Revisiting the production of L(+)-lactic acid from vine shoots: bioconversion improvements by employing thermotolerant bacteria

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Abstract
Vine shoots (Vitis vinifera L.) constitute an abundant lignocellulosic source which is frequently underutilised. Alkaline and acidic pretreatments (with and without washing steps) were compared and optimised to release fermentable sugars from vine shoots. An acidic pretreatment using 1.72% H₂SO₄ at 134 °C for 17 min (with 10% w/w solid biomass), followed by an enzymatic hydrolysis, offered the most cost-effective results, releasing 40.21 g/L sugars. Three thermotolerant strains, namely, Bacillus coagulans DSM 2314, Geobacillus stearothermophilus DSM 2313, and G. stearothermophilus DSM 494, were assessed to produce lactic acid from vine-shoot hydrolysates under aerobic and non-sterile conditions, without the need of detoxification steps. In addition, wine lees were satisfactorily employed as nitrogen sources for the fermentation, providing similar results to yeast extract and being the only nutrient added to vine-shoot hydrolysates. Under optimal conditions, B. coagulans DSM 2314 produced 29.21 ± 0.23 g/L lactic acid in 24 h, with a sugar consumption of 98.74 ± 0.07% and a yield of 96.38 ± 0.76%, when supplemented with red wine lees. The purity of the isomer L(+) reached 97.59 ± 1.35% of the total lactic acid produced. Although G. stearothermophilus was able to transform the hexoses from vine-shoot hydrolysates into lactic acid, it proved to be inefficient for metabolising pentoses, thus obtaining lower lactic acid values (16–18 g/L).

Key points
• Thermotolerant bacteria produced L(+) -lactic acid from vine shoots
• Bacillus coagulans obtained a yield of 96% lactic acid from vine-shoot hydrolysates
• Lactic acid can be produced with wine lees as the only additional nitrogen source

Keywords Bacillus coagulans · Thermotolerance · Biorefinery · Winery wastes · Wine lees

Introduction
In the current challenging environmental context, the necessity of developing new alternatives to conventional petrochemical-based plastics enhances the production of biomolecules that act as the building blocks needed to polymerise biodegradable replacements. One of the most promising polymers corresponds to polylactic acid, which is manufactured through the following three stages: lactic acid production, lactic acid purification followed by lactide production, and, finally, the polymer synthesis by polycondensation and ring-opening polymerisation routes (Hu et al. 2016; Nduko and Taguchi 2021). In addition, lactic acid, especially the L(+) -lactic acid isomer, has many applications in the food, cosmetic, pharmaceutical, and chemical industries (Ajala et al. 2020).

Regarding the first step of the process, the bioproduction of L(+) -lactic acid by microbial fermentation represents a feasible and preponderant method over the chemical synthesis from hydrocarbon sources (Rawoof et al. 2021). In addition, lactic acid, especially the L(+) -lactic acid isomer, has many applications in the food, cosmetic, pharmaceutical, and chemical industries (Ajala et al. 2020).

To cope with this problem, using the inedible and abundant lignocellulosic feedstocks as a source of carbohydrates for microbial fermentation becomes an
essential solution (Nduko and Taguchi 2021). On the other hand, the use of lignocellulosic feedstocks implies several drawbacks mainly associated with their complex and recalcitrant nature, such as the high cost of their pretreatment and hydrolysis process to release fermentable sugars, as well as the simultaneous production of harmful compounds that could inhibit or reduce the microbial productivity of lactic acid (Ajala et al. 2020; Li et al. 2021; Nwamba et al. 2021).

Reduction of the costs associated with lignocellulosic material could be implemented in all the steps of the bioconversion process. For example, at the fermentation stage, the use of thermostolerant bacterial species for lactic acid production could contribute to economic savings related to refrigeration costs, lower risks of microbial contamination, higher yields, and greater ease of performing simultaneous saccharification and fermentation (Poudel et al. 2016).

Lactic acid bioproduction from lignocellulosic biomass has been extensively reviewed in the last decades, but works were mainly focused on the order of Lactobacillales (Abdel-Rahman et al. 2011; Castillo Martinez et al., 2013; Cubas-Cano et al. 2018; John et al. 2007, 2009; Nwamba et al. 2021; Tarraran and Mazzoli 2018; Wang et al. 2015). However, comparatively, the information on thermophilic species producing lactic acid, such as Bacillus coagulans and Geobacillus stearothermophilus, is scarce.

Vine shoots (Vitis vinifera L.) are the most abundant by-product from the winery industry, representing 93% of the solid residues, and they are generated at a rate of 1.4–2.0 ton/ha; therefore, vast amounts of this lignocellulosic feedstock are produced annually in those countries devoted to viticulture, considering that a total of 7,450,000 ha were under vines worldwide in 2018 (Garita-Cambronero et al. 2021). Production of lactic acid from vine shoots was demonstrated in previous works by using various Lactobacilli species as well as to avoid sterile and anaerobic conditions during their bioconversion. Besides, the use of vine lees as an alternative source of nitrogen and micronutrients under the thermophilic conditions assayed was also evaluated.

### Material and methods

#### Description of vine shoots

Vine shoots (pruning residues) were collected at the experimental plots of ITACyL (Finca Zamadueñas, Valladolid, Spain) in May 2019. These winery by-products were dried in an oven at 45 °C for 48 h (until constant weight), ground in a SM100 Comfort rotary mill (Retsch GmbH, Haan, Germany), and sieved to a size of 0.5–1.0 mm. Dry vine shoots were composed of 32.77% cellulose, 11.31% hemicellulose (49.27% total carbohydrates), 2.82% galacturonic acid, 21.30% acid-insoluble lignin, 4.34% protein, 0.52% fat, 7.91% moisture, 2.40% ashes, and 13.3 mg/g total phenolic compounds. Chemical characterisation was performed according to Garita-Cambronero et al. (2021).

#### Description and processing of wine lees

White wine lees and red wine lees were collected in September–November 2020 at the Oenological Station of Castile and Leon—ITACyL (Rueda, Spain). White wine lees had a density of 1.03 g/L and contained 81.8 g/L ethanol, 6.8 g/L total Kjeldahl nitrogen, and 0.43 g/L total phenolic compounds. Red wine lees had a density of 1.05 g/L and contained 99.3 g/L ethanol, 12.2 g/L total Kjeldahl nitrogen, and 1.51 g/L total phenolic compounds. Their chemical composition in terms of anions and cations is provided elsewhere (Hijosa-Valsero et al. 2021).

In order to use wine lees as nitrogen sources for microbial fermentation, they were concentrated and sonicated by modifying previously described methods (Bustos et al. 2004a; del Fresno et al. 2019). Briefly, wine lees were centrifuged at 4000 rpm and 4 °C for 15 min with a Fisherbrand GT 4R centrifuge (Thermo Electron LED GmbH, Osterode am Harz, Germany). The supernatant was discarded and replaced by distilled water, the sample was homogenised by shaking, and it was centrifuged again. This washing/centrifuging step was performed four consecutive times. Finally, the supernatant was discarded and the decanted solid was sterilised at 121 °C for 15 min. Concentrated wine lees were kept at 4 °C until further use. This solid concentrate contained 23.2 g/kg of total nitrogen (TN) in the case of white wine lees and 18.6 g TN/kg in the case of red wine lees. Then, fermentation broths were supplemented with concentrated wine lees in an amount equivalent to 1.1 g TN/L, and they were sonicated for 30 min at 330 W and 80 kHz in an Elmasonic P 180 H ultrasound bath (Elma Schmidbauer GmbH, Singen, Germany) in 500 mL Erlenmeyer flask.
flasks before microbial inoculation (see section “Use of alternative nitrogen sources: wine lees” for more details).

Pretreatment of vine shoots

Two different physicochemical pretreatment methods were assessed for vine shoots, namely, an alkaline pretreatment and an acidic pretreatment. The alkaline pretreatment consisted of treating vine shoots with an aqueous solution of 1.16% NaOH (w/w), at 125 °C for 110 min, as optimised in a previous work (Garita-Cambronero et al. 2021). On the other hand, the acidic pretreatment employed H$_2$SO$_4$ and its working conditions were optimised according to section “Optimisation of the acid pretreatment.” Both pretreatments were performed with a high-pressure 2-L reactor made of alloy Carpenter-20 (Parr Instrument Company, Moline, IL, USA) with a solid-to-solvent ratio of 10% (w/w). In both pretreatments, 40 g of dry vine shoots were placed in the reactor container and 360 g of the corresponding acidic or alkaline aqueous solution were added. The reaction mixture was heated at a rate of about 7.6 °C/min with continuous stirring, until the programmed working temperature was attained. Then, the reactor was kept at that temperature during the required time for each experiment. Time zero was considered at the beginning of the isothermal stage. At the end of the process, the reactor was cooled and the solid/liquid mixture was recovered.

Optimisation of the acid pretreatment

The conditions of this physicochemical pretreatment were optimised in order to obtain a fermentation broth with the maximum concentration of simple sugars (cellobiose, glucose, xylose, rhamnose, and arabinose) and the minimum concentration of inhibitors (formic acid, acetic acid, levulinic acid, furfural, 5-hydroxymethyl furfural, and phenolic compounds). In the case of vine shoots, the variables to be optimised were H$_2$SO$_4$ concentration (range of 0–3% w/w), temperature (range of 100–180 °C), and treatment time (range of 2–30 min). A Box-Behnken design and its related response surface methodology (RSM) experiments were performed for the physicochemical treatments; with 3 factors, 15 runs, 1 block, and 3 central points. A response surface was calculated, and the resulting equations were used to estimate the optimal temperature, time, and sulphuric acid concentration values to obtain the highest amount of total sugars released and the lowest amount of total inhibitors in the broth after the physicochemical treatment in the reactor and the subsequent enzymatic hydrolysis. Afterwards, the mathematically estimated optimal points were validated with experiments.

Enzymatic hydrolysis of acid-pretreated samples

After the physicochemical pretreatment, an enzymatic hydrolysis with Cellec CTe2 (Novozymes, Bagsvaerd, Denmark) at 50 °C was performed for 48 h on the biomass solid/liquid mixture obtained in the reactor, following the method described by Hijosa-Valsero et al. (2017). For all the experiments leading to the optimisation of the acid pretreatment, an enzymatic dose of 3.60 FPU/g biomass was employed (sections “Optimisation of the acid pretreatment” and “Establishment of optimal conditions for the acid pretreatment”). However, it was later observed that this dose was insufficient and, for fermentation experiments, this value was increased to 17.40 FPU/g, as suggested by Garita-Cambronero et al. (2021). After enzymatic hydrolysis, vine-shoot hydrolysates were vacuum-filtered (filter paper No. 1305, Filtros Anoia SA, Barcelona, Spain).

Enzymatic hydrolysis of alkali-pretreated samples

The solid/liquid mixture from the pretreatment reactor was centrifuged in order to separate the solid and the liquid fraction. The solid fraction was washed several consecutive times with distilled water until the pH of the rinse water was approximately 7.0. Then, the sample was centrifuged again, the solids were recovered by filtration, and a volume of water equivalent to that of the liquid fraction removed was added to the solids. This mixture was subjected to hydrolysis with Cellic CTe2 with an enzymatic load of 17.40 FPU/g pretreated solids at 50 °C and pH 5.0 (citrate buffer 50 mM) for 48 h (Garita-Cambronero et al. 2021). Then, hydrolysates were vacuum-filtered (filter paper no. 1305, 73 g/m$^2$, Filtros Anoia SA, Barcelona, Spain). A control experiment without biomass washing before enzymatic hydrolysis was also performed.

Cell growth and inocula preparation

The strains Bacillus coagulans DSM 2314, Geobacillus steaerotherophilus DSM 2313, and G. steaerotherophilus DSM 494 were purchased from Deutsche Sammlung von Mikroorganismen und Zellkulturen (Braunschweig, Germany). Lyophilised cells of B. coagulans were resuspended in 10 mL sterile medium containing Man-Rogosa-Sharpe (MRS) broth (Fisher Scientific SL, Madrid, Spain) and then added to 50 mL MRS medium in glass bottles capped with a rubber septum, where they were incubated at 52 °C. The lyophilised cells of G. steaerotherophilus strains were reactivated in nutrient broth according to Smerilli et al. (2015) and incubated at 60 °C. After a 24-h incubation, 1.5 mL of each strain were transferred to a sterile cryogenic vial and 0.4 mL glycerol (80% v/v) were added. The vials were closed, shaken, and stored at −80 °C until being used.
For inocula preparation, a loopful of the thawed glyc erinate was added to 50 mL of liquid MRS medium for B. coagulans or 50 mL of tryptic soy broth (Becton, Dickinson and Company; Le Pont de Claix, France) for G. stearothermophilus. The media were placed in glass bottles capped with a rubber septum and were incubated for 24 h at 100 rpm at 52 °C (B. coagulans) or 60 °C (G. stearothermophilus) in an Infors HT Ecotron orbital shaker (Infors AG, Bottmingen, Switzerland) until an approximate bacterial density of 5 × 10⁸ cells/mL was attained.

**Fermentation tests for different biomass pretreatment methods**

The three different vine-shoot hydrolysates described in sections “Enzymatic hydrolysis of acid-pretreated samples” and “Enzymatic hydrolysis of alkali-pretreated samples” (alkaline washed, alkaline non-washed and acidic) were supplemented with 10 g/L yeast extract, and their pH was adjusted to 6.0 with concentrated HCl or NaOH aqueous solutions. No sterilisation was applied to fermentation broths. Then, 10 mL inoculum of B. coagulans DSM 2314 were added to 100 mL of each type of hydrolysate. Only this bacterial strain was tested in this preliminary experiment because B. coagulans has been reported to cope with several lignocellulosic hydrolysates (Poudel et al. 2016), which might guarantee a successful lactic acid production with at least one of the vine-shoot hydrolysates. Fermentations were performed at 50 °C and 200 rpm in a Carousel 6 Plus Reaction Station (Radleys, Essex, UK), where pH was maintained at 6.0 with an automatic dispenser loaded with an aqueous solution of NaOH 5 M. These experiments were performed in triplicate. The most cost-effective pretreatment method was chosen for further experiments, based on lactic acid production and ease of operation for the pretreatment.

**Strain comparison**

In order to select the most efficient strain for lactic acid production, the strains B. coagulans DSM 2314, G. stearothermophilus DSM 2313, and G. stearothermophilus DSM 494 were compared to ferment the most adequate hydrolysate resulting from the test described in section “Fermentation tests for different biomass pretreatment methods.” Vine-shoot acidic hydrolysates were supplemented with 10 g/L yeast extract, and their initial pH was adjusted to 6.0 for DSM 2314 or to pH 7.0 for DSM 2313 and DSM 494. No sterilisation was applied to fermentation broths. Then, 10 mL of the respective inoculum were added to 100 mL of acidic hydrolysate. Fermentations were performed at 50 °C for B. coagulans or 60 °C for G. stearothermophilus in a Carousel 6 Plus Reaction Station at 200 rpm, where pH was maintained at 6.0 (B. coagulans) or 7.0 (G. stearothermophilus strains) with an automatic dispenser loaded with NaOH 5 M. In addition, fermentation controls for each strain were prepared with aqueous solutions containing glucose and xylose mixtures at similar concentrations to those of vine-shoot acidic hydrolysates (22 g/L glucose and 14 g/L xylose), and supplemented with 10 g/L yeast extract, in order to study the possible detrimental or beneficial effect of the complex chemical mixture of vine-shoot hydrolysates on lactic acid fermentation. All experiments were performed in triplicate.

**Use of alternative nitrogen sources: wine lees**

After selecting the most proficient lactic acid-producing strain on acidic vine-shoot hydrolysate, concentrated white and red wine lees were tested as alternative nitrogen sources in comparison to yeast extract. Commercial yeast extract (Fluka, Sigma-Aldrich, Buchs, Switzerland) contained 110 g TN/kg. Yeast extract and concentrated wine lees were added to fermentation broths in such an amount that TN concentrations were always 1.1 g/L. Therefore, for fermentation tests, acidic vine-shoot hydrolysates were supplemented with 10 g/L yeast extract, 47.5 g/L white wine lees, or 59.0 g/L red wine lees. In the case of concentrated wine lees, samples were sonicated as explained in section “Description and processing of wine lees.” No sterilisation was applied to fermentation broths. Then, 10 mL inoculum of B. coagulans DSM 2134 were added to 100 mL of each type of hydrolysate and fermentations were carried out as described in section “Fermentation tests for different biomass pretreatment methods.”

**Chemical analyses**

Hydrolysates and fermentation aqueous samples were centrifuged at 13,400×g in a microcentrifuge for 3 min (Minispin, Eppendorf, Hamburg, Germany). The supernatant was filtered through a nylon syringe filter (0.20 µm pore; Agilent Technologies, Santa Clara, CA, USA) and a Whatman No. 1 filter paper. The samples were then acidified with HCl to 1.1 g/L. Total phenolic compounds were analysed according to Folin and Denis (1912) and were expressed as gallic acid equivalents (GAE).

Lactic acid isomers were determined by HPLC–DAD with an Agilent 1100 series HPLC system (Agilent Technologies, Santa Clara, CA, USA) equipped with a G1315B diode array detector (DAD) monitoring at 254 nm. The separation was carried out with a Chirex® 3126 (D)-penicillamine (150×4.6 mm) column and a Chirex® 3216 (D)-penicillamine (30×4.6 mm) guard column (Phenomenex, Torrance, CA, USA) operated at 30 °C. The mobile phase was an aqueous solution of 0.25 g/L CuSO₄·5H₂O, and the flow rate was
1 mL/min. The injection volume was 20 μL, and the run time was 30 min.

Hexose consumption (ΔH), pentose consumption (ΔP), and total sugar consumption (ΔS) during the fermentation process were expressed as a percentage. Lactic acid yield, Y (%), was calculated as the ratio between the mass of lactic acid produced (g) and the mass of total sugars consumed (g). In both cases, volumetric corrections due to the addition of NaOH-solution for pH control were performed. Lactic acid productivity, W (g/(L·h)), was calculated as the concentration of lactic acid in the final broth (g/L) divided by the fermentation time (h).

Statistics

Comparisons among samples were assessed with a one-way ANOVA and Tukey’s HSD test using the software Statistica 7 (StatSoft Inc., Tulsa, OK, USA). Box-Behnken RSM experimental designs were performed with the software Minitab 16 (Minitab Inc., State College, PA, USA).

Results

Establishment of optimal conditions for the acidic pretreatment

The operational conditions of the physicochemical pretreatment of vine shoots (temperature, time, and concentration of H2SO4) were optimised with an RSM experimental design focused on maximum sugar release and minimum generation of fermentation inhibitors. Under the RSM-tested conditions, total sugar concentrations ranged between 12.19 and 31.03 g/L (Table S1), which represents a sugar recovery efficiency of only 22.58–52.81%, taking into account the initial carbohydrate composition of vine shoots. The data from Table S1 were used to calculate mathematical models to estimate the concentration of each response variable from any combination of the three independent variables (Tables S2-S5). Contour plots for total sugar and inhibitor concentrations are given in Fig. S1.

The mathematical model determined that a pretreatment performed at 134 °C, for 17 min and with a concentration of 1.72% H2SO4 (w/w) would produce a hydrolysate containing 30.15 g/L sugars, 0.13 g/L formic acid, 3.90 g/L acetic acid, 0.21 g/L levulinic acid, 0.72 g/L 5-HMF, 0.20 g/L furfural, and 0.69 g/L phenolic compounds (Table S6). These optimal working conditions were validated experimentally by treating vine shoots at the calculated conditions, followed by an enzymatic hydrolysis with 3.60 FPU/g biomass (Table 1).

Fermentability of vine-shoot hydrolysates: effect of pretreatment type

Vine shoots were subjected to two physicochemical pretreatments (alkaline and acidic) and a subsequent enzymatic hydrolysis. In the case of the alkaline pretreatment, two different strategies were applied, namely, washing the pretreated solids with distilled water to remove possible inhibitors or using the unwashed solids. In both cases, the solids underwent an enzymatic hydrolysis thereafter. The alkaline washed hydrolysate contained 1.59 g/L cellobiose, 25.74 g/L glucose, 10.68 g/L xylose, (38.01 g/L total sugars), 0.25 g/L acetic acid, and 0.24 g/L total phenolic compounds, whereas the alkaline unwashed hydrolysate contained 1.98 g/L cellobiose, 30.17 g/L glucose, 10.58 g/L xylose, 0.09 g/L arabinose (42.82 g/L total sugars), 0.55 g/L formic acid, 2.13 g/L acetic acid, and 0.84 g/L total phenolic compounds. The wash water may sweep away small quantities of simple sugars released during the physicochemical pretreatment, which would explain the slightly lower sugar concentration in the washed alkaline hydrolysate in comparison to the unwashed sample. On the other hand, the acidic hydrolysate contained 2.08 g/L cellobiose, 22.43 g/L glucose, 14.09 g/L xylose, 0.48 g/L rhamnose, 1.13 g/L arabinose (40.21 g/L total sugars), 0.11 g/L formic acid, 3.80 g/L acetic acid, 0.11 g/L levulinic acid, 0.40 g/L 5-HMF, 0.13 g/L furfural, and 0.60 g/L total phenolic compounds (Table 1). According to these results, the three pretreatments offered similar total sugar concentrations (38–43 g/L), but the types of sugars produced by each treatment were slightly different.

The three abovementioned hydrolysates were supplemented with 10 g/L yeast extract and were inoculated with B. coagulans DSM 2314 to initially check their fermentability using a known robust and inhibitor-tolerant species (Zhang et al. 2014). After a 24-h fermentation, it was observed that the unwashed alkaline hydrolysate was toxic for microorganisms and thus unsuitable for lactic acid production (Fig. 1), perhaps due to the moderate concentrations of formic acid.
(0.55 g/L) and total phenolic compounds (0.84 g/L) in the hydrolysate or to the presence of non-monitored inhibitors. In addition, the detrimental effect of excess Na⁺ on the unwashed NaOH-treated biomass cannot be discarded. On the contrary, the washed alkaline hydrolysate and the acidic hydrolysate were successfully fermented by *B. coagulans* DSM 2314 (Fig. 1). In fact, this was reflected in a significantly lower \((p < 0.05)\) cell density in the unwashed-alkaline broth (1.13 ± 0.15) in comparison to the washed-alkaline broth (8.27 ± 0.90) or the acidic broth (7.80 ± 1.88, expressed as \(\times 10^8\) cell/mL in all three cases). Consequently, the final concentrations of lactic acid were 31.70 ± 0.06 g/L, 2.32 ± 0.00 g/L, and 29.84 ± 0.47 g/L, for washed-alkaline, unwashed-alkaline, and acidic pretreatments, respectively. This implies that the washed alkaline pretreatment was significantly superior \((p < 0.05)\) to the rest of pretreatments for lactic acid production (Fig. 1).

In spite of the good results obtained in the fermentation of the hydrolysates from the washed alkaline-treated vine shoots, the washing step is time- and water-consuming. In contrast, the acidic pretreatment did not imply solid/liquid separations or biomass washing, making it a more straightforward process. Therefore, the acid pretreatment was preferred over the alkaline pretreatment to obtain vine-shoot hydrolysates destined for lactic acid production in the subsequent tests.

**Effect of microbial strain on lactic acid production**

The behaviour of the thermotolerant strains *B. coagulans* DSM 2314, *G. stearothermophilus* DSM 494, and *G. stearothermophilus* DSM 2313 was studied by employing both synthetic control media and acidic vine-shoot hydrolysates for lactic acid production. Initial sugar concentrations in the broths were about 35 g/L after inoculum addition in all cases.

When using control media, all fermentations were finished in 24 h. In this case, *B. coagulans* DSM 2314 obtained 28.16 ± 0.26 g/L lactic acid, which was significantly higher \((p < 0.05)\) than the concentrations attained by both *G. stearothermophilus* strains, which barely reached 14–17 g/L (Fig. 2a). This can be due to the incomplete sugar consumption of DSM 494 and DSM 2313 (about 99% hexoses and 9% pentoses), hence making the fermentation inefficient, in contrast to the complete sugar consumption of *B. coagulans* DSM 2314 (100% hexoses and 100% pentoses). Therefore, *G. stearothermophilus* DSM 494 and DSM 2313 do not seem adapted to xylose consumption, which is translated into lower lactic acid production values.

When fermenting acidic vine-shoot hydrolysates, *B. coagulans* DSM 2314 was also significantly superior \((p < 0.05)\) to *G. stearothermophilus* DSM 494 and DSM 2313 for lactic acid production (Fig. 2b), since concentrations of 29.84 ± 0.47 g/L, 18.22 ± 1.69 g/L, and 16.29 ± 0.93 g/L were attained, respectively. In addition, both *G. stearothermophilus* strains needed 48 h to finish the fermentation in vine-shoot hydrolysates, as opposed to *B. coagulans* DSM 2314, which only needed 24 h. Remarkably, *B. coagulans* DSM 2134 and *G. stearothermophilus* DSM 494 obtained significantly higher lactic acid concentrations in vine-shoot hydrolysates than in control media \((p < 0.05)\). Moreover, both *G. stearothermophilus* strains (DSM 494 and DSM 2313) consumed significantly larger amounts of pentoses in vine-shoot hydrolysates than in control media \((p < 0.05)\). This behaviour could be related to the complex composition

| Table 1 Composition of vine-shoot hydrolysates under optimised acid-pretreatment conditions (134 °C, 17 min and 1.72% w/w H₂SO₄). Estimated and experimental values are given. EH: enzymatic hydrolysis |
|---------------------------------------------|---------------------------------------------|---------------------------------------------|
| Pretreatment                               | Pretreatment + EH (3.60 FPU/g biomass)      | Pretreatment + EH (17.40 FPU/g biomass)     |
| Concentration (g/L)                        | Experimental validation                      | Mathematical estimation (RSM)               |
|                                            |                                          |                                  | Experimental validation                     |
| Cellobiose                                 | 0.95                                      | 0.60                                    | 2.08                                      |
| Glucose                                    | 11.73                                     | 15.62                                   | 22.43                                     |
| Xylose                                     | 13.55                                     | 13.85                                   | 14.09                                     |
| Rhamnose                                   | 0.36                                      | 0.29                                    | 0.48                                      |
| Arabinose                                  | 0.91                                      | 0.77                                    | 1.13                                      |
| Total sugars                               | 27.50                                     | 30.15                                   | 40.21                                     |
| Formic acid                                | 0.14                                      | 0.13                                    | 0.11                                      |
| Acetic acid                                | 4.03                                      | 3.90                                    | 3.80                                      |
| Levulinic acid                             | 0.17                                      | 0.21                                    | 0.11                                      |
| 5-HMF                                      | 0.80                                      | 0.72                                    | 0.40                                      |
| Furfural                                   | 0.20                                      | 0.20                                    | 0.13                                      |
| Phenolic compounds                         | 0.76                                      | 0.69                                    | 0.60                                      |

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of vine-shoot hydrolysates, some of whose compounds could exert synergistic effects on xylose absorption by bacterial cells.

Therefore, *B. coagulans* DSM 2314 was the most appropriate strain for lactic acid production from acidic vine-shoot hydrolysate and it was employed for further fermentation tests.

**Influence of the nitrogen source on lactic acid fermentation**

After selecting the acidic pretreatment as the best process for sugar release and *B. coagulans* DSM 2314 as the most proficient lactic acid producer, the effect of substituting yeast extract for concentrated white wine lees and concentrated red wine lees (with a dose of 1.1 g/L TN in all cases) was tested.

As shown in Fig. 3, the three nitrogen sources tested resulted in successful fermentations. In fact, there were no significant differences for lactic acid concentrations among the three nitrogen supplements, reaching values of 29.84 ± 0.47 g/L for yeast extract, 29.11 ± 0.23 g/L for white lees, and 29.21 ± 0.36 g/L for red lees. The use of wine lees did not increase fermentation times (24 h), which is advantageous from the economic point of view. However, certain fermentation parameters were significantly (*p* < 0.05) affected by the type of nitrogen source used (Table 2), such as cell density and hexose consumption (higher with yeast extract), or pentose consumption, total sugar consumption, and lactic acid yield (higher with red lees). In any case, both white lees and red lees were excellent nutritional alternatives to yeast extract for lactic acid fermentation.

The nature of the lactic acid isomers produced by *B. coagulans* DSM 2314 in vine-shoot acidic hydrolysates was analysed. As expected, isomer L (+) was predominant in the fermentation broth of *B. coagulans* DSM 2314, regardless of the nitrogen source, constituting 97.59 ± 1.35% of the total lactic acid produced. This high purity facilitates the use of the obtained lactic acid for chemical and food applications.

**Discussion**

**Response surface methodology for the optimisation of the acidic pretreatment**

The RSM-optimised acid pretreatment alone produced 27.50 g/L sugars (Table 1). This amount is slightly inferior to those of previous works where vine shoots were pretreated with dilute sulfuric acid (3% H<sub>2</sub>SO<sub>4</sub>, 130 °C, 15 min, biomass load 11% w/w), reporting values of 9–12 g/L glucose, 17–19 g/L xylose, 4–7 g/L arabinose, and 4–6 g/L acetic acid (Bustos et al. 2004b, 2005a, 2005b; Moldes et al. 2006, 2007; Vecino et al. 2017). This can be due to the lower acid concentration employed in the present work, aiming at inhibitor reduction. In fact, Bustos et al. (2005b) obtained an acidic vine-shoot hydrolysate containing 32.8 g/L total sugars, 4.0 g/L acetic acid, 0.7 g/L furfural, and 0.1 g/L 5-HMF when employing 3% H<sub>2</sub>SO<sub>4</sub> in the pretreatment, but there is no information available about the generation of other potential inhibitors such as formic acid or phenolic compounds.

Regarding the production of total inhibitors according to the RSM model (Fig. S1b), their generation followed a logical pattern: higher temperatures, higher treatment times, and higher acid concentrations resulted in the formation of more inhibitors, which is in agreement with previous theoretical findings (Palmqvist and Hahn-Hägerdal 2000). The experimental concentrations of individual inhibitors were similar to those estimated by the model (Table 1). Although the concentration of acetic acid in the hydrolysate was relatively high (~ 4 g/L), this value
Fig. 2  Fermentative behaviour of different bacterial strains for a synthetic control medium (22 g/L glucose and 14 g/L xylose) and b acidic hydrolysates of vine shoots (n = 3). Nitrogen source: 10 g/L yeast extract. The hours above the graphs indicate fermentation times.

Fig. 3  Effect of different nitrogen sources (1.1 g/L TN) on lactic acid production in a 24-h fermentation of acidic vine-shoot hydrolysates with B. coagulans DSM 2314 (n = 3).
should not suppose a problem for lactic acid fermentation, since Zhang et al. (2014) demonstrated that the strain *B. coagulans* IPE22 was able to maintain its growth in the presence of individual concentrations of 2 g/L furfural, 2 g/L 5-HMF, 1 g/L formate, 30 g/L acetate, 3 g/L vanillin, and 60 g/L sulfate. In addition, Abdel-Rahman et al. (2021) observed that *B. coagulans* Azu-10 produced lactic acid in the presence of individual concentrations of 1–4 g/L furfural, 1–5 g/L 5-HMF, 5–15 g/L acetic acid, 5 g/L formic acid, 7 g/L levulinic acid, and 1 g/L syringaldehyde, *p*-hydroxybenzaldehyde, or vanillin. Therefore, the inhibitor concentrations obtained in this work by RSM optimisation are clearly low and presumably safe for *B. coagulans*.

### Pretreatment types

Although the three pretreatments offered similar total sugar concentrations (38–43 g/L), the types of sugars produced by each treatment were slightly different. In fact, the alkaline unwashed pretreatment yielded the highest glucose values, whereas the acidic pretreatment led to the highest values of xylose and arabinose (pentoses). This was expectable because alkaline pretreatments are usually employed to remove lignin and favour cellulose degradation in order to obtain glucose, while acid pretreatments are known to efficiently hydrolyse hemicellulose, thus releasing its main components, such as xylose, rhamnose, arabinose, or acetyl groups (Hendriks and Zeeman 2009). Another remarkable fact is the effect of each pretreatment on inhibitor generation. When comparing both alkaline pretreatments, it can be observed that the washing step removes formic acid, acetic acid, and total phenolic compounds to a great extent. As for the acidic treatment, it produced the highest amounts of acetic acid, levulinic acid, 5-HMF, and furfural, which is in agreement with the capacity of strong acids to hydrolyse carbohydrate polymers and degrade hexoses and pentoses into inhibitory substances (Palmqvist and Hahn-Hägerdal 2000; Paniagua-García et al. 2019). In any case, it was possible to minimise inhibitor generation during acidic pretreatment, thanks to the RSM performed on vine shoots, especially in terms of formic acid (section “Establishment of optimal conditions for the acidic pretreatment”).

The acidic vine-shoot hydrolysate and the washed-alkaline hydrolysates were readily fermentable by *B. coagulans*, in contrast to the unwashed-alkaline hydrolysate, whose fermentation failed. Therefore, it is essential to wash alkaline-treated biomass to avoid toxic effects during lactic acid fermentation with *B. coagulans* DSM 2314. Conversely, it has been reported that a similar alkaline unwashed pretreatment enabled an efficient ABE fermentation of vine-shoot hydrolysates with *Clostridium beijerinckii* CECT.

### Table 2

| Nitrogen source | Time (h) | Lactic acid (g/L) | Ethanol (g/L) | Acetic acid (g/L) | Formic acid (g/L) | Bacterial density, cells/mL (×10⁸) | Y (%) | W (g/L·h) | ΔS (%) | ΔP (%) | ΔH (%) |
|-----------------|---------|-------------------|---------------|------------------|------------------|-------------------------------|-------|----------|---------|---------|---------|
| Yeast extract   | 24      | 29.94±0.47         | 3.95±0.19     | 0.37±0.23         | 0.12±0.03         | 92.47±0.23                    | 98.98±0.23 | 0.56±0.10  | 96.53±0.05 | 100±0.00 | 98.74±0.07 |
| White wine      | 24      | 29.11±0.23         | 3.88±0.03     | 0.11±0.03         | 0.03±0.04         | 93.82±0.21                    | 97.06±0.09 | 0.30±0.01  | 95.90±0.00 | 100±0.00 | 99.08±0.07 |
| Red wine lees   | 24      | 29.21±0.26         | 3.98±0.02     | 0.36±0.02         | 0.01±0.01         | 92.78±0.29                    | 97.20±0.15 | 0.36±0.04  | 95.03±0.05 | 100±0.00 | 98.74±0.07 |
| White wine lees | 24      | 29.21±0.26         | 3.98±0.02     | 0.36±0.02         | 0.01±0.01         | 92.78±0.29                    | 97.20±0.15 | 0.36±0.04  | 95.03±0.05 | 100±0.00 | 98.74±0.07 |
| Yeast extract   | 24      | 29.94±0.47         | 3.95±0.19     | 0.37±0.23         | 0.12±0.03         | 92.47±0.23                    | 98.98±0.23 | 0.56±0.10  | 96.53±0.05 | 100±0.00 | 98.74±0.07 |
| White wine      | 24      | 29.11±0.23         | 3.88±0.03     | 0.11±0.03         | 0.03±0.04         | 93.82±0.21                    | 97.06±0.09 | 0.30±0.01  | 95.90±0.00 | 100±0.00 | 99.08±0.07 |
| Red wine lees   | 24      | 29.21±0.26         | 3.98±0.02     | 0.36±0.02         | 0.01±0.01         | 92.78±0.29                    | 97.20±0.15 | 0.36±0.04  | 95.03±0.05 | 100±0.00 | 98.74±0.07 |

Note: A different letter between brackets (a, b, c) indicates the existence of significant differences (p<0.05) between nitrogen sources for a given parameter. ΔH: hexoses consumption; ΔP: pentoses consumption; ΔS: total sugar consumption; W: lactic acid productivity; Y: lactic acid yield.
508 (Garita-Cambronero et al. 2021). Bustos et al. (2005b) reported that an alkaline pretreatment of vine shoots (including a washing step), performed at 130 °C with 8% NaOH and 9% biomass load for 75 min, resulted in the production of 24.7 g/L lactic acid in a simultaneous saccharification and fermentation (SSF) process with Lactobacillus rhamnosus. Those working conditions were more severe than the ones used in the present study (125 °C, 1.16% NaOH, 110 min), but yet led to a lower lactic acid production, since 31.70 ± 0.06 g/L lactic acid were generated in the present work. This evidences the importance of pretreatment optimisation to reduce inhibitor induction, although the use of different bacterial species in both works could have also played a key role. Regarding acid pretreatments, Moldes et al. (2007) applied a sulfuric-acid treatment to vine shoots (3% H₂SO₄, 130 °C, 15 min, 11% solid load) without posterior enzymatic hydrolysis and obtained 19.2 g/L lactic acid with Lb. pentosus CECT 4023 in batch fermentation in 16 h. The inclusion of a detoxification step with CaCO₃ and adequate nutrient addition improved lactic acid production up to 26.5 g/L in a 48-h batch fermentation with Lb. pentosus CECT 4023 (Moldes et al. 2006). Accordingly, the RSM strategy applied to vine shoots in the present study to maximise sugar release while minimising inhibitor production proved to be successful, because 29.84 ± 0.47 g/L lactic acid were obtained from the acidic hydrolysate without the need of detoxification steps (Fig. 1).

**Microbial strains**

*Bacillus coagulans* is a thermotolerant aerobic or facultative anaerobic microorganism that can metabolise pentose and hexose sugars by the pentose phosphate pathway and the Embden-Meyerhof-Parnas pathway, respectively, producing L(+)-lactic acid with a yield of 1 g/g for xylose and 1 g/g for glucose (Poudel et al. 2016). This species has been efficiently employed for lactic acid production from several food and agriculture by-products, such as organic kitchen waste (Sakai and Ezaki 2006), sugarcane bagasse (van der Pol et al. 2016), Phragmites australis biomass (Zhang et al. 2018), or wheat straw (Maas et al. 2008; Zhang et al. 2014), obtaining yields of 0.94–0.98 g/L and lactic acid concentrations in the range of 38–86 g/L depending on the initial sugar concentration.

*Geobacillus stearothermophilus* is a thermophilic and amylolytic facultatively anaerobic bacterium which has been reported to produce about 40 g/L lactic acid from substrates containing 50 g/L potato starch under non-sterile conditions, with low nutritional requirements and reaching a complete sugar consumption (Smerilli et al. 2015). Kunasundari et al. (2017) have also described its ability to produce lactic acid from rice starch waste. To the best of our knowledge, this is the first time that *G. stearothermophilus* is tested for the fermentation of lignocellulosic hydrolysates containing hexoses and pentoses. Although *G. stearothermophilus* has been used to produce cellulase enzymes for cellulose hydrolysis (Alrumman 2016), the present results indicate that it is not an adequate species to deal with hemicellulose or pentoses.

In recent years, different species have been tested for lactic acid production from vine-shoot hydrolysates with variable results (Nanni et al. 2021), as summarised in Table 3. For instance, 21.7–24.7 g/L lactic acid have been produced with *Lb. rhamnosus* (Bustos et al. 2005b), 15.6–26.5 g/L with *Lb. pentosus* CECT 4023 (Bustos et al. 2004b, 2005a; Moldes et al. 2006, 2007), or 14.3 g/L with *Lactococcus lactis* CECT 4434 (Rodríguez et al. 2010). The highest lactic acid concentration (36.4 g/L) was reported with a coculture of *Lb. pentosus* CECT 4023 and *Lb. plantarum* CECT 221 dealing with a mixture of acidic hydrolysates and alkaline-treated solids, under simultaneous saccharification and fermentation conditions (Rodríguez-Pazo et al. 2013). In all those cases, Lactobacilli had been supplemented with mineral salts (Man-Rogosa-Sharpe or Mercier nutrients) or corn steep liquor, in addition to yeast extract, for the fermentation of vine shoots. It must be noted that only *Lb. rhamnosus* and *Lactococcus lactis* are known to produce isomer L (+) with a high degree of purity, whereas *Lb. pentosus* and *Lb. plantarum* generate a mixture of isomers L (+) and D (−). Accordingly, *B. coagulans* DSM 2314, with a production of about 30 g/L of optically pure L (+)-lactic acid, seems equally or even more efficient for lactic acid production from vine-shoot hydrolysates than previously reported Lactobacilli strains. In addition, its thermotolerance enabled the work under non-sterile conditions during the whole process, which simplifies the operation.

**Nitrogen sources**

The use of wine lees as nitrogen sources for the fermentation of vine-shoot hydrolysates had been explored previously. However, in those cases, lactic acid production decreased compared to conventional organic nitrogen sources. For instance, Bustos et al. (2005b) worked with *Lb. rhamnosus* and obtained 24.7 g/L lactic acid adding Man-Rogosa-Sharpe nutrients and 21.7 g/L lactic acid adding wine lees (Table 3). Similarly, Moldes et al. (2007) employed *Lb. pentosus* CECT 4023 and reported 19.2 g/L lactic acid with Mercier nutrients and 15.6 g/L with wine lees. Therefore, *B. coagulans* DSM 2314 seems to utilise wine lees more easily than Lactobacilli, since in the present study its performance was similar when fed with yeast extract or with concentrated wine lees. This is probably related to the ability of *B. coagulans* to grow on media containing inorganic nitrogen and low amounts of organic nitrogen, thus supposing an advantage in comparison to Lactobacillales strains, whose nutritional requirements are more demanding (Poudel et al. 2016).
| Strain          | Metabolism (a) | Isomers of LA (b) | Pretreatment | EH  | Detoxification | Initial sugars (g/L) | Nutrients          | Fermentation | $T$ (°C) | $t$ (h) | LA (g/L) | Y (g/g) | Reference                     |
|----------------|---------------|-------------------|--------------|-----|----------------|----------------------|--------------------|--------------|----------|--------|---------|--------|--------------------------------|
| *Lb. pentosus* | 2 Mixture     | $3\%$ $\text{H}_2\text{SO}_4,$ $130^\circ \text{C},$ 15 min, 11% biomass | No | No | 27.8 | 10 g/L YE, 10 g/L CSL | B | 31 | 9 | 21.8 | 0.77 | Bustos et al. (2004b) |
| *Lb. pentosus* | 2 Mixture     | $3\%$ $\text{H}_2\text{SO}_4,$ $130^\circ \text{C},$ 15 min, 11% biomass | No | CaCO$_3$ | 33.7 | 10 g/L YE, 10 g/L CSL | B | 31 | 24 | 21.8 | 0.61 | Bustos et al. (2005a) |
| *Lb. pentosus* | 2 Mixture     | $3\%$ $\text{H}_2\text{SO}_4,$ 15 min | No | CaCO$_3$ | 35 | 10 g/L YE, 10 g/L CSL | B | 31 | 48 | 26.5 | 0.76 | Moldes et al. (2006) |
| *Lb. pentosus* | 2 Mixture     | $3\%$ $\text{H}_2\text{SO}_4,$ $130^\circ \text{C},$ 15 min, 11% biomass | No | No | 32.1 | Mercier | B | 31 | 16 | 19.2 | 0.7 | Moldes et al. (2007) |
| *Lb. pentosus* | 2 Mixture     | $3\%$ $\text{H}_2\text{SO}_4,$ $130^\circ \text{C},$ 15 min, 11% biomass | No | No | 32.2 | 20 g/L wine lees | B | 31 | 16 | 15.6 | 0.58 | Moldes et al. (2007) |
| *Lb. rhamnosus*| 2 L(+)        | $3\%$ $\text{H}_2\text{SO}_4,$ $130^\circ \text{C},$ 15 min, 11% biomass; delignification of the solid fraction with $12\%$ NaOH, $130^\circ \text{C},$ 75 min | Yes | Washing | - | MRS, 2 g/L CaCO$_3$ | SSF | 45 | - | 23.8 | - | Bustos et al. (2005b) |
| *Lb. rhamnosus*| 2 L(+)        | $3\%$ $\text{H}_2\text{SO}_4,$ $130^\circ \text{C},$ 15 min, 11% biomass; delignification of the solid fraction with $12\%$ NaOH, $130^\circ \text{C},$ 75 min | Yes | Washing | - | 20 g/L wine lees, 2 g/L CaCO$_3$ | SSF | 45 | - | 21.8 | - | Bustos et al. (2005b) |
| *Lb. rhamnosus*| 2 L(+)        | $3\%$ $\text{H}_2\text{SO}_4,$ $130^\circ \text{C},$ 15 min, 11% biomass; delignification of the solid fraction with $8\%$ NaOH, $130^\circ \text{C},$ 75 min | Yes | Washing | - | MRS, 2 g/L CaCO$_3$ | SSF | 45 | - | 24.7 | - | Bustos et al. (2005b) |
| *Lb. rhamnosus*| 2 L(+)        | $3\%$ $\text{H}_2\text{SO}_4,$ $130^\circ \text{C},$ 15 min, 11% biomass; delignification of the solid fraction with $8\%$ NaOH, $130^\circ \text{C},$ 75 min | Yes | No | - | 20 g/L wine lees, 2 g/L CaCO$_3$ | SSF | 45 | - | 21.7 | - | Bustos et al. (2005b) |
### Table 3 (continued)

| Strain | Metabolism (a) | Isomers of LA (b) | Pretreatment | EH | Detoxification | Initial sugars (g/L) | Nutrients | Fermentation | T (°C) | t (h) | LA (g/L) | Y (g/g) | Reference |
|--------|----------------|-------------------|--------------|----|----------------|----------------------|-----------|--------------|---------|-------|-----------|---------|-----------|
| *Lb. pentosus*  
CECT 4023 and  
*Lb. plantarum*  
CECT 221 | 2 | Mixture | 3% H₂SO₄, 130 °C, 15 min, 11% biomass; delignification of the solid fraction with 8% NaOH, 130 °C, 120 min; addition of the hemicellulosic liquid | Yes | No | - | MRS, 0.6 g/L phenylalanine | SSF, Co | 31.5 | 144 | 36.4 | - | Rodríguez-Pazo et al. (2013) |
| *Lactococcus lactis*  
CECT 4434 | 2 | L(+) | Hemicellulosic fraction | No | No | - | - | B | - | 74 | 14.3 | - | Rodríguez et al. (2010) |
| *B. coagulans*  
DSM 2314 | 4 | L(-) | 1.72% H₂SO₄, 134 °C, 17 min, 10% biomass | Yes | No | 33.0 (c) | 59 g/L red wine lees | B | 50 | 24 | 29.2 | 0.96 | This study |

(a) 1: homofermentative (only hexoses); 2: facultative heterofermentative (homofermentative for hexoses and heterofermentative for pentoses); 3: obligate heterofermentative (hexoses and pentoses); 4: homofermentative (hexoses and pentoses)

(b) LA lactic acid, EH enzymatic hydrolysis, B batch, SSF simultaneous saccharification and fermentation, Co coculture, Y lactic acid yield, MRS Man-Rogosa-Sharpe's nutrients, YE yeast extract, CSL corn steep liquor

(c) This concentration was measured after adding the inoculum to the fermentation broth. Sugar concentration in the raw hydrolysate was 40.21 g/L.
### Table 4  Other lignocellulosic and food by-products used for lactic acid bioproduction

| Strain                          | Metabolism (a) | Isomers of LA (b) | Substrate and Pretreatment | EH | Detoxification | Initial Sugars (g/L) | Nutrients | Fermentation | T (°C) | t (h) | LA (g/L) | Y (g/g) | Reference                     |
|--------------------------------|----------------|-------------------|-----------------------------|----|----------------|----------------------|-----------|--------------|--------|-------|---------|--------|--------------------------------|
| *B. coagulans* A20-EXA (evolved) | 4              | L(+)              | Gardening residues (steam explosion, 0.33 M H₂SO₄, 180 °C, 10 min; only solid fraction) + pure xylose | Yes | No             | 25                   | MRS       | B, Co (with *S. cerevisiae*) | 52     | 120   | 22.15   | 0.89   | Cubas-Cano et al. (2020)      |
| *B. coagulans* DSM 2314       | 4              | L(+)              | Wheat straw (Ca(OH)₂, 85 °C, 30 rpm, 16 h; solid fraction) | Yes | No             | -                    | 25 g/L YE, 5 g/L (NH₄)₂HPO₄, 8.75 g/L (NH₄)₂SO₄, 0.05 g/L MgCl₂·6H₂O, 0.25 g/L CaCl₂·2H₂O | SSF, FB | 50     | 55     | 40.7   | 0.81   | Maas et al. (2008)            |
| *B. coagulans* NBRC 12,583    | 4              | L(+)              | Kitchen waste, non-sterile | No  | No             | 117                  | -         | B            | 55     | 120   | 86      | 0.98   | Sakai and Ezaki, (2006)       |
| *B. coagulans* DSM 2314       | 4              | L(+)              | Sugarcane bagasse (0.72% H₂SO₄, 170 °C, 15 min, steam explosion) | Yes | No             | -                    | YE        | SSF          | 50     | 90    | 74.6    | 0.94   | van der Pol et al. (2016)     |
| *B. coagulans* DSM 23,183 and DSM 23,184 (c) | 4              | L(+)              | Xylitol by-products (2% H₂SO₄, 121 °C, 90 min, 10% biomass) | No  | No             | 150                  | 10 g/L YE, 60 g/L CaCO₃ | FB           | 50     | 51    | 100–106 | -      | Xu et al. (2013)              |
| *B. coagulans* IPE22 (c)      | 4              | L(+)              | Wheat straw (2% H₂SO₄, 121 °C, 90 min, 10% biomass) | Yes | No             | -                    | 10 g/L CSL | SSF          | 50     | 90    | 38.42   | -      | Zhang et al. (2014)           |
| *B. coagulans*                | 4              | L(+)              | *Phragmites australis* (2% H₂SO₄, 121 °C, 60 min, 10% biomass) | Yes | No             | -                    | MRS       | SSF          | 50     | 55    | 35.05   | -      | Zhang et al. (2018)           |
| *Bacillus sp.* CCTCC M 2011,468 (c) | 4              | L(+)              | Corn stover (steam explosion) | Yes | No             | 94                   | No information | B            | 50     | 42    | 56.37   | 0.5996 | Ouyang et al. (2013)          |
| Strain                        | Metabolism (a) | Isomers of LA (b) | Substrate and pretreatment                                                                 | EH | Detoxification | Initial sugars (g/L) | Nutrients | Fermentation | T (°C) | t (h) | LA (g/L) | Y (g/g) | Reference                                    |
|------------------------------|----------------|-------------------|-------------------------------------------------------------------------------------------|----|----------------|----------------------|-----------|--------------|--------|------|----------|--------|---------------------------------------------|
| *Lb. amylovorus* ATCC 33,620 | 1              | Mixture           | Casava bagasse (80 °C, 40 min, 10% biomass)                                              | Yes| No             | -                    | 8% v/v CSL | B            | 37     | 144  | 29.69    | -      | Carpinelli Macedo et al. (2020)            |
| *Lb. amylovorus* ATCC 33,620 | 1              | Mixture           | Casava bagasse (80 °C, 40 min, 14% biomass)                                             | Yes| No             | -                    | 10% v/v CSL | FB           | 37     | 144  | 66.9     | -      | Carpinelli Macedo et al. (2020)            |
| *Lb. delbrueckii* subsp. bulgaricus ATCC 11,842 | 1              | D(-)              | Beech wood (ethanol:water, 1:1; 160 °C, 2 h, 16 bar O₂, 9% biomass; only solid fraction) | Yes| Washing        | ~65                  | MRS        | SSF          | 44     | 72   | 61.8     | 0.69   | Karnaouri et al. (2020)                    |
| *Lb. delbrueckii* subsp. bulgaricus ATCC 11,842 | 1              | D(-)              | Pine wood (acetone:water, 1:1; 175 °C, 1 h, 16 bar O₂, 9% biomass; only solid fraction) | Yes| Washing        | ~80                  | MRS        | SSF          | 44     | 72   | 36.4     | 0.4    | Karnaouri et al. (2020)                    |
| *Lb. paracasei* KCTC 11710BP (c) | 2              | L(+)              | Ethanol fermentation waste (autolysis, distillation, centrifugation)                   | No | No             | 51.1                 | -          | B            | 37     | 32   | 50.6     | 0.99   | Moon et al. (2013)                         |
| *Lb. pentosus* CECT 4023     | 2              | Mixture           | Distilled grape marc (3.3% H₂SO₄, 130 °C, 30 min, 11% biomass)                      | No | CaCO₃          | 12.5                 | 10 g/L YE, 10 g/L CSL | B            | 31     | 12   | 7.2      | 0.71   | Portilla Rivera et al. (2007)              |
| *Lb. pentosus* ATCC 8041     | 2              | Mixture           | Sugarcane bagasse (acid hydrolysis; hemicellulosic fraction only)                   | No | No             | 61.5                 | MRS        | B            | 37     | 33   | 32.5     | -      | Wischral et al. (2019)                     |
| Strain                        | Metabolism (a) | Isomers of LA metabolism | Substrate and pretreatment | HH Detoxification | Initial sugars (g/L) | Nutrients                                                                 | Fermentation T (°C) | t (h) | LA (g/L) | Y (g/g) | Reference                                                                 |
|------------------------------|----------------|--------------------------|-----------------------------|-------------------|---------------------|---------------------------------------------------------------------------|---------------------|-------|----------|---------|---------------------------------------------------------------------------|
| *Lb. pentosus* ATCC 8041    |                |                          |                              |                   |                     | No, 80, 90, 100% solids, both fractions, 90% solids, NaOH (1 M), 100 °C | Yes, No             | 53    | 106.2    | 0.93    | Wichanal et al. (2019)                                                    |
| *Lb. rhamnosus* B108         |                |                          |                              |                   |                     | 45 mL/g, YE 2 g/L, SSF, Co 37°C, 48 h, 57 g/L LA                          | No, No             | 60    | 28       | 0.64    | Pleissner et al. (2017)                                                   |
| *Lb. rhamnosus* B108         |                |                          |                              |                   |                     | 5 g/L NaOH, 1 M, 90 °C, 1 h, only solid fraction, 10% biomass             | No, No             | 60    | 13.88    | -       | Zhang et al. (2016)                                                      |
| *Streptococcus* sp. A620     |                |                          |                              |                   |                     | ~67, 37°C, 50% Biomass                                                   | No, No             | 60    | 20.95    | 0.70    | Cui et al. (2011)                                                        |
| *Rhizopus oryzae* NLX-M-1    |                |                          |                              |                   |                     | 75 g/L NaOH, 100 °C, 1 h, only solid fraction, 20% biomass, evaporation   | No, No             | 60    | 60.5     | 0.64    | B                                                                          |

(a) 1: homolifermentative (only hexoses); 2: facultative homolifermentative (hexoses and heterolifermentative for pentoses); 3: obligate heterolifermentative (hexoses and pentoses); 4: homolifermentative (hexoses and pentoses)
(b) LA: lactic acid, EH: enzymatic hydrolysis, B: batch, FB: fed-batch, SSF: simultaneous saccharification and fermentation, Co: coculture, Y: lactic acid yield, MRS: Man-Rogosa-Sharp’s nutrients, YE: yeast extract, CSL: corn steep liquor
(c) Not commercially available
Prospects for the use of thermotolerant bacteria in bioprocesses

Through pretreatment optimisation and strain screening, it was possible to ferment vine-shoot hydrolysates with *B. coagulans* without the need of detoxification steps, yet obtaining higher L(+)-lactic acid concentrations than previously reported with strains of the order Lactobacillales. The use of the thermotolerant bacteria *B. coagulans* implied several advantages, such as working under aerobic and non-sterile conditions or the efficient use of wine lees as single additional nutrient to vine-shoot hydrolysates. Although the lactic acid concentration obtained in the present work (~30 g/L) is insufficient from the economical and industrial point of view, this study opens the way to new microbiological processes where other lignocellulosic or agri-food biomasses could be employed as feedstocks for L(+)-lactic acid production. Numerous non-edible feedstocks have been studied recently for lactic acid production (Table 4). According to López-Gómez et al. (2020), the use of kitchen wastes and certain food wastes could allow reaching about 100 g/L lactic acid, whereas the application of non-sterile conditions could reduce process costs by 10–15%. Alternative methods for lignocellulosic biomass pretreatment have been recently proposed for lactic acid biosynthesis, including the use of ionic liquids (Grewal and Khare 2018). Therefore, future research on lactic acid production by thermotolerant bacteria from different feedstocks is still needed to improve available pretreatment and fermentation methods and increase lactic acid concentrations and yields with the objective of implementing viable biorefineries.

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Data availability The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

Conflict of interest The authors declare no competing interests.

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