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Fermented butter aroma for plant-based applications

Liuyan Gu[a], Belay Tilahun Tadesse[a], Shuangqing Zhao[a], Jesper Holck[b], Ge Zhao[a], Christian Solem[a*]

[a] National Food Institute, Technical University of Denmark, DK-2800 Kgs. Lyngby, Denmark.

[b] Protein Chemistry and Enzyme Technology Section, DTU Bioengineering, Department of Biotechnology and Biomedicine, Technical University of Denmark, DK-2800 Kgs. Lyngby, Denmark.

[*]Corresponding authors:

National Food Institute, Technical University of Denmark, Kemitorvet Building 201, DK-2800 Kgs. Lyngby, Denmark.

Tel: (+45) 30585533

E-mail address: chso@food.dtu.dk

ABSTRACT

Plant-based dairy alternatives are gaining increasing interest, e.g. alternatives to yoghurt, cheese and butter. In all these products butter flavor (diacetyl + acetoin) plays an important role. We previously have reported efficient butter flavor formation from low value dairy side streams using a dairy isolate of *Lactococcus lactis* deficient in lactate dehydrogenase. Here we have tested the ability of this strain, RD1M5, to form butter flavor in plant milks based on oat and soy. We found that oat milk, with its high sugar content, supported more efficient production of butter aroma, when compared to soy milk. When supplemented with glucose, efficient butter aroma production was achieved in soy milk as well. We also carried out an extended adaptive laboratory evolution of the dairy strain in oat milk. After two months of adaptation, we obtained a strain with enhanced capacity for producing butter aroma. Despite of its high sugar content, RD1M5 and its adapted version only metabolized approximately 10% of the fermentable sugars available in the oat milk, which we found was due to amino acid starvation and partly starvation for vitamins. The study demonstrates that dairy cultures have great potential for use in plant-based fermentations.

Keywords

butter aroma, plant-based, lactic acid bacteria, diary culture, adaptive laboratory evolution

Introduction

Plant-based dairy alternatives are becoming increasingly popular, with a global market expected to reach a staggering USD 47.95 Billion by 2028 (Fior Markets, 2021). Many consumers, however, are reluctant to change from dairy to plant-based alternatives, mainly because of a lower flavor and sensory acceptability of these products (Sethi et al, 2016; Harper et al., 2022). Fermented dairy products like cheese and yoghurt derive much of their flavor from various lactic acid bacteria (LAB), which are either intentionally added to or naturally occur in the milk. While growing in the milk, these LAB generate metabolites which contribute to flavor and shelf life. In ripened products like cheese, post-growth events like proteolysis and lipolysis also play a significant role for flavor development (Crow et al., 1993). In butter, cheese and yoghurt, diacetyl and acetoin are important flavor compounds, which confer a buttery flavor (Cheng, 2010). Diacetyl is much more potent than acetoin, and only a few mg/kg are needed to impart an intense buttery flavor (Clark & Winter, 2015; Shibamoto, 2014), however, since acetoin reduces the harshness of diacetyl, the presence of both compounds is desired (Cheng, 2010). Diacetyl and acetoin are generated by certain LAB, which are capable of metabolizing the citric acid present in milk (< 2 g/L) (Hugenholtz, 1993). *Lactococcus lactis* subsp. *lactis* biovar diacetylactis together with various *Leuconostoc* strains have traditionally been used to generate butter flavor in products like cheese and butter, but various lactobacilli also have this capacity (Branen & Keenan, 1971; Cardenas et al., 1985; Benito de Cardenas et al., 1989). In citric acid metabolizing LAB, after citrate is taken up, it is cleaved into oxaloacetate and acetate by citrate lyase, and oxaloacetate is subsequently transformed into pyruvate, by oxaloacetate decarboxylase (Magni et al., 1999). Acetolactate synthase, which in *L. lactis* has rather low affinity for pyruvate (Km 30-50 mM) (Monnet et al., 1994), then condenses two pyruvates into α-acetolactate, an inherently unstable compound, which spontaneously, but slowly, decomposes into either diacetyl or acetoin, depending on the conditions (de Man, 1959). However, strains with high acetolactate decarboxylase activity rapidly decarboxylate most of the α-acetolactate into acetoin, and thus strains low in acetolactate decarboxylase are needed if high diacetyl titers are to be obtained (Monnet et al., 2000) (Fig. 1).

It is also possible to generate diacetyl and acetoin when citric acid is not available, e.g. when oxygen is used as an electron acceptor (Liu et al., 2016). In the presence of hemin, *L. lactis* can obtain a fully functional electron transport chain, which can oxidize NADH and thus regenerate NAD^+ (Sijpesteijn, 1970). Since lactic acid formation requires NADH, the pyruvate pool is boosted, thus allowing for α-

acetolactate and the derived acetoin and diacetyl to be formed (Sijpesteijn, 1970). *L. lactis* also possesses a water forming NADH oxidase, which likewise can oxidize NADH using oxygen as electron acceptor (Lopez de Felipe et al., 2001). We have previously demonstrated that a lactate dehydrogenase deficient *L. lactis* strain efficiently can produce acetoin and diacetyl when cultivated with aeration (Liu et al., 2020). Here we test the capacity of this strain to produce butter aroma in oat and soy milk. Then we adapt the strain to growth in oat milk and find that growth and butter aroma forming capacity can be enhanced substantially. Finally, we analyze and determine the carbohydrate composition of oat milk, and determine the factors that restrict growth of RD1M5 in this particular plant-based beverage.

Materials and methods

Microorganisms and media

L. lactis subsp. *lactis* biovar diacetylactis RD1 was isolated after evolution at constant high temperature in ultrahigh-temperature (UHT)-pasteurized milk (Dorau et al., 2021). *L. lactis* RD1M5 derived from RD1, deficient in lactate dehydrogenase activity was isolated after chemical mutagenesis (Liu et al., 2020). *L. lactis* Ge001 was obtained after transferring the nisin gene cluster from *L. lactis* subsp. *lactis* ATCC 11454 by conjugation into *L. lactis* RD1M5 (Zhao et al., 2021). Oat milk (Oatly, Malmø, Sweden) was bought in a local supermarket as was the soy milk (Naturli, Vejen, Denmark). The strains were grown aerobically in M17 medium (Oxoid, Darmstadt, Germany) supplemented with different sugars.

Acidification profiles

A single colony of *L. lactis* RD1 grown on M17-Agar with 1% glucose (GM17) was inoculated into GM17-Broth and incubated at 30°C, with 200 rpm shaking overnight. The overnight cell culture was harvested by centrifugation at 5000 *g* for 5 min and washed with 0.9% NaCl. The washed cells were re-suspended in oat milk or soy milk with and without glucose (desired initial OD600 = 0.05) and incubated at 30°C. The acidification was monitored using an iCinac (AMS Alliance, Barsanti 17/a Room, Italy).

Colony forming unit (CFU)

A single colony of *L. lactis* RD1 grown on M17-Agar with 1% glucose (GM17) was inoculated into GM17-Broth and incubated at 30°C, with 200 rpm shaking overnight. The overnight cell culture was harvested by centrifugation at 5000 *g* for 5 min and washed with 0.9% NaCl. The washed cells were re-suspended in oat milk or soy milk with and without glucose (desired initial OD600 = 0.05) and incubated at 30°C. Samples for CFU per ml were taken at 0 h, 4 h, 8 h and 24 h. The cells were diluted in 0.9% NaCl and cultured on GM17 agar.

Fermentation in shake flasks

Soy milk fermentations. *L. lactis* RD1M5 or Ge001 was grown on M17-Agar supplemented with either 1% glucose (GM17) or 2% sucrose (SM17), respectively. Then a single colony of *L. lactis* RD1M5 or Ge001 was inoculated into GM17-Broth or SM17-Broth, respectively. Both strains were incubated at 30°C, with 200 rpm shaking overnight. The cells in the overnight culture were harvested by centrifugation at 5000 *g* for 5 min and washed with 0.9% NaCl, and then re-suspended in 100 mL soy milk (desired initial OD_{600} = 0.2) in a 1000 mL shake flask for fermentation, and cultivation was carried out at 30°C, with 200 rpm shaking.

Oat milk fermentations. A single colony of *L. lactis* RD1M5 or $RD1M5_{ALE}$ grown on GM17-Agar was inoculated into GM17-Broth and incubated at 30°C, with 200 rpm shaking overnight. Cells in overnight culture were harvested by centrifugation at 5000 *g* for 5 min and washed with 0.9% NaCl. The washed cells were re-suspended in 50 mL oat milk (desired initial OD600 = 0.2) in a 500 mL shake flask, and cultivation was carried out at 30°C, with 200 rpm shaking.

Adaptive laboratory evolution (ALE)

The ALE was carried out in 100 ml conical flasks containing 25 ml oat milk. Incubation was at 30°C with 200 rpm shaking. After reaching a stationary phase, 10% cell culture was transferred into a fresh oat milk. The ALE of *L. lactis* RD1M5 was continued about 2 months.

Carbohydrate analysis of plain and fermented oat milk

Oat milk fermented by *L. lactis* RD1M5 and its oat milk adapted version $RD1M5_{ALE}$ for 48 h was collected by centrifugation at 5000 *g* for 30 min. At the same time, plain oat milk was also centrifuged. The pellets were removed and the supernatant was collected for analysis. Individual linear α-1,4-glucan structures of DP1 to DP8 were quantified using high-performance anion-exchange chromatography with pulsed amperometric detection (HPAEC-PAD; Dionex ICS-5000 system (Dionex Corp, Sunnyvale, CA, USA)) equipped with a CarboPac PA1 column (Thermo Fisher Scientific, Waltham, MA, USA). Glucose and maltooligosaccharides of DP2-DP8 were used as external standards. Putative β-glucan oligosaccharides were quantified as their respective α-glucan oligosaccharide equivalents. The carbohydrate analysis was carried out by the Carbohydrate Analytics Core at the Technical University of Denmark.

Determining the growth limiting components of oat milk

A single colony of *L. lactis* RD1M5 grown on GM17-Agar was inoculated into GM17-Broth and incubated at 30°C, with 200 rpm shaking overnight. The overnight cell culture was harvested by centrifugation at 5000 *g* for 5 min and washed with 0.9% NaCl. The washed cells were re-suspended in oat milk with different combinations of 0.5% casein peptone, vitamins normally contained in defined Synthetic Amino acid medium (SA medium), salts from SA medium and 0.05% ascorbic acid (desired initial OD600 = 0.2). Vitamins from SA: biotin, folic acid, riboflavin, niacinamide, thiamine, calcium pantothenate and pyridozal. Salts from SA: 3-(*N*-morpholino) propanesulfonic acid (MOPS) buffer, tricine, $FeSO_4$, $CaCl_2$, $MgCl_2$, NaCl, $(NH_4)_6(MO_7)_{24}$, H_3BO_3, $CoCl_2$, $CuSO_4$, $MnCl_2$ and $ZnSO_4$. The amounts of vitamins and salts added were the same as in SA medium (Jensen et al., 1993). For experiments with an initial OD_{600} of 10, the washed cells were re-suspended and concentrated in oat milk. Samples for measurement of maltose under different conditions were taken at 0 h and 48 h.

Quantification of metabolites

Quantification of sugars and acetoin was carried out using an Ultimate 3000 high-pressure liquid chromatography system (Dionex, Sunnyvale, USA) equipped with an Aminex HPX-87H column (Bio-Rad, Hercules, USA) and a Shodex RI-101 detector (Showa Denko K.K., Tokyo, Japan). The column oven temperature was set at 60°C and 5 mM H_2SO_4 was used as the mobile phase at a flow rate of 0.5 mL/min. The detection of diacetyl was performed by method described by Benson et al. (1996).

Results

Preliminary assessment of growth and acidification of *Lactococcus lactis* in oat and soy milk

When characterizing growth of microorganisms in liquid media, it is common to rely on absorbance measurements, as most standard laboratory media are transparent in nature. For lactic acid bacteria, a simple alternative to measuring absorbance is to measure the drop in pH over time, as growth for these bacteria, to a great extent, is coupled to lactic acid production (Andersen et al., 2001). Since our objective here is to study the butter aroma forming capacity of a non-acid forming *L. lactis* strain RD1M5 in two opaque plant milks, based on soy and oat, we decided to first characterize acid formation, and hence growth, of the lactic acid forming RD1, from which RD1M5 is derived. Soy milk is known to contain small amounts of sucrose and other sugars (Yazdi-samadi et al., 1977) that not all dairy lactococci are

able to metabolize, and we therefore included experiments where the plant milks were supplemented with 1% glucose. The initial pH of soy milk was 6.8 whereas oat milk had a slightly lower pH of 6.0. In the plant milks supplemented with glucose, acidification was quite rapid (Fig. 2A) and cell density (CFU per ml) increased quickly within 4 h (Fig. 2B), indicating presence of all the nutrients needed for fast growth. RD1 grew quickly in oat milk supplemented with glucose and the CFU per ml reached 2.39×10^8 at 4 h, which was the highest. Without added glucose, acidification and growth was slower, in particular for soy milk, which is known to mainly contain sucrose, which RD1 is unable to metabolize. After 4 h, the CFU per ml of RD1 in oat milk with/without glucose started to drop quickly, while the CFU per ml of RD1 in soy milk with/without glucose continued to increase slowly. Nonetheless, the results clearly demonstrate that *L. lactis* can grow in both soy and oat milk, which thus potentially could serve as fermentation substrates for the butter aroma producing RD1M5.

Butter aroma production in plain soy milk

The butter aroma forming *L. lactis* RD1M5 was inoculated into shake flasks containing soy milk and incubated with shaking. After 48 hours of incubation, the diacetyl content was quantified, and found to be almost undetectable (data not shown). Soy milk mainly contained sucrose, approximately 6.5 mM, and this sugar cannot be metabolized by RD1M5. Recently we constructed a natural derivative of RD1M5, which is able to metabolize sucrose (Zhao et al., 2021), which we decided to test in soy milk. This strain, Ge001, generated small amounts of diacetyl (0.09 mM) and acetoin (5.81 mM), but formation of these compounds, as well as consumption of sucrose was quite slow (Fig. 3A). Interestingly not all sucrose was used up, and 1.27 mM sucrose remained in the soy milk after a 75 h fermentation.

Butter aroma production in glucose supplemented soy milk

The sucrose content of soy milk was apparently insufficient to support high level formation of the butter aroma compounds diacetyl and acetoin. We therefore decided to supplement the soy milk with 1% glucose (55 mM), a sugar that is readily metabolized by *L. lactis* RD1M5. Indeed, glucose was rapidly consumed, resulting in formation of 0.22 mM diacetyl and 37 mM acetoin (Fig. 3B). By supplementing the soy milk with glucose, the fermentation could be accelerated and most glucose was used up in the first 24 h.

Butter aroma production in oat milk

In soy milk, even when supplemented with glucose, growth of *L. lactis* was slow, and more than 24 h were needed for RD1M5 to reach the final titer of diacetyl and acetoin. In contrast, in oat milk, which contained 126.85 mM maltose, almost 0.3 mM diacetyl could be formed in only 8 h, and in 48 h reached 0.36 mM, while 42 mM acetoin was formed (Fig. 4A). In total, 9.5 mM of maltose and 1.69 mM glucose had been consumed during the fermentation, indicating the presence of other fermentable carbohydrates as one hexose is required for each diacetyl/acetoin formed.

Adaptive laboratory evolution (ALE) of *L. lactis* RD1M5 in oat milk and its impact on butter aroma production

L. lactis RD1M5 is derived from a dairy isolate of *L. lactis* and is adapted to growth in milk. Oat milk apparently supports growth of *L. lactis*, albeit it may be slow. There could be several reasons for the latter, e.g. difficulties obtaining the required amino acids from the oat proteins, and inadequate amounts of vitamins and minerals. It should be possible to enhance growth in oat milk by supplementing it with various nutrients, however, consumers increasingly demand foods and food ingredients produced using less additives. Therefore, instead of fortifying the oat milk to accommodate good growth of *L. lactis*, we decided to investigate whether it would be possible to adapt *L. lactis* into growing better in plain oat milk. Thus, we carried out ALE, where RD1M5 was grown in oat milk for more than two months, and tested the performance of one of the resulting ALE isolates ($RD1M5_{ALE}$). Surprisingly, it was possible to enhance the performance of the strain, and $RD1M5_{ALE}$ generated 0.44 mM diacetyl in 48 h, while

producing similar amounts of acetoin as RD1M5. The adapted strain furthermore consumed 20% more maltose (11.4 mM) and 80% more glucose (3.0 mM) than RD1M5 (Fig. 4B).

In detail carbohydrate analysis of plain and fermented oat milk

The glucose and maltose consumed by RD1M5 growing in oat milk was insufficient for generating the amounts of acetoin and diacetyl formed (1 maltose can result in 2 acetoin/diacetyl), which indicated that other fermentable carbohydrates were present in the oat milk.

We therefore analyzed the carbohydrate content of oat milk, as well as oat milk fermented with RD1M5 and $RD1M5_{ALE}$. The oat milk used in our experiments mostly contained maltose (g2), although small amounts of glucose (g1), maltotriose (g3) and higher oligomers were identified as well (Fig. 5). Compared with RD1M5, the $RD1M5_{ALE}$ derivative consumed 0.47 g/L more maltose (1.38 mM), which is similar to what we found in our initial HPLC analysis. The imbalance in sugar consumption/product formation which we found earlier, could be explained from the consumption of maltotriose (g3) and higher oligomers.

Bottlenecks of maltose consumption by *L. lactis* RD1M5 in oat milk

Although *L. lactis* RD1M5 can produce butter aroma in oat milk, the overall consumption of sugars in oat milk by *L. lactis* RD1M5 was low, especially a large amount of maltose remains. To determine the factors limiting sugar consumption, we characterized sugar consumption in oat milk supplemented with different components (peptone, salts, vitamins and ascorbic acid) (Fig. 6). With the addition of 0.5% casein peptone, maltose consumption increased to 57.6 mM. By only adding vitamins, maltose consumption increased by 5.1 mM. However, when adding salts from SA medium and ascorbic acid, no increase in maltose consumption was observed. The combination of casein peptone, vitamins and salts effectively increased maltose consumption to 87.7 mM, which is approximately 10 times the amount metabolized without any additions. Thus, insufficient amounts of amino acids and vitamins appeared to explain the limited maltose consumption of *L. lactis* RD1M5 in oat milk.

We also tested the effect of using a higher inoculum, as it was speculated that RD1M5 might suffer from oxidative stress after prolonged aeration. However, increasing the inoculum only had a minor effect on maltose consumption, and when the initial OD_{600} was 10, maltose consumption increased by 7.2 mM.

Discussion

Plant-based butter spreads and margarine often contain diacetyl, which is added to confer a butter-like flavor to the product. The diacetyl used for this purpose is often manufactured chemically from sugar cane bagasse using high temperatures and oxygen limiting conditions, under which a series of radical reactions take place, ultimately resulting in formation of diacetyl (Zeitsch, 2000). Although the diacetyl obtained in this way is classified as being natural, the harsh conditions used to produce it can hardly be claimed as such. By-products are undoubtedly formed, the safety aspects of which surely have not been assessed. It therefore makes a lot of sense to explore fermentation-derived butter aroma made using plant substrates. Many LAB are able to metabolize citric acid and produce butter aroma (Drinan et al., 1976). It has been argued that LAB isolated from plants are preferred candidates for fermenting plant substrates (Ruiz Rodríguez et al., 2019), e.g. due to their capacity to metabolize certain plant sugars (Kelly et al., 1998; Passerini et al., 2013). However, for generating butter flavor, our dairy isolate and its adapted version performed well. Dairy LAB have been used by humans for thousands of years, are safe and well-characterized and cultures in use today have been selected for their excellent industrial performance. In contrast, LAB isolated from plants have been less optimized for human applications, and potentially could produce unwanted or even toxic compounds like biogenic amines (Barbieri et al., 2019). One potential challenge when using dairy LAB in plant fermentations could be a poor ability to degrade plant proteins. Plant proteins often have large, multimeric and globular structures which make them less accessible to the cell envelope bound protease of LAB, whereas caseins found in milk, with their open and disordered

structure, are more easily hydrolyzed (Savijoki et al., 2006; Harper et al, 2022). For oat and soy milk, this was not a significant problem, and RD1M5 grew well in these substrates. Despite the relatively high protein content of the oat milk used, we found that RD1M5 starved for amino acids, and that this restricted the amount of maltose that could be metabolized. Supplementation with peptone helped overcome this limitation. RD1M5 possesses a cell-envelope bound protease which ensures good growth in milk, but which apparently is not optimal for degrading the non-soluble proteins in oat milk, which are in suspension, and can easily be recovered by mild centrifugation. By adding commercially available proteases, e.g. Flavourzyme®, it is possible to degrade the oat proteins to peptides that RD1M5 readily can use. Besides amino acids, we found that the vitamin content of oat milk was insufficient to support efficient growth, something that has been reported previously for plant based substrates (Van Niel et al., 1999). For example, thiamine is a cofactor for the pyruvate dehydrogenase complex, which is necessary for generating acetyl-CoA. Here we found that extra supply of vitamins, mainly B vitamins could promote the sugar fermentation of RD1M5.

Diacetyl is produced from α-acetolactate in a spontaneous manner, and this reaction requires oxygen, and oxygen is also needed for NAD^+ regeneration (Liu et al., 2016). It is known that many of the reactions involving oxygen lead to formation of reactive oxygen species and oxidative stress, which eventually halt growth. We found that using a higher inoculum, to a small extent, could enhance sugar consumption, which may have been due to reduced oxidative stress.

Furthermore, we demonstrated that the performance in oat milk readily could be improved by simple adaptation. As mentioned above, many *L. lactis* plant isolates are able to metabolize sugars like sucrose, raffinose and stachyose (Passerini et al., 2013), an ability dairy strains, in general, appear to lack. Dairy strains able to metabolize sucrose, however, are common (Siezen et al., 2011), and stachyose metabolism can easily be transferred from plant strains by natural conjugation (Machielsen et al., 2011), and the resulting transconjugants are considered natural, i.e. have non-GMO and QPS (Qualified Presumption of Safety) status. Most crops consumed by humans, however, do contain carbohydrates that dairy LAB easily can ferment like sucrose, maltose, maltotriose, fructose or glucose (Yazdi-samadi et al., 1977; Passerini et al., 2013), or if necessary, can easily be supplemented with these sugars.

In conclusion, this is the first report describing the use of diary *L. lactis* RD1M5 for the production of butter aroma in plant-based milks. Sugars (e.g. glucose, maltose, maltotriose and higher oligomers) in oat milk are easier for RD1M5 to utilize, and the final concentrations of diacetyl and acetoin were 0.36 mM and 42 mM, respectively. However, the main carbon source sucrose in soy milk was not suitable for RD1M5, however adding another fermentable sugar can easily solve this problem. Starvation for amino acids was caused by difficult-to-hydrolyze plant proteins, and vitamins were also demonstrated to be a limiting factor for growth of RD1M5 in oat milk. In addition, we demonstrated that ALE was an efficient method for improving the performance of *L. lactis* in plant milks. Overall, this study could lay the foundation for efficient butter aroma formation in plant milks by dairy cultures.

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The featured image was created with BioRender.com.

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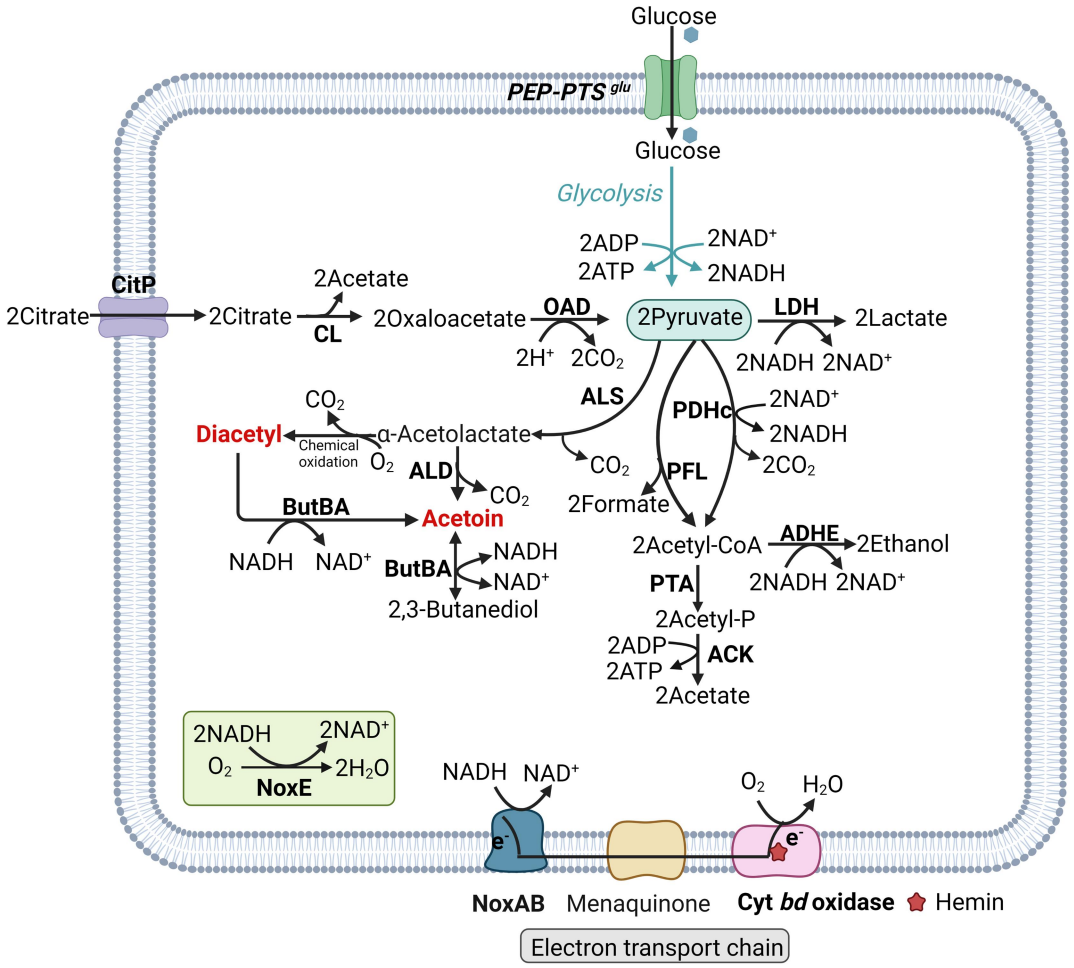

Fig. 1 Central metabolism of *Lactococcus lactis*. Abbreviations for enzymes are: CitP: citrate permease; CL: citrate lyase; OAD: oxaloacetate decarboxylase; PEP-PTS^{glu}: phosphoenolpyruvate-dependent glucose phosphotransferase system; LDH: lactate dehydrogenases; ALS: α-acetolactate synthase; ALD: α-acetolactate decarboxylase; ButBA: 2,3-butanediol dehydrogenase; PDHc: pyruvate dehydrogenase complex; PFL: pyruvate-formate lyase; ADHE: acetaldehyde dehydrogenase and alcohol dehydrogenase; PTA: phosphate acetyltransferase; ACK: acetate kinase; NoxE: NADH oxidase; NoxAB: NADH dehydrogenase; Cyt bd oxidase: cytochrome *bd* oxidase.

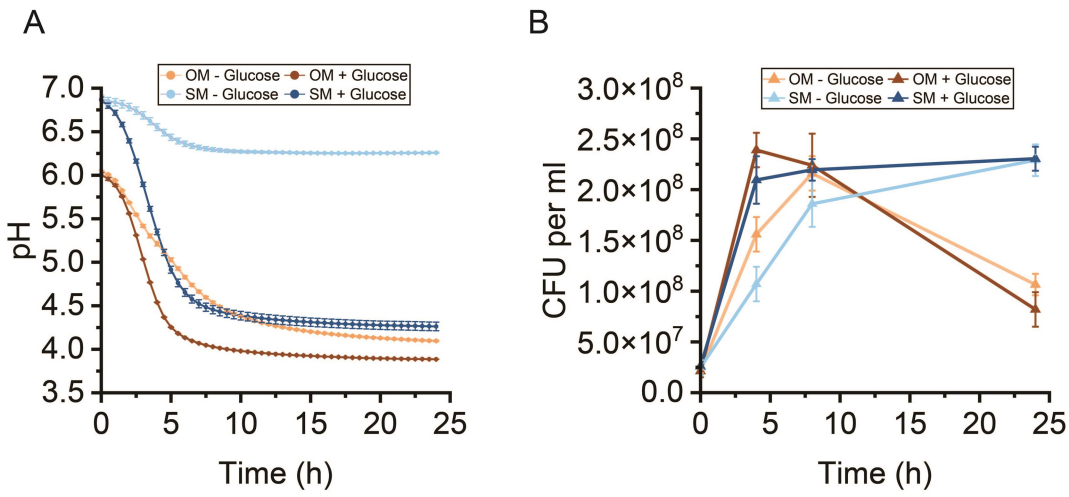

Fig. 2 Performance of RD1 in oat milk (OM) and soy milk (SM), with or without added glucose (1%). (A) Acidification profiles for RD1 and corresponding (B) colony forming units (CFU) at four time points during the fermentation. OM and SM were inoculated with RD1 cells to an initial OD_{600} of 0.05 (as measured in M17 broth). Incubation was done at 30°C, without active aeration, and the acidification profiles were recorded using an iCinac. Error bars indicate standard deviations.

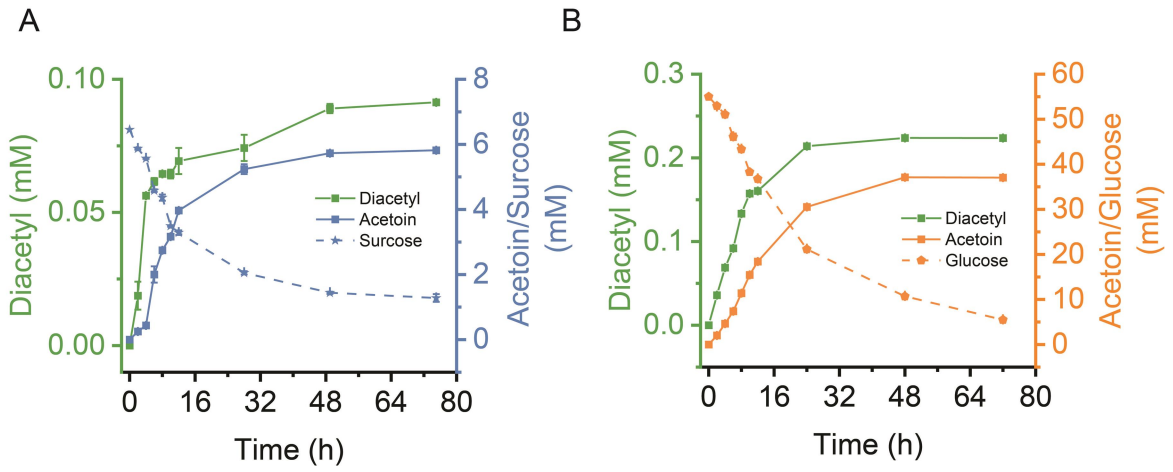

Fig. 3 Formation of the butter aroma compounds diacetyl and acetoin in plain and glucose supplemented soy milk by using *L. lactis*. (A) Formation of the butter aroma compounds diacetyl and acetoin in soy milk (SM) using *L. lactis* Ge001. The initial OD_{600} was 0.2. The green square: diacetyl concentration; the blue square: acetoin concentration; the blue pentagram: sucrose concentration. (B) Formation of the butter aroma compounds diacetyl and acetoin in glucose supplemented SM using *L. lactis* RD1M5. The initial OD_{600} was 0.2. The green square: diacetyl concentration; the orange square: acetoin concentration; the orange pentagon: glucose concentration. Error bars indicate standard deviations.

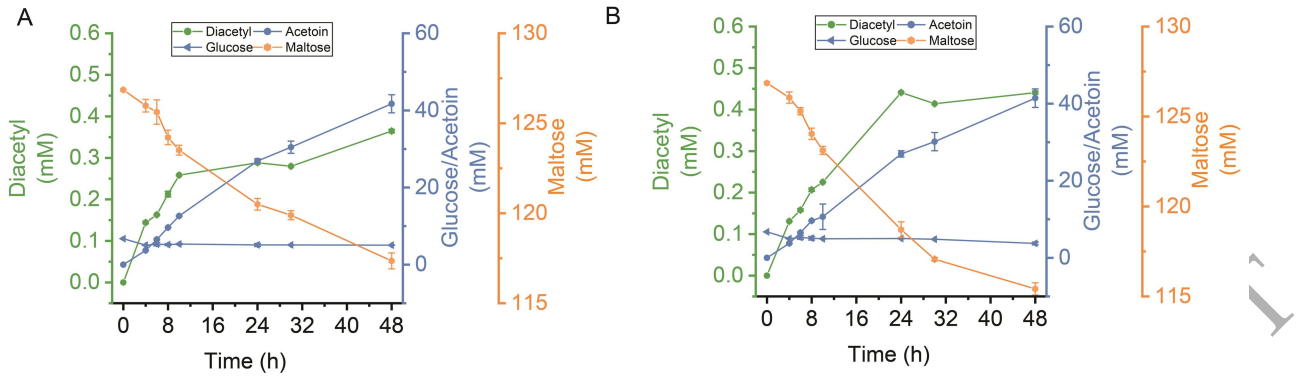

Fig. 4 Formation of the butter aroma compounds diacetyl and acetoin in oat milk (OM) using *L. lactis* RD1M5 (A) and *L. lactis* RD1M5$_{ALE}$ (B). The initial OD_{600} was 0.2. The green circle: diacetyl concentration; the blue circle: acetoin concentration; the blue triangle: glucose concentration; the orange circle: maltose concentration. Error bars indicate standard deviations.

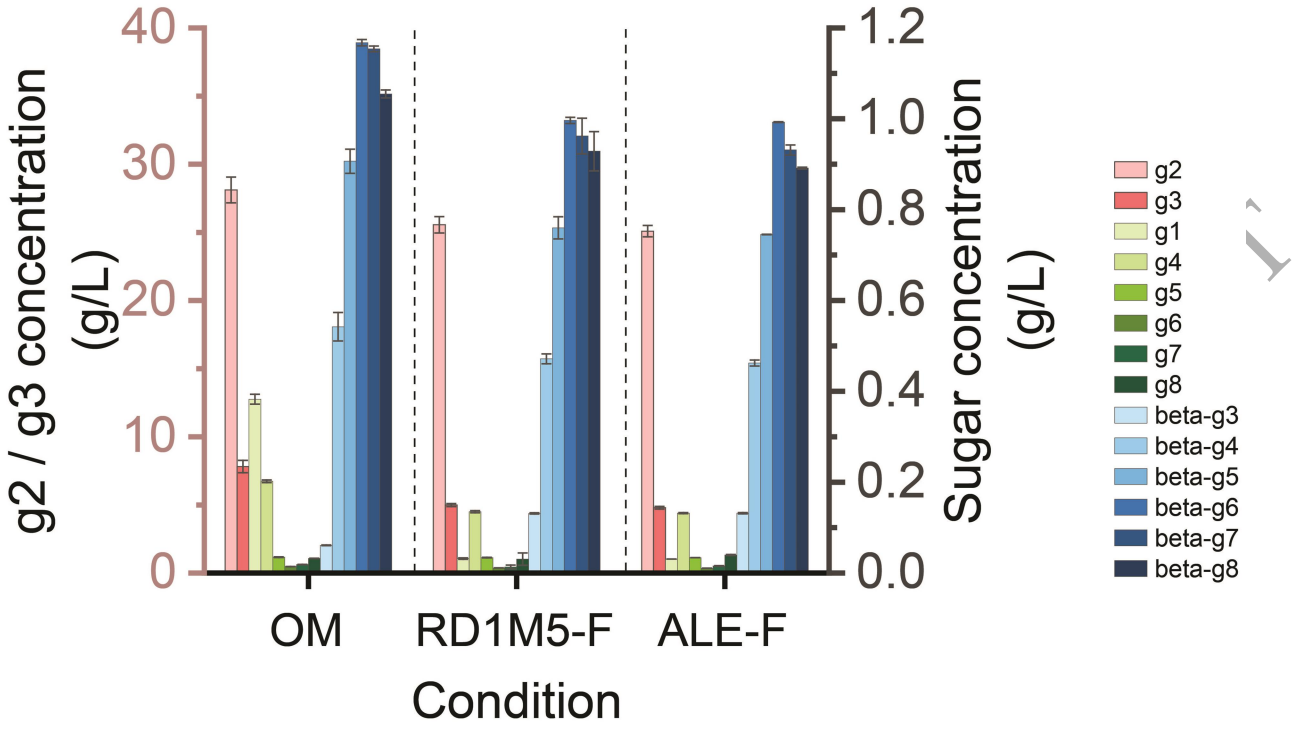

Fig. 5 Carbohydrate composition of plain and fermented oat milk (OM). Oat milk was fermented by two different *L. lactis* strains, RD1M5 (RD1M5-F) and its oat milk adapted version $RD1M5_{ALE}$ (ALE-F). g1 to g8: different numbers of oligomers of glucose. The samples were taken at 48 h. Error bars indicate standard deviations.

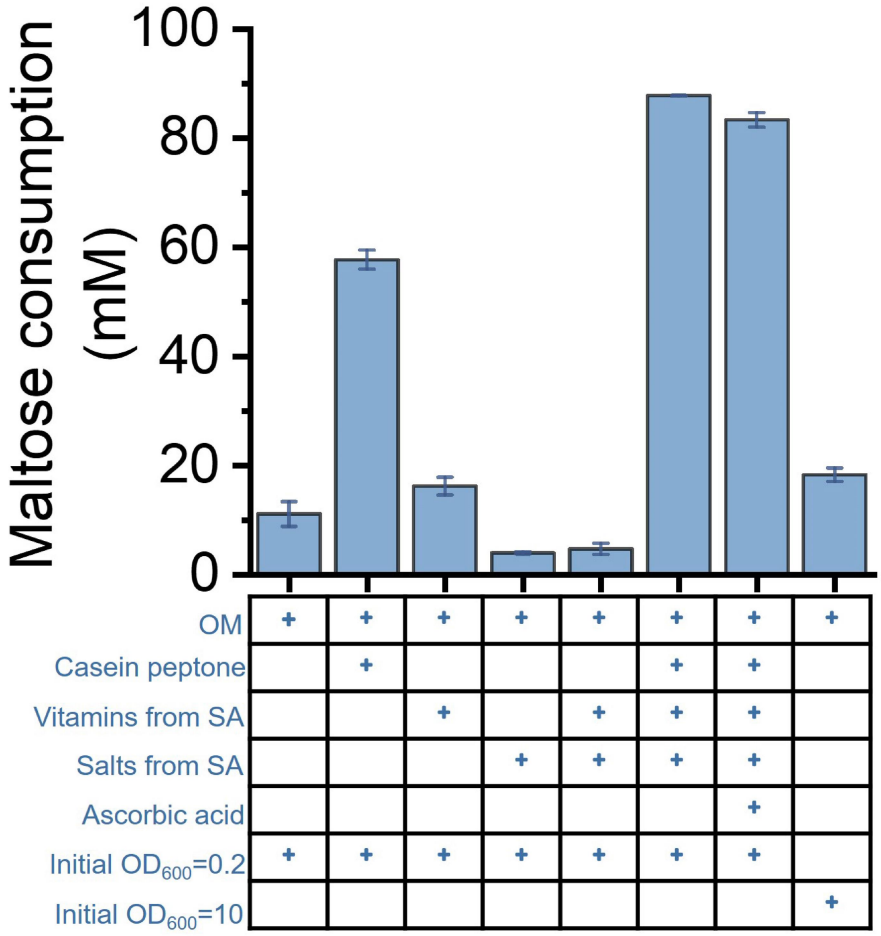

Fig. 6 Maltose consumption in oat milk (OM) with different additions and initial OD_{600}. The + in the table indicates the addition of the corresponding compounds or the relevant initial OD_{600}. SA: synthetic amino acid medium. Maltose consumption (mM) = maltose concentration at 0 h (mM) - maltose concentration after 48 h fermentation (mM). Error bars indicate standard deviations.