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A novel approach for accelerating smear development on bacterial smear-ripened cheeses reduces ripening time and inhibits the growth of *Listeria* and other unwanted microorganisms on the rind

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**ABSTRACT**

Bacterial smear-ripened cheeses are commonly contaminated with fungi and the potentially dangerous pathogen *Listeria monocytogenes*, which compromises food safety and quality. Here, we present new approach for accelerating smear development on these cheeses, while reducing growth of unwanted microorganisms. We grew the smear microorganism *Brevibacterium linens* in acid whey (AW) supplemented with a by-product of whey protein hydrolysate production (FCH). *B. linens* grew to a high cell density (10.27 log$_{10}$ CFU/mL), and alkalized the AW-FCH medium. We found that the fermented AW-FCH, with its high pH, rapidly killed *L. monocytogenes* and yeasts. When culture medium was smeared on the cheese surface, the surface pH was rapidly neutralized, and in just 7 days, the cheeses developed a dense orange color and growth of spoilage fungi was inhibited. Summing up, by growing *B. linens* to high cell density on low-value dairy byproducts, and applying dense suspensions onto cheeses, we can reduce the time needed for establishing the smear. Added benefits are increased food safety and less spoilage, as quick smear development inhibits growth of unwanted microorganisms on the rind. Preliminary tests at a dairy show that flavor development is comparable to traditional bacterial surface-ripened cheeses with far more complex surface microflora.

1. Introduction

Bacterial smear-ripened cheeses, such as Limburger, Münster, Brick, Tilser, Appenzeller, and the Danish Danbo cheeses, are characterized by an orange to red-brown rind color and a special aroma/flavor that distinguish them from non-surface ripened cheeses (Brennan, Cogan, Loessner, & Scherer, 2004; Matera et al., 2018). This characteristic color is generated by *Brevibacterium linens* and other smear bacteria that produce carotenoids (Giuffrida et al., 2020). *B. linens* is an obligate aerobe that can tolerate high concentrations of NaCl (up to 150 g/L), and which can metabolize lactic acid and amino acids (El Soda & Awad, 2014). *B. linens* is acid sensitive and grows in the pH range 5.5–10, with an optimum around 7. When it metabolizes amino acids, ammonia is generated, which gradually increases the pH of its growth substrate (Forquin & Weimer, 2014). *B. linens* produces several enzymes that are considered important for the ripening of smear-ripened cheeses, e.g., proteinases that can hydrolyze both α$_{S1}$- and β-casein to peptides, and peptidases that are active on dipeptides with leucine as the N-terminal amino acid (Cogan, 2016).

Normally, the freshly brined cheeses are inoculated with dilute smear culture, either washed off from cheeses with an established smear (old-young smearing) or obtained from culture providers (often a combination is used). Freshly brined cheeses are slightly acidic and have a pH of around 4.9–5.3 depending on cheese type (Watkinson et al., 2001). This low pH is a challenge for *B. linens*, which is why acid tolerant, and lactic acid metabolizing yeasts such as *Geotrichum candidum* and *Debaryomyces Hansenii* initially dominate the cheese surface (Beresford, Fitzsimons, Brennan, & Cogan, 2001). The yeasts consume lactic acid and hydrolyze protein to generate ammonia, which raises the pH of the rind (Beresford et al., 2001), thus allowing for growth of *Brevibacterium* and other bacteria (Wadhawan, Steinberger, Rankin, Suen, & Czuprynski, 2021; Wilhelm & Tobias, 2001). Overcoming this low-pH barrier thus makes smear cheese ripening a slow process. To facilitate growth of the surface smear, special temperature and moisture-controlled facilities are needed, which are costly to run, and since the cheeses have to be flipped regularly to ensure even development of the smear, a lot of manual labor is involved (Mounier, Coton, Irlinger, Landaud, & Bonnarme, 2017). A serious drawback of the...
traditional “old-young” smearing method, is that it introduces an unnecessary risk: pathogenic and spoilage microorganisms often present in the environment easily can end up in the smear.

Listeria monocytogenes is an important example of a biological threat to the cheese industry (Le, Bazger, Arthur, & Wilcock, 2014). In Europe, L. monocytogenes-contaminated dairy products are responsible for almost half of all food-related listeriosis outbreaks (Melo, Andrew, & Faleiro, 2015). Listeria induced human listeriosis can cause septicemia and meningitis with high mortality rates (Yildiz et al., 2007). A challenge with L. monocytogenes is that it can grow at a wide pH range, has a high salt tolerance, and thrives at refrigeration temperatures. The origin of L. monocytogenes is mainly inadequately pasteurized milk and the cheese manufacturing environment, which makes it difficult to control (John et al., 2022; Lomonaco et al., 2009).

Fungal growth is also one of the main issues affecting cheese quality and safety in all varieties of cheese. Although some fungi are accepted in certain cheese-making, unwanted fungal growth significantly impacts cheese safety and quality. In the cheese context, Penicillium is the most dominant genus, followed by Aspergillus (Kure & Skaar, 2019). These mold species can produce mycotoxins such as ochratoxin A, aflatoxin, and cyclopiazonic acid (Dobson, 2017). Mucor, is also a frequent cheese contaminant, which grows quickly, forming fluffy white tufts on the surface of the cheese, sometimes known as “Cat hair” defects (Plascencia-Jatomea, Vinegра, Olayo, Castillo-Ortega, & Shirai, 2003). Thus, measures to avoid unwanted microbial growth on cheeses are urgently needed to ensure their safety and to avoid economic losses.

In this study we investigate a novel approach for overcoming the slow smear development on red-smeared ripened cheeses. Our model strain, B. linens, is grown in a bioreactor on acid whey (AW) supplemented with a nitrogen source to a high cell density, and then applied to the cheese surface. We follow smear development over time and the effect on the growth of unwanted microorganisms, e.g. various fungi and Listeria, is assessed.

2. Material and methods

2.1. Reagents

FCH 110 (FCH), a by-product of Arla Foods Ingredients whey protein hydrolysate, was provided by Arla Foods, Sonderhoj, Denmark. AW was obtained from Arla Foods. Glucose, glycerol, lactic acid, acetic acid, chloramphenicol, ammonium hydroxide, sodium hydroxide, sodium chloride, yeast extract, Luria-Bertani (LB) broth, and Listeria selective agar were purchased from Sigma-Aldrich, St. Louis, Missouri, USA. Brain heart infusion (BHI) broth, potato dextrose agar (PDA), peptone, and agar were purchased from Merck Millipore (Darmstadt, Germany). Yeast glucose chloramphenicol agar (YGCA) was prepared by adding 5 g of yeast extract, 20 g of glucose, and 15 g of agar to 1 L of water and adjusting the final pH (at 25°C) to 6.6 ± 0.2 with 4 mol/L NaOH and then autoclaved, after cooling down to 45°C, 0.1 g/L chloramphenicol was added. All culture media and reagents were sterilized prior to use. Ribberhus cheese (Arla Foods, Copenhagen, Denmark) was purchased from a local supermarket.

2.2. Strain cultivation

B. linens CX3 was isolated from CLO protective culture (CLO, Sacco, Italy) and cultivated in 50 mL of LB at 30°C and 200 rpm for 3 day as a preculture. After that, 10% preculture was inoculated into AW plus 30 g/L FCH (w/v) and incubated with shaking at 200 rpm for 3 day as a seed culture. To determine colony forming units (CFU), the B. linens CX3 culture was decimally diluted, plated on LB agar, and incubated for 5 day at 30°C. To assess antimicrobial effect, thirteen target organisms were used. Five molds were purchased from DSMZ, including Penicillium commune DSM 2211, Penicillium crustosum DSM 62837, Mucor sp. DSM 1222, Aspergillus niger DSM 737, Aspergillus ochraceus DSM 824. Two types of yeasts, Geotrichum. candidum DSM 1240 and Debaryomyces Hansenii DSM 70590 were also obtained from DSMZ. Debaryomyces Hansenii LAF3 was obtained from Mammen dairy. Five Listeria monocytogenes strains were kindly provided by Arla Foods Innovation center, including four strains isolated from Cheese: Listeria monocytogenes 0107.0111, Listeria monocytogenes 0107.0489, Listeria monocytogenes 0107.0513, Listeria monocytogenes 0107.0243, and one strain from milk: Listeria monocytogenes 0107.0263. All the molds were grown on PDA plates, incubated at 28°C for ten days, and then the spores were harvested. A hemocytometer was used to check the concentration of the harvested spores, which was adjusted to a concentration of 10³ spores/mL using 0.1% sterile peptone water. Yeasts were grown in YPD medium at 30°C and 200 rpm for 48 h. L monocytogenes was grown in BHI broth at 30°C and 200 rpm for 24 h. All fungi spores, yeasts, and bacteria were stored at −80°C in culture broth containing 325 g/L glycerol as cryoprotectant. Yeasts and bacteria were subcultured twice from frozen stocks before the experiments.

2.3. Optimization of B. linens fermentations in shake flasks

Media composed of AW supplemented with different amounts of FCH (0 g/L, 10 g/L, 30 g/L, 50 g/L, 70 g/L, 100 g/L) and with/without pH adjustment were tested. Shake flasks for 250 mL containing 50 mL medium were inoculated with 1% or 10% B. linens culture grown in LB medium and incubated with 200 rpm shaking at 30°C for three days. The pH was measured every 24 h with a pH meter (Lab 845, SI Analytics, Denmark). To determine cell density, plate counting was applied.

2.4. Bioreactor fermentations

For the bioreactor fermentations, the growth substrate consisted of AW supplemented with 30 g/L FCH. One-liter bioreactors (Sartorius, Germany) containing 450 mL sterilized medium were inoculated with 50 mL seed culture. The initial pH was 5.6 ± 0.5, and the temperature was maintained at 30°C. The dissolved oxygen level was monitored with electrodes (Mettler Toledo) and controlled at 50% by automatic adjustment of stirring speed and airflow. The fermentation lasted for three days, and the final pH was about 9.

2.5. Time-kill assays for assessing antimicrobial activity of the B. linens CX3 culture

G. candidum DSM 1240, D. hansenii DSM 70590 and D. hansenii LAF3 were cultured in YPD at 30°C and 200 rpm for 48 h. Cell density was adjusted to about 10⁸ CFU/mL by decimal dilution in 9 g/L NaCl solution. Five L. monocytogenes strains were grown on BHI agar plate at 30°C for 24 h. Single colonies were picked and inoculated in a 100 mL shake flask containing 10 mL of BHI broth at 30°C with 200 rpm shaking for 24 h. The outgrown cultures were decimally diluted in 9 g/L NaCl solution, and cell density was adjusted to 10⁵ CFU/mL. The cells in 1 mL of the diluted yeast and L. monocytogenes suspensions were harvested by centrifugation (10,000 rpm for 3 min), the pellet resuspended in 1 mL of 9 g/L NaCl solution, and re-centrifuged to harvest cells. These cells were mixed with 1 mL of B. linens culture (pH 9), the unfermented substrate (AW-30 g/L FCH) supplemented with ammonium hydroxide (pH 9) and unfermented substrate where acid whey had been replaced with water supplemented with corresponding concentrations of organic acid (122.88 mmol/L lactic acid and 16.08 mmol/L acetic acid in AW) (mAW-30 g/L FCH), and subsequently adjusted to pH 9 with ammonium hydroxide. All the treatments were incubated for either 0.5, 1, 3, or 6 h at room temperature. After this, the suspensions were decimally diluted in 9 g/L NaCl solution. Yeast cells were spread on YGCA plates and incubated at 30°C for four days (Leclercq-Perlat, Oumer, Bergere, Spinnler, & Corrieu, 2000). Listeria cells were plated on Listeria selective agar and incubated at 30°C for three days. The mean number of colonies (CFU/mL) was counted and compared with those recorded in the control.
assay. The percentage of the dead yeast or *Listeria* cells was calculated using the following formula:

\[
\text{Dead Cell} (\%) = \left( \frac{C_{\text{control}} - C_{\text{sample live cells}}}{C_{\text{control}}} \right) \times 100
\]

Where \( C_{\text{control}} \) represents the number of yeast or *Listeria* colonies without treatment. \( C_{\text{sample live cells}} \) means the yeast and *Listeria* colony count in sample groups treated with *B. linens* culture or ammonium hydroxide treated media for different time periods.

### 2.6. Assessing antifungal activity of *B. linens* on the cheese surface

Cheeses were sliced into \( 3 \times 4 \times 0.7 \) cm pieces and placed separately in the Petri dish. One mL of *B. linens* culture was smeared onto the top surface of the cheese slices using a sterilized cell spreader. As a control, cheeses were smeared with 1 mL of 9 g/L NaCl solution. The smeared cheeses were allowed to dry on the lab bench for 60 min. After this, one \( \mu \)L of different fungal spore suspensions containing 100 spores per \( \mu \)L, were spotted on the middle of the cheese top surface. Then, the cheeses were transferred to a 16 °C storage room, where they were cultivated for 12 days. Fungal growth over time was recorded by taking photos. The Inhibitory effect was calculated based on the area of the cheese covered by the fungi using the following formula:

\[
\text{Inhibitory Effect} (\%) = \left( \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \right) \times 100
\]

Where \( A_{\text{control}} \) is the area of fungal growth on the cheese without treatment, and \( A_{\text{sample}} \) is an area of fungal growth on cheeses smeared with *B. linens* culture.

A similar procedure was used for the cheeses to observe the color changes, except these were not inoculated with fungi spores and cultivated for 7 days at 16 °C. Color changes were recorded for day 1, 3, 5 and 7 by taking photos. Non-treated cheese was used as control.

### 2.7. Statistical analysis

Each analytical result is presented as the mean of triplicate sample measurements. One-way ANOVA followed by Tukey’s test (\( p = 0.05 \)) was used to analyze the results. All analysis were performed using Origin 2021 software (version 9.8.0.200 (Academic); OriginLab, Northampton, United States).

### 3. Results and discussion

#### 3.1. *B. linens* can de-acidify and grow in acid whey supplemented with a protein source

Since AW is readily available at a low cost, we decided to explore whether AW could be used as a growth substrate for *B. linens* CX3. However, when 1% of *B. linens* CX3 was inoculated into AW and AW supplemented with 30 g/L FCH, the cell count greatly decreased (\( p < 0.05 \)) over 3 days (Fig. 1(b)), and the pH remained constant with no significant difference (\( p > 0.05 \) ) (Fig. 1(a)). This effect was probably due to the low pH of AW (4.6) and AW supplemented with 30 g/L FCH (5.2). Indeed, after pH adjustment to 7, both substrates supported good growth of *B. linens* CX3 (Fig. 1(b)). An extended growth lag period of 24 h was observed for the pH-adjusted AW, which we hypothesize is due to a low free amino acid content. It is reported that *B. linens* can metabolize both lactic acid and proteins, where the latter leads to formation of ammonia, which increases the pH of the growth substrate, as does the consumption of lactic acid (Adamitsch, Karner, & Hampel, 2003; Forquin & Weimer, 2014). This effect was observed both for the pH 7 adjusted AW and the FCH supplemented (30 g/L) AW cultures. For both cultures, the pH increased gradually over 3 days, in particular for the culture supplemented with FCH (Fig. 1(a)). In an attempt to avoid the need for pH adjustment, we increased the inoculum from 1% to 10%, which indeed allowed growth to occur, albeit with some delay (Fig. 1(b)).

Acid whey is generally a challenge for the dairy industry as it has no obvious applications and is costly to dispose of due to its high ash level, low pH, and a high organic acid content (Wherry, Barbano, & Drake, 2019). In the US, each year, the treatment of acid whey consumes 2 billion kilowatts of electrical energy and results in generation of 500 million kilograms of CO\(_2\) and pollutes land and water (Lindsay, Walker, Dumesic, Rankin, & Huber, 2018). With our strategy, acid whey can be reused as a growth substrate for *B. linens* used for ripening cheeses, which is a far more sustainable and eco-friendly option for the dairy industry.

#### 3.2. Optimizing growth of *B. linens* by adjusting FCH concentration

*B. linens* CX3 is sensitive to low pH and only grows above pH 5.6. As shown above, by using a higher inoculum (10%), it was possible to overcome this limitation in the AW-FCH medium. When AW is supplemented with FCH, the initial pH increases with the amount of FCH added (Fig. 2). After 72 h of fermentation, pH reaches 9 with no significant difference, except these were not inoculated with fungi spores and cultivated for 7 days at 16 °C. Color changes were recorded for day 1, 3, 5 and 7 by taking photos. Non-treated cheese was used as control.

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difference \((p > 0.05)\) when AW is supplemented with FCH in the range of 30 g/L to 100 g/L. Without supplementation with FCH, in contrast to when using a 10% inoculum, no growth was observed and the cell count decreased to 7.5 log \(_{10}\) CFU/mL by 72 h (Fig. 2 (b)), and the pH kept stable at 5.2 \((p > 0.05)\) (Fig. 2 (a)). We also found 10 g/L FCH supported good growth to a high final cell density of 9.9 log \(_{10}\) CFU/mL, however, a substantial fraction of the cells died within the first 24 h because of the low initial pH. As AW supplemented with 30 g/L FCH supported fast growth of \textit{B. linens} CX3 without pH adjustment, we decided to use this amount of FCH for the remaining experiments.

### 3.3. Ammonia and high pH effectively kill yeast

It is often mentioned in the literature that \textit{B. linens} and other smear
bacteria exert an inhibitory effect on various unwanted microorganisms, an effect that has been attributed to different compounds such as bacteriocins and volatile sulphur compounds (Rudolf & Scherer, 2001). Inspired by the self-inhibitory effect of the high pH on B. linens observed above, a simpler hypothesis was raised: could the high pH have an inhibitory effect on other microorganisms as well? To test this hypothesis, we first tested this effect on different yeast cells. The results for G. candidum and D. hansenii are shown in Fig. 3. All the tested yeast strains were sensitive to the fermented AW-FCH and unfermented AW-FCH adjusted to the same pH with ammonia. Within 30 min, G. candidum was almost completely eliminated after exposure to the high pH, and there was no significant difference (p < 0.05) between treatment with B. linens culture and the pH adjusted AW-FCH medium (Fig. 3(a)). D. hansenii LAF3 was less sensitive to the high pH, although about 85% of the cells were killed after a 6-h exposure, again without any significant difference (p > 0.05) between fermented B. linens culture and medium containing ammonia (Fig. 3(b)). As shown in Fig. 3 (c), D. hansenii DSM 70590 was the most robust strain, and only 25% and 36% of the cells were killed after a 60 min exposure. Ammonia is known to serve as a general quorum sensing signaling molecule for some D. hansenii strains, regulating colony survival and development (Dam, Gori, Mortensen, Arneborg, & Jespersen, 2007). It is also known that the presence of fermentable sugars allows D. hansenii to generate ATP needed for coping with stressful conditions (Chamba & Irlinger, 2004).

To test if the sugars present in the broth made D. hansenii more resilient to high pH, we prepared modified broth without added sugars (“mAW-30 g/L FCH”). As shown in Fig. 3, (b) and (c), when D. hansenii cells were treated with this modified medium at pH 9 for 6 h, 97% and 98% of the cells were killed, which indeed demonstrated that the sugars present in AW/FCH helped D. hansenii to survive the high pH. On smear ripened cheeses, these yeasts have been reported to provide growth factors for the smear bacteria (Beresford et al., 2001; Cogan, 2016). However, uncontrolled yeast growth is not beneficial as this can lead to off-flavors, slick rinds, and toad skin deformities (Westall & Filtenborg, 1998; Siposová, Konuchová, Valík, Trebichavská, & Medved’ová, 2021). Here, we have demonstrated that the anti-yeast effect of B. linens CX3, at least partially, can be attributed to its ammonia production.

3.4 Ammonia and high pH effectively kills Listeria monocytogenes

Bacterial smear-ripened cheeses can be challenging in terms of food safety. During 2010–2017, 6.3% of listeriosis incidents were linked to eating contaminated cheese (Gérard, 2021). We therefore tested the effect of the B. linens CX3 culture and the ammonia containing medium against five strains of L. monocytogenes. As seen in Fig. 4, at least 74% of L. monocytogenes cells were killed within 30 min. For strains except L. monocytogenes 0107.0513, more than 90% of cells were killed in 6 h without significant difference (p > 0.05) between treatment with B. linens culture or ammonia containing medium (Fig. 4 (a),(b),(c),(d)). L. monocytogenes 0107.0513 was the most resistant strain (Fig. 4 (e)), and for this particular strain treatment with B. linens culture initially (at 0.5 and 1 h) was more efficient than broth containing ammonia (p < 0.05). After 6 h treatment, however, the ammonia containing broth was more efficient at killing this strain (87% killing rate). Our results thus showed that the sensitivity of L. monocytogenes to high pH is strain-dependent. As we have shown for certain yeasts, survival may be linked to the presence of fermentable sugars, which help reduce ammonia stress and thereby increase survival of L. monocytogenes (Roberts, Chakravarty, Gardner, Ricke, & Donaldson, 2020).

In previous research, the anti-listeria effect of ammonia has been clearly demonstrated. E.g., when chicken manure is exposed to ammonia gas, this leads to a more than 4-log<sub>10</sub> reduction of L. monocytogenes (Himathongkham & Riemann, 1999). For wheat straw, corn grains, and cottonseed, ammonia treatment can eliminate 99.999% of Listeria and other contaminants present (Tajkarimi et al., 2007).

To test the antifungal effect of the surface smear, different fungal spores were inoculated in the smear suspension together with B. linens CX3, and the inhibitory effect on the fungi was assessed. As seen in Fig. 5, (a), the cheese smeared with B. linens CX3 culture developed a dense orange smear at a relatively low temperature (16 °C) in just 7 days.

Fig. 4. Time-kill assays for (a) L. monocytogenes 0107.0111 (b) L. monocytogenes 0107.0243 (c) L. monocytogenes 0107.0263 (d) L. monocytogenes 0107.0489 and (e) L. monocytogenes 0107.0513 for treating with B. linens culture (AW · 3% FCH). □ broth (AW · 3% FCH) adjusted to pH 9 with ammonia. Means among different substrates with capital letters are significantly different (P < 0.05); Means among different treating time with small letters are significantly different (P < 0.05).

3.5 Accelerated smear development and antifungal activity on cheeses smeared with dense B. linens CX3 suspensions

Surface-ripened cheeses are usually ripened much faster than nonsurface ripened ones, but can still take as much as one year; soft Limburger cheese is ripened for at least two weeks, semi-soft Tilsit 1–5 months, Danbo usually 1–2 months, and Gruyère for 4–12 months (Bockelmann, 2011). As shown in Fig. 5. (a), the cheese smeared with B. linens CX3 culture developed a dense orange smear at a relatively low temperature (16 °C) in just 7 days. To test the antifungal effect of the surface smear, different fungal species were able to grow on the cheeses. Mucor and Penicillium displayed robust fungal growth, whereas Aspergillus displayed poor growth.
even after 12 days. As shown in Table 1, the development of all the selected fungi on the cheese surfaces was effectively inhibited by the *B. linens* CX3 smear, and growth of *P. commune*, *A. ochraceus*, *A. niger*, and *Mucor* were strongly inhibited. *P. crustosum* was 87% inhibited in its growth. The inhibition of the molds could be due to a combination of things, e.g., competition for space and nutrients and the inhibitory effect of metabolites produced by *B. linens* such as ammonia and methanethiol. It has been reported that 400 mg/L of ammonia ultimately kills fungi (Samapundo et al., 2007; Veverka, Stolcová, & Růžek, 2007). Yet another reason for the antifungal effect observed could be the ability of *B. linens* CX3 to convert methionine to the volatile compound methanethiol (CH₃SH). This compound has been demonstrated to have antifungal effects (Veverka et al., 2007). It also was reported that *B. linens* could generate 2 mg/L of methanethiol, an amount that is enough to inhibit *P. expansum*, *A. niger*, *Rhizopus nigricans* and *Mucor racemosus* (Weimer, 1999).

4. Conclusions

The results obtained from this study clearly show the benefits of using high-cell density cultures of *B. linens* when smearing surface-ripened cheeses. Not only is it possible to establish a deep orange surface smear in days, in contrast to 3–4 weeks when using the traditional approach. Applying dense suspensions thus should significantly shorten the smear ripening period and reduce associated costs, e.g., manual labor for flipping cheeses, temperature, and moisture control of the ripening facilities. An added bonus was the strong antimicrobial effects observed. Five spoilage fungi were efficiently inhibited, as was *L. monocytogenes*, an effect that most likely was due to ammonia production. Finally, we have demonstrated a novel application of acid whey, a challenging by-product of Greek yoghurt Skyr and acid coagulated cheese production, which is costly to dispose of and currently has no real value to dairies.

CRediT authorship contribution statement

Shuangqing Zhao: Conceptualization, Methodology, Formal analysis, Investigation, Writing – original draft. Ge Zhao: Validation, Methodology, Writing – review & editing. Liuyan Gu: Writing – review & editing. Christian Solem: Conceptualization, Methodology, Supervision, Writing – review & editing, Validation.

<table>
<thead>
<tr>
<th>Fungal Species</th>
<th>Inhibitory effect (%)</th>
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<tbody>
<tr>
<td><em>P. commune</em></td>
<td>100 ± 0.0</td>
</tr>
<tr>
<td><em>P. crustosum</em></td>
<td>87.0 ± 3.2b</td>
</tr>
<tr>
<td><em>Mucor sp.</em></td>
<td>99.6 ± 0.2a</td>
</tr>
<tr>
<td><em>A. ochraceus</em></td>
<td>100 ± 0.0a</td>
</tr>
<tr>
<td><em>A. niger</em></td>
<td>100 ± 0.0a</td>
</tr>
</tbody>
</table>

Values are presented as mean ± SD N (n = 3). Different lowercase superscripts indicate significant different in different fungal species growth area on cheese (p < 0.05).

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

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