live *O. oeni* cells caused strong inhibition in the growth of all the indicator microorganisms. This suggests a strong competition effect and implies that, during malolactic fermentation, *O. oeni* has great potential to maintain prevalence and to control the process. Besides, some of the extracellular metabolites can cleave to the microbial cell wall and in this case they could be removed together with the alive cells. However, when evaluating the native supernatants without the live *O. oeni* cells which can serve as a model of the storage and maturation time that follows malolactic fermentation, the antimicrobial activity was found to be strain-dependent and had a significantly lower intensity (Table 2).

Next, the dynamic of the exogenous biosynthesis of metabolites by *O. oeni CECT* 217T, as well as the maximum activity of these antimicrobial compounds, were

Table 3 Maximal activity of exogenous metabolites of *Oenococcus oeni* CECT 217T against selected malolactic bacteria isolates

Selected indicator MLB isolates	All exogenous metabolites		BLIS	
	Max activity (AU/ml)	Cultivation time (h)	Max activity (AU/ml)	Cultivation time (h)
Lactobacillus brevis CECT 4121T	200	15	–	–
Lactobacillus brevis CECT 216	200	15	–	–
Leuconostoc mesenteroides CECT 219T	800	15	200	15
Leuconostoc mesenteroides CECT 394	1600	15	800	15
Pediococcus pentosaceus CECT 4695T	3200	20	1600	20
Pediococcus pentosaceus CECT 923	1600	15	200	15

considered. We observed that the metabolites are produced at the end of *O. oeni's* exponential growth phase and that the maximum activity fell between hours 15 and 20 of cultivation (Table 3). This observation is in line with those of other authors, who showed that biosynthesis of bacteriocins begins at the end of the exponential phase [19, 26, 27, 29, 30] or at the beginning of the stationary phase [17, 19] of the producer's growth.

The maximum antimicrobial activity of the secreted metabolites was strain-specific and ranged from 200 to 3200 AU/ml. The highest bactericidal activity was observed against *Pediococcus pentosaceus* CECT 4695T (Table 3). The BLIS synthetized by *O. oeni* isolate were also characterized with high specific activities. The antagonistic properties of 800 and 1600 AU/ml were shown against *Leuconostoc mesenteroides* CECT 394 and *Pediococcus pentosaceus* CECT 4695T, respectively (Table 3).

To date, only one research group has presented data on the antimicrobial activity of *O. oeni* strains. Knoll et al. [25] screened a broad spectrum of lactic acid bacteria of enological origin for bacteriocin-encoding genes. Among the strains they evaluated were strains of *O. oeni* (wine isolates and commercial starter cultures), which were found to be able to inhibit wine-associated bacteria such as *Lactobacillus hilgardii, Lactobacillus plantarum, Lactobacillus brevis, Lactobacillus paracasei, Lactobacillu pentosus, Lactobacillus fermentum, Lactococcus mesenteroides, Pediococcus pentosaceus*, as well as *Listeria monocytogenes* and *Enterococcus faecalis*. The inhibiton zones were in the range of 1–5 mm in a colony-overlay test. However, this very useful report focused on the genomic site of bacteriocin production mechanisms, and not on the characteristics of these active metabolites.

Physiochemical characteristics of the synthetized BLIS

A high sensitivity of different bacteriocin substances to proteolytic enzymes has been noted by researchers evaluating their physiochemical properties [19, 26, 27]. The active metabolites produced in our study by *Oenococcus oeni* were also sensitive to proteolytic enzymes, with their complete inactivation being observed after treatment with pronase E, proteinase K, trypsin, pepsin and α-chymotrypsin. On the other hand, no changes were noted in antimicrobial activity when other enzymes (such as α-amylase, lysozyme, catalase, and lipase) were applied (Table 4). This may indicate that the antimicrobial activity of the tested *O. oeni* metabolites is relevant to the proteinaceous part of the bacteriocin molecules. Rojo-Bezares et al. [27] and Navarro et al. [26] also studied the possibility of using lysozyme in the microbial control of malolactic process and to increase the microbial stability of wine. The active metabolites they investigated, like those produced in our study, remained stable when treated with lysozyme, which clearly shows that the combination of antimicrobial enzyme (lysozyme) and antimicrobial metabolites synthetized by bacteria of enological origin, especially *O. oeni*, may be useful in vinification procedures for controlling malolactic fermentation in wineries, and also as a method of reducing sulfitation dosages.

Table 4 Influence of enzyme, temperature and pH on the antimicrobial activity of bacteriocin-like inhibitory metabolites of *Oenococcus oeni* CECT 217T

Treatment	Activity (AU/ml)[a]
Before treatment	1600
Enzymes	0
Pronase E	0
Proteinase K	0
Trypsin	0
Pepsin	0
α-chymotrypsin	1600
α-amylase	1600
Lysozyme	1600
Catalase	1600
Lipase	
Heating	1600
10 min. 80 °C	1600
20 min. 80 °C	1600
30 min. 80 °C	1600
10 min. 100 °C	1600
20 min. 100 °C	800
30 min. 100 °C	0
15 min. 121 °C	
pH	1600
2.0–8.0 (Δ = 0.5)	400
8.5–9.5	200
10.0	

[a]Activity evaluated by critical dilution method against *Pediococcus pentosaceus* CECT 4695T

We next tested the antimicrobial activity under different heating regimes. The bioactive metabolites of *O. oeni* showed good resistance to increased temperatures. Conditions such as 100 °C for 20 min did not alter the antibacterial potential of the tested metabolites. Longer exposure (100 °C for 30 min) significantly decreased metabolite activity from 1600 to 800 AU/ml. Complete loss of antimicrobial activity was observed after 15 min at 121 °C (Table 4). This relatively high thermostability (for proteinaceous compounds) of bacteriocinic substances may arise from their low-complexity structure (perhaps due to the absence of a tertiary structure) or from the presence of covalent bonds that help stabilize the protein globular structure [26, 31].

The bioactive compounds we examined, like most bacteriocins and bacteriocin-like substances described in the literature, were stable over a wide pH range. After 2 h of incubation in environments with pH values ranging from 2.0 to 8.0, no changes in the antibacterial activity were detected.

The metabolites lost their bacteriostatic properties at pH values above 8.0. At pH 10, only very low activity was seen (200 AU/ml) (Table 4).

After these tests, it can be posited that the bioactive metabolites synthetized by *Oenococcus oeni* CECT 217T are heat-resistant proteins with their highest activity at low pH values. Very similar properties, such as high protease sensitivity, thermostability, thermoresistance and higher activity at lower pH values, have been also reported by authors studying other bacteriocins, such as pediocin PA-1 [32], pediocin N5p [19], brevicin 37, caseicin 80 and lactarin F [33, 34], plantaricin A [35], plantaricin S and T [30], plantaricin C [36], plantaricin D [37], plantaricin F [38], and others [19, 20, 26, 27, 31]. Navarro et al. [26] and Rojo-Bezares [27] have additionally stated that bacteriocin activity remained stable at storage temperatures (4 and − 20 °C), and following treatment with organic solvents, such as chloroform and ethanol.

Dynamics of BLIS biosynthesis at different temperatures

The efficiency of BLIS biosynthesis was monitored at temperatures ranging from 10 to 37 °C. Bacteriocin-like inhibitory substances were synthetized by the *O. oeni* isolate at all tested temperatures, but with the effectiveness of biosynthesis strongly depending on the temperature (Fig. 1). The highest biosynthesis dynamics and the greatest activity on the part of the metabolites produced were seen at 20 °C. The highest activity (3200 AU/ml) was noted from hour 20 of cultivation onwards, and did not change until hour 60. The lowest level of synthesis was recorded at 37 °C (100–200 AU/ml). It was also observed that the incubation temperature determined the stability of the bacteriocin-like metabolites that we tested. At 10 and 20 °C, the activity peaked in hour 20 and thereafter maintained a stable level. On the other hand, a gradual decrease in bactericidal activity was seen at higher temperatures (Fig. 1).

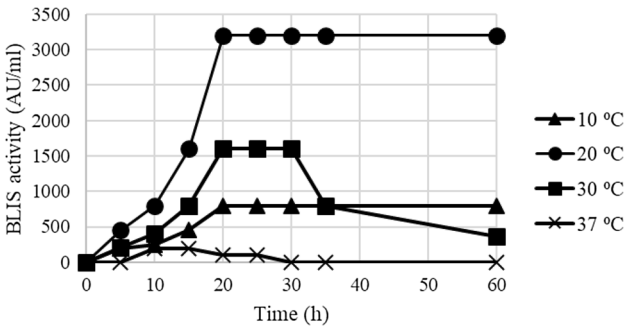

Fig. 1 Dynamics of BLIS biosynthesis at different temperatures; indicator strain: *Pediococcus pentosaceus* CECT 4695T

Navarro et al. [26] evaluated the dynamics of bacteriocin production by *Lactobacillus plantarum* at 25 and 30 °C. In that study, the temperature also affected the rate of bacteriocin biosynthesis. The detectable antimicrobial activity was noted to be faster when synthetized at 30 °C. The beginning of the biosynthesis process was observed at the end of the exponential phase and the beginning of the stationary phase and the activity remained stable for the following next 10 h.

Effect of BLIS on the growth of *Pediococcus pentosaceus*

To evaluate the effect of BLIS, an indicator microorganism, *Pediococcus pentosaceus* CECT 4695T was cultivated in MRS medium for 4 h and thereon 200, 400, and 800 AU/ml of BLIS active extracts were added to the growing culture. It was observed that the addition of the BLIS to the culture environment significantly altered the growth dynamic of the bacteria. Even the lowest dosage of BLIS (200AU/ml) resulted in a significant decrease in the growth dynamics of *Pediococcus pentosaceus* (Fig. 2).

The application of 400–800 AU/ml of BLIS led to a reduction of 1–2 log units during first four hours of incubation, and afterwards halted the growth of these bacteria, keeping their numbers stable. Similarly, a significant decline in the growth curve was recorded when the BLIS synthetized by *Leuconostoc mesenteroides* subsp. *cremoris* was added to the growth medium of *Lactobacillus delbrueckii* [20].

Conclusion

In recent years in the wine industry, special attention has been paid to the need for alternative preservatives to replace the traditional sulfitation procedure. Some alternatives have been investigated, and there have been indication that it might be possible to use natural antimicrobial agents such

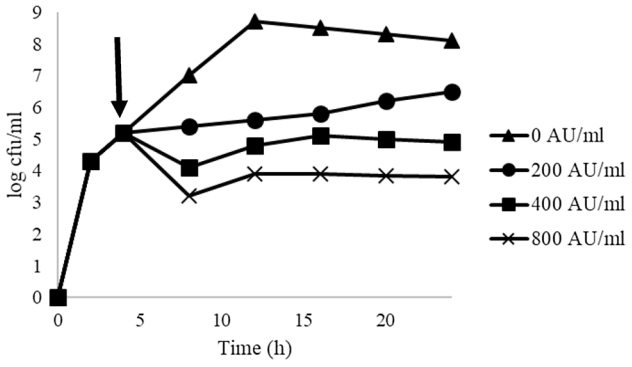

Fig. 2 Influence of BLIS on *Pediococcus pentosaceus* CECT 4695T growth (arrow indicate the time of AE addition, 0 AU/ml—without BLIS addition, as a control culture)

as lysozyme, nisin, lacticin, polyphenols, or bovine lactoferrin [26, 29, 39–41]. As products of natural origin, these are perceived as much more "friendly" than sulfities, but they nonetheless remain materials added to the process, and thus classified as food additives. They have also been found to inhibit not only unfavorable wine microflora but also the growth and activity of *O. oeni*—bacteria essential to the successful malolactic process.

In our study, we found that *O. oeni* itself is capable of synthetizing certain protein compounds with antimicrobial activity, these can be classified as bacteriocin-like inhibitory substances. This is the first study to present and evaluate the BLIS synthetized by the *O.oeni* wine isolate, so further investigations should be undertaken to screen other strains and to assess their growth inhibitory activities, which may be of enological importance under winemaking conditions.

The use of a bacteriocin-producing *O. oeni* starter culture may prove to be a good enological practice for the wine industry. Our promising results suggest that the use of an *O. oeni* starter culture with the appropriate antimicrobial potential may not only help malolactic bioconversion to succeed without undesirable metabolites, but may also maintain microbial stability and prevent wine spoilage problems.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Compliance with ethics requirements This article does not contain any studies with human or animal subjects.

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