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Mutually stimulating interactions between lactic acid bacteria and *Saccharomyces cerevisiae* in sourdough fermentation

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ABSTRACT

Interactions between microorganisms are key to their performance in food habitats. Improved understanding of these interactions supports rational improvement of food fermentations. This study aimed at identifying interactions between lactic acid bacteria and yeast during sourdough fermentation. Therefore, the lactic acid bacteria *Lactobacillus plantarum* and *Lactobacillus sanfranciscensis* were co-cultured with the yeast *Saccharomyces cerevisiae* in a newly developed medium, as well as *in situ* in a sourdough-like environment. *L. sanfranciscensis* was found to be stimulated by a secreted factor of *S. cerevisiae* in any tested *in vitro* situation, whereas *L. plantarum* and *S. cerevisiae* stimulated each other only in the presence of glucose, fructose and lactose as carbon source, but not with galactose, maltose, sucrose and starch. Moreover, it was demonstrated that *L. sanfranciscensis* is stimulated by CO_2 and another yet to be identified factor produced by yeast in a sourdough-like environment. In conclusion, *S. cerevisiae* produces growth factors stimulatory to lactic bacteria. The nature and the efficacy of these growth factors depend on the target species and on the supplied carbon source.

1. Introduction

Although many food fermentation processes are typically performed by complex consortia of microbes, most fermentation research describes pure cultures. However, recent studies showed that associations within complex consortia are important for the performance of each member and, consequently, for the microbial community as a whole (Franco & Pérez-Díaz, 2013; Sieuwerts, de Bok, Hugenholtz, & van Hylckama Vlieg, 2008a), thereby influencing the characteristics such as taste and texture of the fermented end-product (Irlinger et al., 2012; Smid & Lacroix, 2013). Antagonistic interactions such as competition for a substrate and the production of organic acids have been responsible for improved shelf-life of foods for millennia (Tamime, 2002), whereas positive interactions have been shown to promote desired product characteristics such as flavour formation in wine (Alexandre, Costello, Remize, Guzzo, & Guilloux-Benatier, 2004) and increased viscosity in yoghurt (Sieuwerts et al., 2010). Yeast and lactic acid bacteria are known to co-exist and co-operate in fermented beverages and food products such as wine (Alexandre et al., 2004; Capozzi et al., 2012; Spano & Massa, 2006), kefir (Cheirsilp Shoji, Shimizu & Shioya, 2003), different types of cheese (Corsetti, Rossi, & Gobbetti, 2001; Mounier et al., 2005; Viljoen, 2001), and sourdough (De Vuyst & Neysens, 2005; Gobbetti, Corsetti, & Rossi, 1994a; Gobbetti & Corsetti, 1997). Typically, the composition of the microbial community in these products is stable (De Vuyst & Neysens, 2005; Sieuwerts et al., 2008a), which indicates that there are underlying mechanisms that prevent exclusion by competition. However, information about these exact mechanisms is scarce. It is assumed that often fine-tuned mutually beneficial interactions between the interacting species occur, providing a solid basis for co-existence and preventing the introduction of other microbial species into the habitat. Hence, it is highly relevant to elucidate the interactions between fermenting microbes in industrially relevant food matrices.

Type I sourdoughs are maintained by backslopping, and typically consist of microbial consortia of lactic acid bacteria (LAB) and yeasts. The exact microbial composition of these doughs depends on the type and quality of the flour used (De Vuyst & Neysens, 2005). Sourdoughs have been documented to contain *Saccharomyces cerevisiae* combined with either *Lactobacillus plantarum* or *Lactobacillus sanfranciscensis*. The microbial stability of these doughs is, at least in part, caused by multiple trophic interactions between the consortium members (Gobbetti et al., 1994a; Gobbetti & Corsetti, 1997) that depend on the availability of components in the environment. One example is that *S. cerevisiae* was

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Abbreviations

CDM	Chemically defined medium
LAB	Lactic acid bacteria

shown to secrete valine and leucine to the benefit of several LAB tested, among which *L. plantarum* (Gobbetti, Corsetti, & Rossi, 1994b). In addition, sourdough-associated LAB and yeasts were shown to stimulate each other's growth by maltose hydrolysis (to the benefit of *S. cerevisiae* strains which are unable to hydrolyse and utilize maltose) and sucrose hydrolysis (to the benefit of *L. plantarum* that grows more optimal on glucose) (Gobbetti et al., 1994a). Notably, maltose and sucrose are both major constituents of flour (USDA National Nutrient Database for Standard Reference 25, 2012). However, evidence for mutualistic interactions in dough that are not based on carbon source and amino acid exchange in sourdough is largely unavailable (De Vuyst et al., 2016).

In the present study, we provide evidence for the presence of mutualistic interactions between two LAB species, *L. plantarum* and *L. sanfranciscensis*, and *S. cerevisiae* that go beyond the above-described interactions. Evidence is presented for the existence of a non-proteinaceous component produced by *S. cerevisiae* that stimulates *L. sanfranciscensis*. Mutualistic interactions between *L. plantarum* and *S. cerevisiae* that only occur when these species are co-cultured on specific carbon sources were also found. In addition, it was demonstrated by using a non-respiring *S. cerevisiae* mutant that *L. sanfranciscensis* is stimulated by CO_2 produced by yeast in a sourdough-like environment.

2. Materials and methods

2.1. Strains and culture methods

Lactobacillus plantarum WCFS1 and *Lactobacillus sanfranciscensis* TMW 1.1304 were grown in MRS broth (Oxoid, Basingstoke, England) at 30 °C.

The beer and bakery yeast *Saccharomyces cerevisiae* CEN.PK113-7D (Nijkamp et al., 2012) was grown in YPD broth ((10 g l^{-1} yeast extract (Difco, Franklin lakes, NJ), 10 g l^{-1} peptone (Difco), 20 g l^{-1} glucose (Scharlau)) at 30 °C. Cultures were maintained as frozen stocks at −80 °C cell cultures containing 22% glycerol (Scharlau, Sentmenat, Spain). Cell counts were assessed by spot-plating serial dilutions of lactic acid bacteria and yeast on MRS agar (Oxoid) and YPD agar (YPD broth supplemented with 20 g l^{-1} agar (Sigma-Aldrich, Zwijndrecht, the Netherlands)) at 30 °C as described in (Sieuwerts, de Bok, Mols, de Vos & van Hylckama Vlieg. 2008b), respectively.

Chemically defined medium (CDM) that supports growth of lactic acid bacteria (LAB) and yeasts was developed by combining the highest concentration of each component from a medium described by Chervaux and co-workers for *Lactobacillus bulgaricus* (Chervaux, Ehrlich, & Maguin, 2000) and a medium described by Verduyn and co-workers for yeasts (Verduyn, Postma, Scheffers, & Van Dijken, 1992), except that no ergosterol and carbon source were added to the medium. A full description of the CDM can be found in Table S1. Media consisting of 0.8 vol CDM without carbon source and 0.2 volume of solutions with a range of carbohydrates (glucose, galactose, fructose, maltose, sucrose, lactose (all Scharlau) and starch (Sigma-Aldrich)) were prepared, resulting in final concentrations of 0–20 g l^{-1} of carbon source in the experiments.

A batch of full-grain wheat flour was purchased at a local mill (Wageningen, the Netherlands) and stored in air-tight plastic bags, followed by gamma irradiation (25 kGy) (Isotron, Ede, the Netherlands). In order to produce dough, sterile flour was mixed with autoclaved deionised water in a ratio of 1 g–1.17 ml, giving a dough yield of 217. This is a higher dough yield than normally, which is for the purpose of fast, efficient and homogeneous mixing of the micro-organisms in the dough.

2.2. Assessment of colony size on agar and dough plates

Serial dilutions of a 24 h culture were spotted on MRS agar plates and on anopore slides (Ingham, van den Ende, Pijnenburg, Wever, & Schneeberger, 2005) that were positioned on top of dough plates after the dough was flattened with a spatula. After growth, the colonies in the appropriate dilution (10–50 colonies in a 10 µl spot with a surface of 0.25 cm^2) were photographed and their average sizes determined using ImageJ 1.46 (2012) as described before (Sieuwerts, de Bok, Mols, de Vos, & van Hylckama Vlieg, 2008b). Using the 'analyze particles' function in ImageJ, a list was acquired that contained the number of colonies and their sizes.

2.3. Assessment of cell counts by quantitative PCR

Mixed cultures of *L. plantarum* and *S. cerevisiae* (0.5 ml) in deep-well 96-wells plates were centrifuged at 2000 × *g* for 10 min, followed by removal of the supernatants. The pellets were resuspended in 100 µl InstaGene Matrix (Bio-rad, Hercules, CA, USA). DNA isolation was performed according to the manufacturers protocol for bacteria. Quantitative PCR was performed in a C-1000 thermal cycler (Bio-rad) using SYBR green master mix (Applied Biosystems, Warrington, UK) and primer sets (Sigma-Aldrich) TGATCCTGGCTCAGGACGAA/TGCAAGCACCAATCAATACCA for *L. plantarum* (Marco, Bongers, de Vos, & Kleerebezem, 2007) and ACATATGAAGTATGTTTCTATATAACGGGTG/TGGTGCTGGTGCGGATCTA for *S. cerevisiae* (Martorell, Querol, & Fernandez-Espinar, 2005). The PCR program contained cycles of 5 s melting at 94 °C and 30 s annealing/elongation at 60 °C.

The output of the qPCR, the Ct values, were calculated back to initial cell counts by using calibration curves with known amounts of *L. plantarum* and *S. cerevisiae* as benchmarks.

2.4. Assessment of optical density, pH, sugar content and fermentation product

Optical density of cultures at 600 nm (OD_{600}) was measured in a Lambda 2 photospectrometer (Perkin Elmer, Waltham, MA, USA) with 1 cm path length. The pH was recorded using a porotrode (Metrohm, Herisau, Switzerland). For fermentation end product determination, proteins and fat were precipitated with 0.1 mol l^{-1} perchloric acid (BDH, Poole, UK). After filtration using a Spartan 30/B (Schleicher & Schuell) filter, the liquid was used for high-pressure liquid chromatography. 25 µl of the solution was injected into an HPX-87H column (BioRad, 300 × 7.8 mm) at 60 °C with a flow of 0.6 ml min^{-1} of 0.005 mol l^{-1} sulphuric acid (Sigma-Aldrich). For sugar determination, 1 g of culture was mixed with 10 ml of a solution composed of 4.55 g l^{-1} $Zn(CH_3COOH)_2.2H_2O$ (BDH), 2.73 g l^{-1} $H_3[P(W_3O_{10})]$ 4.xH_2O (BDH) and 2.9 ml l^{-1} 99.8% acetic acid (BDH). Solutions were filtered with a Spartan 30/B filter prior to chromatography analyses. 25 µl of the solution was injected into an HPX-87H column with a flow of 0.4 ml min^{-1} 0.1 g l^{-1} NaCl (BDH). Components were detected using a RID-10A (Shimadzu, Kyoto, Japan) and ERC-7510 (Erma optical works Ltd, Tokyo, Japan) refraction index meter at 40 °C for organic acids and sugars, respectively. Data were compared to standard series of components and analysed using Chromeleon (Dionex, CA, USA).

Where appropriate, data were compared between experimental conditions or cultures by means of two-tailed T-tests ($P < 0.05$).

3. Results

3.1. L. sanfranciscensis is stimulated by a secreted factor of S. cerevisiae

We first assessed whether growth of *L. sanfranciscensis* and *L. plantarum* was enhanced in the presence of *S. cerevisiae*. This appeared to be the case for *L. sanfranciscensis*, as colonies on MRS agar plates were larger in the vicinity of a colony of *S. cerevisiae* (Fig. S1). By contrast, a

similar effect could not be observed for *L. plantarum* growing simultaneously on plates with *S. cerevisiae*. It was hypothesized that the stimulation of *L. sanfranciscensis* was either due to de-acidification of the medium that results from lactic acid consumption by *S. cerevisiae* (Cheirsilp, Shoji, Shimizu, & Shioya, 2003), or due to the secretion of a growth-stimulating factor by *S. cerevisiae*. Measuring the pH of the agar in different parts of the plate did not provide evidence for local de-acidification. Subsequently, 5 µl droplets from a serial dilution of a culture of *L. sanfranciscensis* were spotted (i) around a yeast colony, (ii) around a well cut out of the agar plate and filled with MRS broth, and (iii) around a well containing cell-free MRS medium in which *S. cerevisiae* had grown. After one, two and three days of incubation, colony sizes were determined. No differences were observed between the colonies in the reference condition (spotted on plain MRS agar) and those spotted around the well filled with MRS broth, but both the spent medium and the live *S. cerevisiae* colony clearly led to increased *L. sanfranciscensis* colony size (Fig. 1). The stimulatory effect was not affected by either proteinase K (Sigma-Aldrich) treatment (3 h, 37 °C) and/or heat treatment (10 min, 85 °C) of the spent medium. This indicates that the stimulatory compound of *S. cerevisiae* is thermostable and probably not proteinaceous.

As *S. cerevisiae* and *L. sanfranciscensis* co-occur in sourdough, it was tested whether this stimulatory effect also occurs on dough plates. A serial dilution of a *S. cerevisiae* culture was spotted on dough plates, resulting in 0 to approximately 10^6 cells per cm^2. Anopore strips were placed on top of the dough plates and serial dilutions of a *L. sanfranciscensis* culture were spotted onto the strips. This showed that the bacterium grew best at intermediate concentrations of *S. cerevisiae* (Fig. S1) and indicated that bacterial growth is stimulated on dough as well.

3.2. Effect of CO_2 on growth of *L. sanfranciscensis* in dough

It is established that *S. cerevisiae* produces substantial amounts of CO_2 during growth, responsible for leavening of the dough (Coppola, Pepe, & Mauriello, 1998). Similarly, many lactic acid bacteria are stimulated by CO_2 (Stevens et al., 2008) and inhibited by the presence of reactive oxygen species that are produced in an aerobic environment (An et al., 2011). Therefore, we hypothesized that the production of CO_2 and/or removal of O_2 by *S. cerevisiae* may improve the performance of *L. sanfranciscensis* in sourdough. To test this hypothesis, doughs of 10 g were inoculated with 10^5 *L. sanfranciscensis* cells and incubated at 30 °C under five conditions: (i) a standard spherical dough placed under aerobiosis, (ii) the dough spread out on a Petri dish to allow optimal influx and efflux of gasses under aerobiosis, (iii) the spread dough under anaerobiosis (85% N_2, 10% H_2, 5% CO_2), and (iv) the standard dough inoculated with *L. sanfranciscensis* together with 10^6 *S. cerevisiae* or (v) its non-respiring variant *rip1*Δ. Growth of *L. sanfranciscensis* was assessed by plating after 8 h. Both aerobic doughs showed comparable cell numbers (LOG 5.7 ± 0.1 g^{-1} in the standard dough and LOG 5.4 ± 0.1 g^{-1} in the spread dough) and significantly lower than the anaerobic dough (LOG 6.4 ± 0.2 g^{-1}) and the mixed culture dough containing the respiring yeast (LOG 6.7 ± 0.1 g^{-1}). The mixed culture dough containing the *rip1*Δ strain showed an intermediate result (6.0 ± 0.1 g^{-1}). Taken together, these experiments indicate that CO_2 production and/or O_2 consumption by *S. cerevisiae* plays a role in stimulation of *L. sanfranciscensis* in the early stages of dough fermentation when O_2 is still present.

3.3. *L. plantarum* and *S. cerevisiae* stimulate each other dependent on the carbon source

HPLC analysis of the dough indicated that it contained detectable amounts of the carbohydrates glucose (5,68 ± 0,05 mg g^{-1}), galactose (0,21 ± 0,02 mg g^{-1}), fructose (2,90 ± 0,09 mg g^{-1}), maltose (1,67 ± 0,05 mg g^{-1}) and starch (230 ± 8,02 mg g^{-1}). In order to identify medium conditions that stimulate co-cultivation lactic acid bacteria and yeasts, CDM supplemented with these sugar sources as well as sucrose and lactose was inoculated with 10^5 cells of *L. plantarum*, *L. sanfranciscensis*, *S. cerevisiae* or combinations thereof. After propagating cultures twice at 30 °C for 24 h, final cell counts were assessed by qPCR. Unfortunately, the specific nutritional requirements of *L. sanfranciscensis* did not allow growth in this medium. In none of the mixed cultures of *L. plantarum* and *S. cerevisiae* one of the two species was outcompeted. Moreover, in certain conditions either growth of *L. plantarum* or *S. cerevisiae* was clearly enhanced in the mixed culture compared to the corresponding mono culture (Fig. 2). *L. plantarum* was stimulated by *S. cerevisiae* in presence of glucose (> 2 g l^{-1}) or fructose (any concentration tested) as carbon source and this effect was dose dependent in the cultures containing fructose. *S. cerevisiae* displayed higher cell numbers in cultures with higher fructose concentrations and this was comparable in mono and mixed culture. For each additional cell of *S. cerevisiae* at the higher fructose concentrations, however, there were a factor five additional *L. plantarum* cells in the mixed culture compared to the mono culture at the same fructose concentration (Fig. 3). To test whether the specific stimulation also occurred on *L. sanfranciscensis*, the plate assay was performed with *S. cerevisiae* spent MRS medium with galactose, fructose, sucrose or maltose as carbon source, additional to the already tested glucose. *L. sanfranciscensis* colony size was stimulated by all the spent media (Fig. 1) instead of only by those with glucose or fructose, suggesting that the factor that is

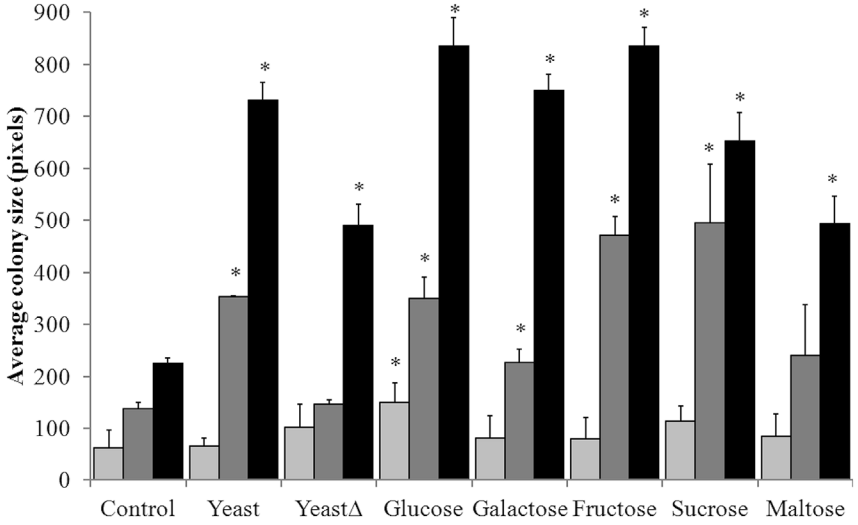

Fig. 1. Average colony size of *L. sanfranciscensis* TMW 1.1304 expressed in number of pixels after one (light), two (middle) and three (dark) days of growth on MRS agar under standard conditions (control), in the proximity (< 5 mm) of an *S. cerevisiae* colony (Yeast), a non-respiring *S. cerevisiae* variant (YeastΔ) and MRS medium spent by *S. cerevisiae* with glucose, galactose, fructose, sucrose or maltose as carbon source. Error bars indicate standard deviations of duplicate measurements of ~30 colonies. Asterisks represent significant differences between the condition and the control using pair-wise T-tests ($P < 0.05$).

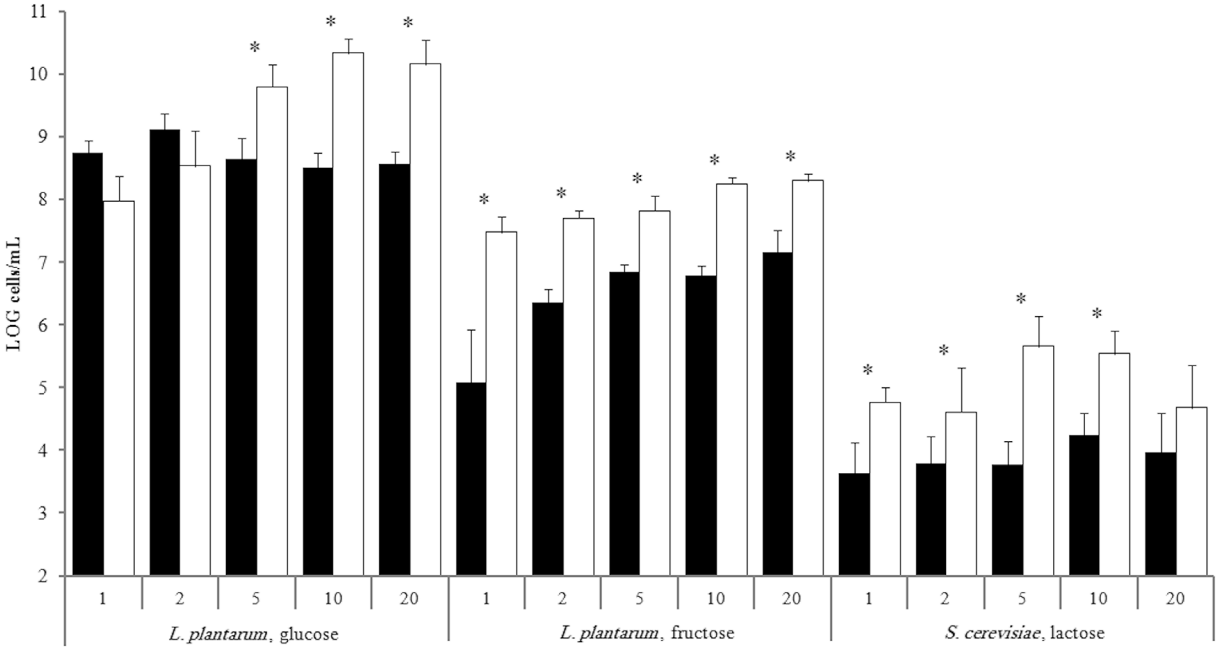

Fig. 2. Cells per mL of *L. plantarum* WCFS1 and *S. cerevisiae* CEN.PK113-7D grown in mono culture (black) and mixed culture (white) in a defined medium containing 1, 2, 5, 10 or 20 g l^{-1} of glucose, fructose or lactose. Error bars represent standard deviations of quadruplicate qPCR measurements on triplicate cultures. Asterisks represent conditions at which the mixed culture is significantly ($P < 0.05$) more than one log different from the mono culture by means of pair-wise T-tests on log transformed data.

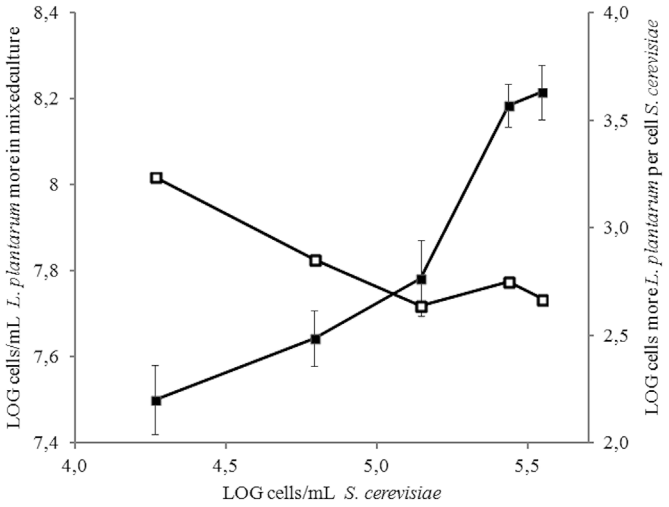

Fig. 3. Number of additional cells of *L. plantarum* WCFS1 grown in mixed culture with *S. cerevisiae* CEN.PK113-7D as compared to growth in mono culture relative to the number of *S. cerevisiae* cells in the mixed culture (black squares) and the number of cells of additional *L. plantarum* cells divided by the number of *S. cerevisiae* cells (white squares).

secreted by *S. cerevisiae* and stimulatory to *L. sanfranciscensis* is different from the factor that stimulated *L. plantarum* in the CDM assay.

S. cerevisiae was only stimulated by *L. plantarum* in CDM when lactose was the carbon source. This yeast strain cannot utilize lactose, but may benefit from the lactic acid produced by *L. plantarum* and from traces of glucose and galactose that may be released by the bacterial β-galactosidase activity. In order to provide evidence that *S. cerevisiae* growth is enhanced at the concentrations of lactic acid present in these experiments, growth and lactate consumption were tested by cultivation for 48 h at 30 °C in YPD broth set to pH 6.6 and supplemented with either 0.2% (w/v) glucose or 0.22% (w/v) lactic acid, or both. As lactic acid metabolism requires oxygen, cultures were grown both anaerobically and aerobically. From turbidity and pH measurements (Fig. S2), it was evident that in the aerobic condition both glucose and lactic acid were used, while in the anaerobic condition growth occurred solely on glucose.

To test whether growth on lactic acid also occurs in a sourdough-like fermentation, 10 g dough containing 0.22% lactic acid pH 6.5 and 10 g without lactic acid were prepared and inoculated with 10^6 *S. cerevisiae* cells. After 24 h of fermentation, the pH of the lactic acid dough had increased to 8.0 while the control dough had a pH of 6.1, indicating that the yeast indeed consumed lactic acid.

4. Discussion

It was established that *L. sanfranciscensis* growth is higher in an anaerobic environment containing CO_2 than under aerobiosis and that *S. cerevisiae* is able to provide such an environment during co-fermentation. This also means that *S. cerevisiae* may provide the right atmosphere containing sufficient CO_2 to favour *L. plantarum* growth (Stevens et al., 2008). Similarly, *S. cerevisiae* was shown to consume lactic acid in a dough environment, thereby slowing down the acidification resulting from lactic acid production by the LAB, allowing extended growth of LAB in a sourdough consortium. Moreover, evidence is presented that growth of *L. sanfranciscensis* is stimulated by a factor secreted by *S. cerevisiae*, both on MRS and dough agar media and in an actual sourdough-like environment. The lack of local pH increase in the agar plate indicated that the stimulation cannot be explained by de-acidification due to the consumption of lactic acid. The stimulant is probably not proteinaceous, as shown by retention of the stimulatory effect in protease-treated and heat-treated spent medium, although small proteinase K insensitive and heat resistant peptides cannot be ruled out. It is therefore not unlikely that the stimulation is based on a vitamin or cofactor provided by *S. cerevisiae*, as this yeast is very versatile in vitamin biosynthesis and many specialised LAB with small genomes, such as *L. sanfranciscensis*, are not (Vogel et al., 2011). Wheat flour does not contain vitamin B_{12}, C, D and only traces of vitamin A, K and folic acid (USDA National Nutrient Database for Standard Reference 25, 2012), but not all may be necessary for *L. sanfranciscensis* growth. However, the essential folic acid cannot be produced *de novo* by *L. sanfranciscensis* while *S. cerevisiae* does have this capability. In addition, *L. sanfranciscensis* TMW 1.1304 is (i) auxotroph for 12 amino acids (Vogel

et al., 2011), (ii) one of the non-proteolytic *L. sanfranciscensis* strains (Vermeulen, Pavlovic, Ehrmann, Gänzle, & Vogel, 2005), and (iii) the content of free amino acids in whole-grain wheat flour is low (USDA National Nutrient Database for Standard Reference 25, 2012). In contrast, *S. cerevisiae* is prototroph for all amino acids and therefore stimulation of *L. sanfranciscensis* in a sourdough-like environment may also partly be based on the availability of amino acids. Similar cross-feeding interactions have been described in sourdough containing different yeasts (De Vuyst & Neysens, 2005; Gobbetti & Corsetti, 1997; Gobbetti et al., 1994a) and in other cultures consisting of *S. cerevisiae* and a LAB (Mendes et al., 2013). However, this latter interaction does not explain the stimulation on MRS agar, in which peptone and yeast extract provide all the amino acids necessary. A further exploration of the aforementioned components (folic acid, vitamins and amino acids) on *L. sanfranciscensis* performance and their effect on the stimulation by *S. cerevisiae* will be necessary to demonstrate the exact nature of this interaction.

Stimulatory interactions between lactic acid bacteria and yeasts occur mutually as was shown by the screening of growth on variable carbon sources in CDM. *L. plantarum* was stimulated by *S. cerevisiae*, but not in all conditions. Apparently, the component stimulatory to *L. plantarum* is only secreted by *S. cerevisiae* in the presence of specific carbon sources, i.e. of the tested carbon sources the stimulatory effect was displayed only in the presence of fructose (all concentrations) and glucose (above concentrations of $2\,g\,l^{-1}$). *In silico* analysis of the *S. cerevisiae* primary metabolism did not yield any obvious differences between the routes of fructose and glucose on the one hand and the other sugars on the other hand, towards glycolysis with respect to molecules that are required and released in the reactions. In order to acquire a deeper understanding of this interaction, a transcriptomic, proteomic or full metabolomics approach may be necessary. In contrast to *L. plantarum*, growth of *L. sanfranciscensis* was stimulated in all the conditions involving live *S. cerevisiae* or spent medium, indicating that the production of a compound stimulatory to this species is independent of the carbon source. This indicates that the modes of interaction between *S. cerevisiae* and the two LAB differ.

The explanation for the stimulatory effect of *L. plantarum* on *S. cerevisiae* in CDM containing lactose is more straightforward. The yeast is not able to utilize lactose as carbon source, but it can grow on galactose and lactic acid. Indeed, growth on galactose secreted by a LAB species has been demonstrated before (Mendes et al., 2013) and growth on lactic acid was shown here. Lactic acid production by LAB and subsequent consumption by yeasts has been described before in kefir (Cheirsilp et al., 2003).

In this study, evidence is presented for the presence of novel mutualistic interactions between LAB and yeasts that occur in a laboratory environment as well as in an industrial setting. It remains to be elucidated which components are responsible for these mutual stimulations and which mechanistic basis underlies the observed carbon source-dependency of mutual growth stimulation.

Conflict of interest

The authors declared no conflict of interest.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.lwt.2017.12.022.

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