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ORIGINAL PAPER

Homo- and heterofermentative lactobacilli differently affect sugarcane-based fuel ethanol fermentation

Thiago Olitta Basso · Fernanda Sgarbosa Gomes · Mario Lucio Lopes · Henrique Vianna de Amorim · Gillian Eggleston · Luiz Carlos Basso

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Abstract Bacterial contamination during industrial yeast fermentation has serious economic consequences for fuel ethanol producers. In addition to deviating carbon away from ethanol formation, bacterial cells and their metabolites often have a detrimental effect on yeast fermentative performance. The bacterial contaminants are commonly lactic acid bacteria (LAB), comprising both homo- and heterofermentative strains. We have studied the effects of these two different types of bacteria upon yeast fermentative performance, particularly in connection with sugarcane-based fuel ethanol fermentation process. Homofermentative *Lactobacillus plantarum* was found to be more detrimental to an industrial yeast strain (*Saccharomyces cerevisiae* CAT-1), when compared with heterofermentative *Lactobacillus fermentum*, in terms of reduced yeast viability and ethanol formation, presumably due to the higher titres of lactic acid in the growth medium. These effects were only noticed when bacteria and yeast were inoculated in equal cell numbers. However, when simulating industrial fuel ethanol conditions, as conducted in Brazil where high yeast cell densities and short fermentation time prevail, the heterofermentative strain was more deleterious than the homofermentative type, causing lower ethanol yield and out competing yeast cells during cell recycle. Yeast overproduction of glycerol was noticed only in the presence of the heterofermentative bacterium. Since the heterofermentative bacterium was shown to be more deleterious to yeast cells than the homofermentative strain, we believe our findings could stimulate the search for more strain-specific antimicrobial agents to treat bacterial contaminations during industrial ethanol fermentation.

Keywords Yeast · Lactic acid bacteria · Bacterial contamination · Homofermentative · Heterofermentative · Fuel ethanol

T. O. Basso (✉)
Novozymes Latin America Ltda., Rua Professor Francisco Ribeiro 683, Araucária 83707-660, Brazil
e-mail: to.basso@gmail.com

F. S. Gomes · L. C. Basso
Departamento de Ciências Biológicas, Escola Superior de Agricultura Luiz de Queiroz, Universidade de São Paulo, Av. Pádua Dias, 11, Piracicaba, SP CEP 13418-900, Brazil

M. L. Lopes · H. V. de Amorim
Fermentec, Av. Antônia Pazzinato Sturion, 1155, Piracicaba, SP CEP 13420-640, Brazil

G. Eggleston
SRRC-USDA-ARS, 1100 Robert E. Lee Boulevard, New Orleans, LA 70124, USA

Introduction

Energy crises and environmental concerns have turned bioethanol into an attractive renewable fuel source. Brazil, using sugarcane as substrate, is one of the

largest ethanol biofuel producers with the most favourable energy balance (Basso et al. 2008; Della-Bianca et al. 2013). Nevertheless, due to the nature of the industrial process and the large volumes of processed substrate, aseptic conditions are never achieved. For this reason, industrial fermentations proceed with microbial contamination, predominantly caused by bacteria. This is considered a major drawback that deviates sugars away from ethanol formation and brings detrimental effects upon yeast fermentative performance, such as reduced ethanol yield, yeast cell flocculation, and low yeast viability (Serra et al. 1979; Amorim and Oliveira 1982; Oliva-Neto and Yokoya 1994; Narendranath et al. 1997; Bayrock and Ingledew 2004).

The majority of the bacterial contaminants encountered during ethanol fermentation comprise lactic acid bacteria (LAB) (Gallo 1990; Lucena et al. 2010), most likely because of their higher tolerance towards acidic pH and high ethanol titres when compared to other microorganisms (Kandler 1983; Kandler and Weiss 1986; Skinner and Leathers 2004). Studies that investigated the identity of these contaminants during yeast fermentation in Brazilian ethanol plants found that *Lactobacillus* was the most abundant genus (Gallo 1990; Lucena et al. 2010). LAB are traditionally classified in two metabolic sub-groups according to the pathway used to metabolise hexose sugars: homo- and heterofermentative (Kandler 1983). Moreover, bacteria isolates from industrial fermented sugarcane substrates have shown to include both homo- and heterofermentative strains (Costa et al. 2008).

Homofermentative bacteria dissimilate hexoses through glycolysis, where fermentation of 1 mol of hexose results in the formation of 2 mol of lactic acid and 2 mol of ATP. In comparison, in heterofermentative bacteria another pathway is active (Kandler and Weiss 1986), and hexoses are converted to equimolar amounts of lactic acid, ethanol or acetate, and carbon dioxide, yielding 1 mol of ATP per mol of hexose fermented (Cogan and Jordan 1994; Axelsson et al. 1993). With the conversion of acetyl phosphate to acetate instead of ethanol, an additional ATP can be produced. Then, regeneration of surplus NAD^+ must be achieved by means of an alternative electron acceptor. Under aerobic conditions, oxygen may serve as the electron acceptor (Condon 1987), but under anaerobic or even oxygen-limited conditions, fructose may be reduced to mannitol (von Weymarn et al. 2002). Therefore fructose seems to be an important co-substrate for heterofermentative lactobacilli, but most of the available data reporting on the bacterial effects on yeast fermentation deal with glucose-based substrates.

Mannitol is considered a very sensitive indicator of sugarcane and sugar beet deterioration and bacterial contamination during industrial fuel ethanol fermentation (Steinmetz et al. 1998; Eggleston et al. 2007). It is well known that both substrates presented an 1:1 ratio of glucose:fructose, since sucrose is the prevalent sugar in the aforementioned feedstocks. This way mannitol can predict sucrose losses due to bacterial dextran synthesis resulting in problems such as viscosity, evaporation, crystallization and, to a lesser extent, poor filterability in sugarcane factories (Eggleston et al. 2004). Another important indicator of bacterial contamination is lactic acid. This organic acid was considered a reliable indicator of bacterial contamination during industrial ethanol fermentation (Alves 1994; Narendranath et al. 1997). However, the formation of D- and L-lactic acids by industrial lactobacilli isolates (Costa et al. 2008), as a result of varying proportions of enzymatic activities (Garvie 1980; Kandler 1983; Viana et al. 2005; Goffin et al. 2005), may bring confusing results depending on the technique employed to quantify this by-product. This is because most of the commercial enzymatic kit usually employed in the routine analysis of lactic acid, normally detect only the L-form.

Due to the increasing evidence of the harmful effects of lactobacilli upon yeast industrial fermentations, and the recent observation of the co-existence of bacterial strains with different types of metabolism in fermentation environments, we deemed it desirable to evaluate the microbial interactions between yeast and lactobacilli in a sugarcane-based substrate. Thus, the aim of the present study was to evaluate the effects of LAB with both metabolic types (homo- and heterofermentative) on yeast fermentation, using sugarcane-related substrates. It is expected that the presence of fructose in such substrate may stimulate heterofermentative bacteria growth during the prevailing anaerobic condition of ethanol fermentation.

Materials and methods

Microorganisms

The bacterial strains used in this work were FT-025B (homofermentative *Lactobacillus plantarum*) and FT-230B (heterofermentative *Lactobacillus fermentum*), both isolated from fermented molasses in industrial ethanol plants located in Brazil by Fermentec Ltda (Piracicaba, Brazil). An industrial yeast strain (CAT-1) of *Saccharomyces cerevisiae* (Basso et al. 2008; Stambuk et al. 2009) also supplied by Fermentec Ltda (Piracicaba, SP) was used in the experiments of co-cultivation with the two *Lactobacillus* strains.

Preparation of bacterial and yeast inocula

Lactobacilli were grown in 250 ml Erlenmeyer flasks containing 50 ml of Mann, Rogosa and Sharpe (MRS) broth. The flasks were incubated in an incubator-shaker at 150 rpm and 32 °C for 48 h. Growth of the microorganisms was estimated by means of viable cell counting using light microscopy with differential staining, as described by Oliveira et al. (1996). An appropriate volume was used to inoculate fresh media to give ca. 10^6 viable cells ml^{-1}. The yeast strain was grown in 250 ml Erlenmeyer flasks with 50 ml of YPD media, containing (per l): 20 g yeast extract, 20 g peptone and 20 g glucose. The flasks were incubated with shaking at 150 rpm and 32 °C for 24 h. Growth of the microorganism was estimated by viable cell counting with differential staining, according to Zago et al. (1989). An appropriate yeast volume suspension was used to inoculate fresh media to give ca. 10^6 viable cells ml^{-1}.

Bacterial growth experiments

Aliquots of bacterial inocula were transferred to 250 ml Erlenmeyer flasks containing 50 ml of growth media to give an initial concentration of 10^6 viable cells ml^{-1}. The growth media consisted of (per l): 5 g yeast extract; 5 g peptone; 10 g fructose; 10 g glucose; 2 g K_2HPO_4; 0.2 g $MgSO_4$; 0.01 g $MnSO_4$ (von Weymarn et al. 2002). The flasks were incubated at 150 rpm and 32 °C for 48 h. Culture samples from appropriate time intervals were collected for cell counting, as described above, and metabolite analysis.

Co-cultivation experiments with yeast and bacteria

Each bacterium was co-cultivated with the yeast strain at a starting cell number of 10^6 viable cells ml^{-1} for each microorganism in screw-capped tubes containing 10 ml of the medium described above and incubated at 32 °C at 150 rpm. Samples were taken at pre-determined intervals for metabolite and sugar analysis. Samples were also analysed for cell viability and cell counts, for both bacterial and yeast cells, as described above.

Co-cultivation experiments simulating the industrial fermentation conditions with cell recycle

Fermentation trials using both bacteria and yeast cells were performed at 33 °C in 150 ml centrifuge vials, simulating the industrial fermentation process as far as possible (Basso et al. 2008). A substrate containing 20 % (w/v) total sugar (composed of sugarcane juice and molasses—50 % of the sugar obtained from each source) was added to a yeast suspension in three equal portions spaced by 1.5 h intervals. The yeast suspension with 33 % yeast biomass (w/v, wet weight) represented 30 % of the total fermentation volume, similar to industrial conditions. The vials were inoculated with the bacterial strains (homo- or heterofermentative bacteria, separately) to give a final concentration of 10^7 viable cells ml^{-1}. After fermentation had ceased (determined gravimetrically), yeast cells were collected by centrifugation ($800\times g$ for 20 min), weighed and reused in a subsequent fermentation, comprising five fermentation cycles. Cell counting and viability were performed in the fermented medium and the supernatant was used for metabolite and sugar analysis.

Kinetic experiments simulating the industrial fermentation conditions with cell recycle

Fermentation trials using strain CAT-1 were also undertaken at 33 °C in 500 ml shake flasks, simulating the fed-batch process of industrial ethanol fermentation (Basso et al. 2008). Fermentation must (60 ml) containing 20 % (w/v) total reducing sugars (composed of sugarcane juice and molasses—50 % of the sugar obtained from each source) was fed into the yeast slurry (30 ml, containing 9 g yeast fresh weigh

or 2.25 g dry weigh) at a constant rate using a peristaltic pump. Flasks were fed for 4 h and fermentation was terminated after 6 h, comprising a total fermentation time of 10 h, similarly as is performed in industrial conditions. After fermentation had ceased, yeast cells were collected by centrifugation (800×*g* for 20 min), weighed and reused in a subsequent fermentation cycle. Samples were taken at regular time intervals and used for metabolite and sugar analysis.

Quantification of extracellular metabolites

Culture samples from appropriate time intervals were collected and centrifuged (800×*g* for 10 min) and the supernatant was filtered on nitrocellulose membranes with pore size of 0.22 microns (Millipore, Bellerica, MA). Organic acids, ethanol and glycerol were quantified by HPLC, on a HPX-87H column, using 5 mM H_2SO_4 as the mobile phase at a flow rate of 0.6 ml min^{-1} and refractive index detection. Sugars (glucose, fructose and sucrose), glycerol and mannitol were determined by HPAEC (high-performance anion exchange chromatography), using Dionex (Sunnyvale, CA) DX-300 equipment, on a Carbo-Pac PA1 4 × 250 mm column, flushed with 100 mM NaOH as the mobile phase at a flow rate of 0.9 ml min^{-1} and detected by pulsed-amperometric detection as described elsewhere (Basso et al. 2008).

Results and discussion

Growth of bacterial strains on glucose/fructose mixtures

During industrial fuel ethanol fermentation, conducted with molasses and sugarcane juice, the substrate is composed of sucrose and near equal amounts of glucose and fructose; the main sugar, sucrose, is continuously hydrolysed by yeast cell invertase during fermentation. When growing bacteria in a mixture of equal amounts of glucose and fructose, i.e., what normally occurs with industrial substrates, homo- and heterofermentative strains showed different patterns of sugar consumption. In cultures of the homofermentative strain, glucose uptake was faster than fructose, while the heterofermentative strain consumed fructose much faster than glucose (Fig. 1). It has been previously shown that mannitol is produced when fructose or sucrose is used as substrates (von Weymarn et al. 2002) and the present data show that fructose was completely exhausted by the heterofermentative strain. Since this sugar may act as an electron acceptor during oxidative metabolism (in order to regenerate oxidised cofactors), mannitol was generated at nearly equivalent amounts of the consumed fructose (Fig. 1). Lactic acid was the only metabolite produced by the homofermentative strain FT-025B, whereas the heterofermentative strain FT-230B produced mannitol as the major metabolite, followed by lactic and acetic acids, respectively. The production of lesser amounts of lactic acid and in particular acetic acid confirms the results of Huet (2011) who grew the heterofermentative *Leuconostoc mesenteroides* on sugar beet juice as a substrate. These results confirmed their metabolism

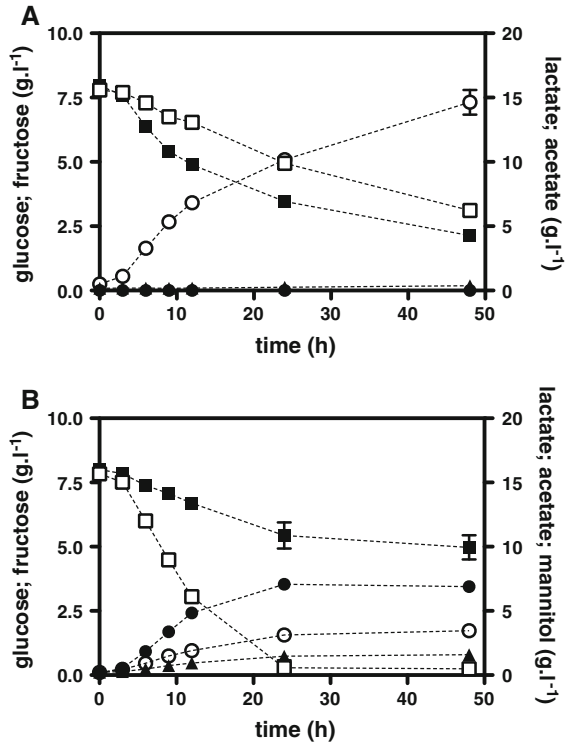

Fig. 1 Distinct pattern of sugar utilisation by homo- and heterofermentative lactobacilli. Glucose (*filled square*), fructose (*open square*), mannitol (*filled circle*), lactic acid (*open circle*), and acetic acid (*filled triangle*) extracellular concentrations during growth of strain FT-025B (*L. plantarum*; homofermentative) (**a**) and FT-230B (*L. fermentum*; heterofermentative) (**b**) on a medium containing equal amounts of glucose and fructose (around 7.5 g l^{-1} each). Results are the average of triplicate experiments and *error bars* correspond to the standard deviations

Table 1 Total cell count and viabilities of yeast and bacteria, and medium pH after 24 h co-cultivation experiments

Yeast and bacterial strains	Yeast cell count at 24 h (10^7 cell ml^{-1})	Yeast viability (%)	Bacterial cell count at 24 h (10^7 cell ml^{-1})	Bacterial viability	Medium pH after 24 h
CAT-1	6.37 ± 1.08	99.0 ± 0.5	na	na	4.94 ± 0.08
CAT-1 + homofermentative FT-025B	6.51 ± 0.45	65.0 ± 5.6	70.8 ± 12.5	22.4 ± 0.7	3.53 ± 0.01
CAT-1 + heterofermentative FT-230B	6.52 ± 0.30	97.5 ± 2.0	47.70 ± 3.91	53.0 ± 5.9	4.19 ± 0.05

The media were inoculated with $\sim 10^7$ viable cells ml^{-1} of each bacteria and yeast and the experiments were done in triplicate. Results are the average ± standard deviation of three replicates. Initial inoculum size at 0 h in number of viable cells 10^6 ml^{-1}: CAT-1 = 1.12; FT-025B = 0.96; FT-230B = 0.58

na not applicable

types when growing on hexoses, as being homofermentative (strain *L. plantarum* FT-025B), and heterofermentative (strain *L. fermentum* FT-230B).

Mixed cultures of lactobacilli and *S. cerevisiae*

To investigate the interactions between homo- and heterofermentative lactobacilli and *S. cerevisiae* at the physiological level, three types of cultures were compared: pure cultures of *S. cerevisiae* and two binary mixed cultures in which both species (*S. cerevisiae* and homo- or heterofermentative bacteria) shared the same environment. When co-cultivating these two bacteria with a widely employed industrial *S. cerevisiae* strain CAT-1 (Basso et al. 2008), yeast cell viabilities drastically decreased in the presence of the homofermentative bacterium, whereas no effect was observed with the heterofermentative strain (Table 1). The deleterious effects of the homofermentative bacterium towards yeast cells may be due to the higher lactic acid content (Table 2) in the mixed culture media with this strain, which led to much lower pH values (Table 1) when compared to the control treatment (yeast alone) or to the heterofermentative strain. This suggests that these conditions (higher acidity coupled with high levels of lactic acid) were toxic to the homofermentative bacterium itself, which is evidenced by its lower viability after 24 h cultivation (Table 1) and also when cultivated alone (data not shown). Although it is possible that lactic acid is the main metabolite for decreasing both yeast and bacteria cell viabilities, it was suggested that ethanol is the main factor negatively affecting interactions between *Lactococcus lactis* and *S. cerevisiae* (Maligoy et al. 2008).

Table 2 Concentrations of ethanol, glycerol, acetic acid, lactic acid, and mannitol (in g l^{-1}) in the growth medium, after 24 h growth of mixed cultures of bacteria and yeast cells

Metabolite	CAT-1	CAT-1 + FT-025B	CAT-1 + FT-230B
Ethanol	8.16 ± 0.75	7.36 ± 0.53	7.71 ± 0.60
Glycerol	0.56 ± 0.06	0.51 ± 0.04	0.91 ± 0.09
Acetate	0.10 ± 0.01	0.11 ± 0.01	0.37 ± 0.03
Lactate	0.00 ± 0.00	3.12 ± 0.22	0.83 ± 0.14
Mannitol	0.00 ± 0.01	0.01 ± 0.01	0.40 ± 0.03

Results are the average ± standard deviation of three replicates

It is well known that bacterial contamination increases the formation of glycerol by fermenting yeasts (Alves 1994; Thomas et al. 2001), even though the reason for this phenomenon is still unclear. Suggestions have been made that it is a stress response due to the presence of bacterial metabolites, such as lactic and acetic acids (Thomas et al. 2001). This was observed in this work only when mixed cultures were employed with the heterofermentative strain (Table 2). Surprisingly, in the presence of the homofermentative strain, the glycerol content was slightly lower than the control treatment (inoculated with yeast alone).

Ethanol concentrations were slightly lowered in the presence of both bacterial metabolic types (Table 2). Acetic acid was produced by both yeast and heterofermentative strain. Lactic acid was produced by both bacterial types but to a greater extent by the homofermentative strain (Table 2). Mannitol, in comparison, was only detected when the heterofermentative strain was present (Table 2). These results imply that caution is necessary when utilising mannitol as a chemical index for bacterial contamination in fuel ethanol fermentations as pointed by Eggleston et al.

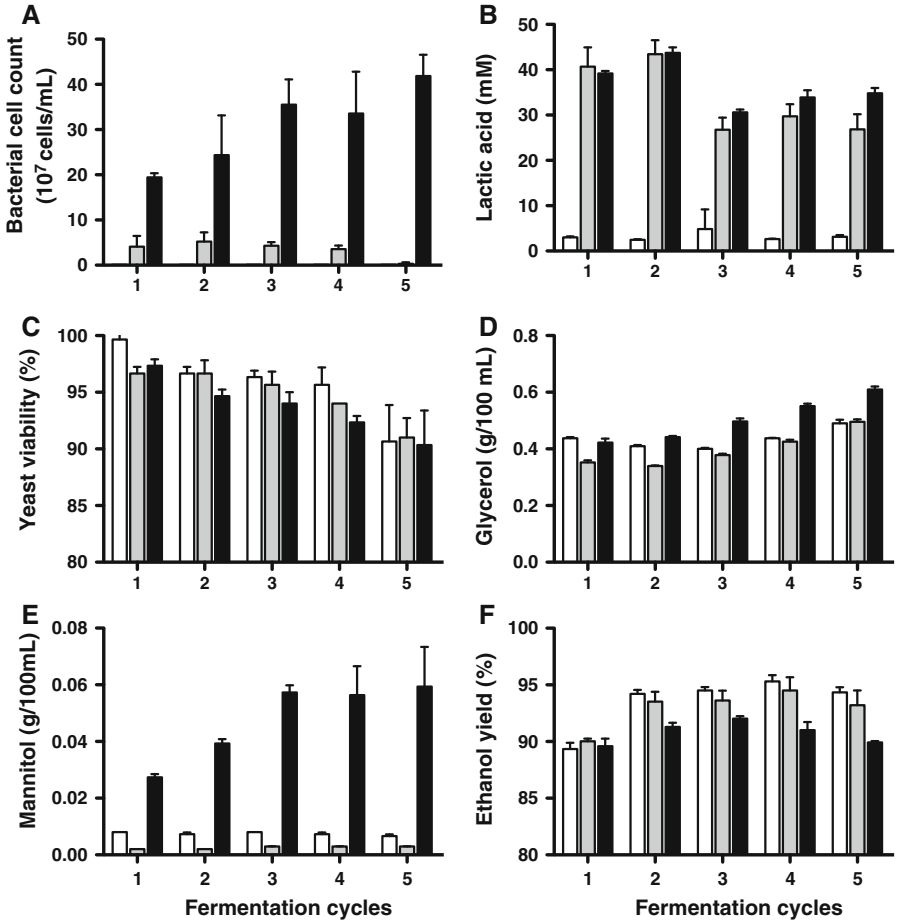

Fig. 2 Influence of lactic acid bacteria on yeast fed-batch fermentation of sugarcane substrate. Bacterial cell numbers (**a**); lactic acid concentration (**b**); yeast viability (**c**); glycerol concentration (**d**); mannitol concentration (**e**); and overall ethanol yield (expressed as the sugar fraction converted into ethanol) (**f**), at the end of each successive yeast fermentation cycle in a fed-batch fermentation of strain *S. cerevisiae* CAT-1 without contamination (*white bars*), co-inoculated with the homofermentative strain (*L. plantarum* FT-025B) (*grey bars*), and co-inoculated with the heterofermentative strain (*L. fermentum* FT-230B) (*black bars*) in sugarcane substrate (cane juice and molasses). In the experimental, the medium was inoculated at the same time with yeast and the bacterial strains. Results are the average of triplicate experiments and *error bars* correspond to the standard deviations

(2007), as bacterial contaminations are due to both homo- and heterofermentative strains. Nevertheless, as the deterioration of both sugarcane (Eggleston et al. 2004, 2007) and sugar beet (Huet 2011) is well known to be caused by contamination with heterofermentative *L. mesenteroides*, the presence of mannitol is still a useful indicator for sugar deterioration.

Effect of lactobacilli on yeast alcoholic fermentation in fuel ethanol conditions

When simulating the industrial fermentation conditions employed in Brazil, i.e., with a very high yeast cell density ($\sim 5 \times 10^8$ yeast cells ml^{-1}), different results were observed. While homofermentative *Lactobacillus* had a higher inhibitory effect upon yeast when at similar cell number (laboratory conditions; Table 1), in industrial fuel ethanol conditions, where higher yeast cell densities occur, the heterofermentative strain was more detrimental, since they succeeded in competing with yeast during fermentation. Figure 2a illustrates that the heterofermentative population increased steadily with cell recycle (yeast cell reuse from one fermentation to another, as employed industrially), whereas the homofermentative population remained at a lower concentration.

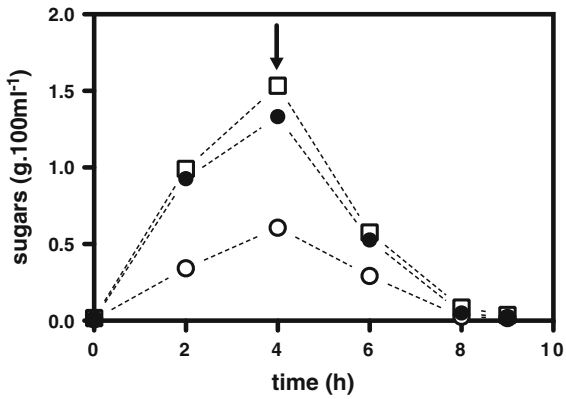

Fig. 3 Residual sugars in the course of fed-batch sugarcane fermentation. Sucrose (*open square*); fructose (*filled circle*); and glucose (*open circle*) concentrations (g/100 ml) in the course of one yeast fermentation cycle in a fed-batch fermentation of strain *S. cerevisiae* CAT-1 without contamination in sugarcane substrate (cane juice and molasses). Results are the average of triplicate experiments and *error bars* correspond to the standard deviations. The *arrow* indicates the time-point in which must feeding was terminated

During yeast fermentation of sugarcane substrates fructose is not only always at a higher concentration than glucose, but also typically the last sugar to be consumed by the fermenting yeast. This is corroborated by the fed-batch fermentation performed with CAT-1 strain, in which fructose concentration remained always higher than glucose concentration in the time course of the fermentation (Fig. 3). Both sugars peaked at 4 h, when must feeding was discontinued. It is possible that such conditions may favour the heterofermentative bacterial strain as observed in this study. Although homo- and heterofermentative strains have been isolated from industrial ethanol fermentations in Brazil (Costa et al. 2008), no data on the prevalence of these bacterial types are currently available.

The reduced growth of the homofermentative bacteria (the greater producer of lactic acid) under industrial fermentation conditions, in comparison to the heterofermentative strain, resulted in similar lactic acid contents for both strains (Fig. 2b). This most likely caused similar toxic effects towards yeast and explains the similar declines in cell viability. For fermentation run with CAT-1 alone, the average pH in the fermented must at the end of each fermentation cycle was around 4.9. The drop in pH was similar for both bacterial strains, being around 4.6. Of particular interest is the observation that yeast cell viability was not affected by the presence of both bacteria in a five-cycle span (Fig. 2c), even when lactic acid titres were higher than 3 g l^{-1}, when a viability drop is normally observed with other yeast strains (Oliva-Neto and Yokoya 1994; data not shown). This suggests a higher stress tolerance of CAT-1 strain of *S. cerevisiae*.

Whilst none of the lactobacilli growing alone produced glycerol (Fig. 1), the production of this compound by yeast increased in the presence of the heterofermentative strain (Fig. 2d). Similar observations have been made in corn mash fermentations contaminated with lactobacilli (Thomas et al. 2001). However, for the homofermentative *Lactobacillus*, the opposite trend was observed. Glycerol levels were lower than the treatment when yeasts were growing alone (Fig. 2d). These results were also observed during co-cultures of yeast and lactobacilli (Table 2). It is likely that the homofermentative strain consumes the glycerol produced by yeast under anaerobic fermentation conditions.

As previously discussed, mannitol production was only significant in the presence of the heterofermentative bacteria (Figs. 1b, 2e), and mannitol concentrations paralleled growth of the bacterial population, which makes this metabolite a sensitive indicator bacterial contamination (Eggleston et al. 2007).

Ethanol yield was decreased in the presence of both bacteria, but to a greater extent with the heterofermentative strain, when a higher amount of sugar was diverted into the production of glycerol and mannitol. As a result of the higher growth rate and higher concentration of metabolites formed by the heterofermentative strain (*L. fermentum*), the ethanol yield was more severely affected when the fermentations were contaminated with this type of bacteria (Fig. 2f). It is frequently observed in industrial fuel ethanol plants that when the number of bacterial cells increases in the fermentor, ethanol yields are negatively affected. Although other factors may vary along with the bacterial population, a significant correlation exists between the number of cells and the ethanol yield at an industrial scale. For example, it has been calculated that around 20,000 litres of ethanol are lost per day (in a medium size Brazilian distillery) when bacterial contamination increases from 10^7 to 10^8 cell ml^{-1} (Amorim et al. 2009).

The experiments presented in this study were also performed with other fuel ethanol yeasts in combination with others homo- and heterofermentative

bacterial strains (previously isolated from industrial fermentations), and similar trends were observed (data not shown). To the best of our knowledge, this is the first report on the differential effects of homo- and heterofermentative lactobacilli upon yeast and ethanol yield in conditions of biofuel production from sugarcane substrates.

While homofermentative lactobacilli presented higher inhibitory effects upon yeast when present in equal cell numbers, in industrial fuel ethanol fermentations using sugarcane and high yeast cell densities, heterofermentative lactobacilli were more deleterious, due to their success in competing with yeast for sugars during fermentation. Both bacterial metabolic types caused reduced ethanol yield during yeast fermentation, but this effect was more pronounced with the heterofermentative strain under fuel ethanol conditions. Increased glycerol production by yeast coupled to higher concentration of bacterial produced metabolites (lactic and acetic acids, plus mannitol) and higher bacterial growth, all led to decreased ethanol yield in fuel ethanol fermentation contaminated with the heterofermentative strain. Studies are in progress on the prevalence of homo- and heterofermentative lactobacilli in Brazilian fuel ethanol plants and on the differential effect of these two types of bacteria on glycerol production by yeast.

Overall, we observed that when simulating the industrial fermentation process, heterofermentative bacterium was more deleterious than the homofermentative strain, causing lower ethanol yield and out competing yeast cells during cell recycle. These observations are in agreement with previous studies that investigated separately these two types of bacterial strains in sugarcane-based substrates (Oliva-Neto and Yokoya 1994; Souza et al. 2012). In view of these results, we believe our findings could stimulate the search for more strain-specific antimicrobial agents to treat bacterial contaminations during industrial ethanol fermentations. Antibiotics are regularly used in the industrial process increasing the ethanol production cost and more recently residual levels of such antibacterial compounds in dried yeast (a distillery by-product) are restricting their use as a protein source for animal or human consumption. This work suggests that a selective heterofermentative LAB population control could result in a lower dosage of antimicrobials (and lower residual levels), thus decreasing significantly the overall ethanol production costs.

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

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