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Interactions between *Lactococcus lactis* and *Streptococcus thermophilus* strains in Cheddar cheese processing conditions

C.P. Champagne [a,*], D. Gagnon [b], D. St-Gelais [a], J.C. Vuillemard [b]

[a] *Food Research and Development Centre, Agriculture and Agri-Food Canada, 3600 Casavant, St. Hyacinthe, QC, Canada J2S 8E3*
[b] *Institut des nutraceutiques et des aliments fonctionnels, Centre de recherche STELA, Université Laval, Québec, QC, Canada G1V 0A6*

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ABSTRACT

The growth of pure and mixed cultures of *Lactococcus lactis* and *Streptococcus thermophilus* under simulated Cheddar cheese manufacture was examined. Cell-free wheys (CFW) of the cultures were prepared for analysis by automated spectrophotometry (AS). The maximal growth rate of the lactococci in *S. thermophilus* R0083 CFW was 13% higher than that noted in their own CFW and three lactococci also gave higher biomass levels (OD_{max}). During simulated Cheddar cheese fermentations with four paired cultures, one *L. lactis* strain grew 20% less when paired with *S. thermophilus* R0083, and an increase in colony forming units (cfu) was found with one other lactococcal strain. Viable counts of *S. thermophilus* in mixed cultures varied by less than 0.1 log cfu mL^{-1}. The AS data on OD_{max} in CFW were useful in predicting the evolution of cfu in the fermented mixed cultures. As a function of strain, the presence of *S. thermophilus* in a Cheddar fermentation process can enable extended growth of the lactococci.

1. Introduction

Starter cultures used in cheese manufacturing are generally composed of many strains of one or more species. It is well known that interactions occur between the various strains of a culture. Antibiosis is often encountered (Babel, 1977; Hugenholtz & Veldkamp, 1985), particularly due to bacteriocins (Bruno & Montville, 1993), but cooperation (Bellengier, Richard, & Foucaud, 1997; Moon & Reinbold, 1976) and symbiosis occur as well (Driessen, Kingma, & Stadhouders, 1982).

In traditional cheesemaking, blends of mesophilic and thermophilic cultures are rarely used. However, *Streptococcus thermophilus* cultures are increasingly being mixed with mesophilic starters, particularly to control acidification during production, moulding, pressing or storage. Furthermore, *S. thermophilus* can be useful in an anti-bacteriophage culture rotation strategy (Stokes, Ross, Fitzgerald, & Coffey, 2001). As a result, its use is extending to Cheddar cheese manufacturing (Michel & Martley, 2001; Morgan, O'Sullivan, Ross, & Hill, 2002). Data on interactions between strains in either mesophilic or thermophilic starters are available (Beal & Corrieu, 1991; Hemme & Foucaud-Scheunemann, 2004; Juillard, Furlan, Foucaud, & Richard, 1996; Moon & Reinbold, 1976), but little is known about interactions between *Lactococcus lactis* and *S. thermophilus*.

Automated spectrophotometry (AS), also called microplate or turbidimetry systems, has been used extensively to develop kinetic models of bacterial growth (Begot, Desnier, Daudin, & Lebert, 1996; Dalgaard, Ross, Kamperman, Neumeyer, & McMeekin, 1994). AS can ascertain the presence of inhibitory compounds in a medium (Skyttä, Haikara, & Mattila-Sandholm, 1993), or the presence of growth factors such as vitamins (Danish Standard, 2003). Although its value has been demonstrated in predicting the levels of colony forming units (cfu) in pure cultures (Dalgaard et al., 1994; Gaudreau, Renard, Champagne, & Van-Horn, 2002) it has never been used to predict populations resulting from interactions between lactic dairy cultures.

The aims of this study were therefore to examine interactions between *L. lactis* and *S. thermophilus* cultures in a simulated Cheddar cheese fermentation process and to ascertain the value of AS in predicting the evolution of viable counts during milk fermentations.

2. Materials and methods

2.1. Bacterial strains and cultures

Cultures used were *S. thermophilus* R0083 (Abiasa Inc., St. Hyacinthe, QC, Canada) and the following strains of *L. lactis*: RBL10, ULAAC I16, ULAAC I15, ULAAC I11, ULAAC I09, ULAAC I02, ULAAC C14, ULAAC C08, ULAAC C02, ULAAC B15, ULAAC A26 and ULAAC A08 (STELA culture collection; Laval University, Québec, Canada). The ULAAC strains were isolated from 13 old-style starters collected

* Corresponding author. Tel.: +1 450 768 3238; fax: +1 450 773 8461.
E-mail address: claude.champagne@agr.gc.ca (C.P. Champagne).

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and lyophilized since 1968 (Gagnon, 2006; Lacroix, 2008) and selected for their ability to produce high amounts of diacetyl. The RBL10 strain, a diacetyl producing strain, was isolated from the commercial starter MD089 (Ezal line, Rhône Poulenc, Dangé Saint-Romain, France). Stock cultures were prepared by mixing fresh cell suspensions with CRYOBANK™ beads (Copan Diagnostics, Inc., Corona, CA, USA) and freezing at −80 °C. A first transfer was carried out by adding one bead to 10 mL of M17 broth (Merck, Darmstadt, Germany) and incubating 16 h at 30 °C (for lactococcal strains) or 8 h at 37 °C (for *S. thermophilus* R0083).

2.2. Preparation of cell-free wheys

The inocula were obtained from M17-grown cultures centrifuged (5000 × *g* for 30 min) and resuspended in a 0.2 M potassium phosphate (BDH Inc, Toronto, ON, Canada). The cell suspensions were standardized to an optical density (OD) having an equivalent of 2.0 at 600 nm using a Beckman 7400 spectrophotometer (Coulter, Fullerton, CA, USA). The milk fermentation was carried out in a fashion that simulated some of the manufacturing practices (rennet addition, temperature profile) which occur during Cheddar cheese production. For each strain, a test tube containing 40 mL of microfiltered and pasteurised skimmed milk (Pûr Filtre, Lactantia™, Parmalat, Victoriaville, QC, Canada) adjusted to 31 °C was inoculated at 0.5% (v/v) with the fresh cell suspension. Immediately after inoculation, rennet (Maxiren double strength; DSM Food Specialties, Seclin, France) was added at 0.01% (v/v). The contents were blended by carrying out tube inversions and then placed into a temperature-programmable water bath (VWR Scientific, Bridgeport, NJ, USA). The temperature profile applied is described in Fig. 1.

At the end of the incubation, tubes were cooled in an ice-water bath, centrifuged at 5000 × *g* for 30 min at 4 °C (GS6R rotor, Beckman Coulter, Fullerton, CA, USA), and the supernatant (whey) was recovered. To enable a second fermentation in the fermented whey, 5 M KOH was added to the supernatants, but this generated the precipitation of phosphate minerals, which was undesirable for the subsequent spectrophotometry analysis. Fermented whey samples were therefore adjusted to pH 7.5 with 5 M KOH, incubated for 3 h at 50 °C, centrifuged at 5000 × *g* for 30 min, the supernatants adjusted to pH 6.5 with 1 M HCl and sterilized by filtration (22 μm pore size, Millex® GP, Millipore, Billerica, NY, USA). A Checkmate Laboratory (Beauport, QC, Canada) pH meter served for pH readings. These neutralised cell-free fermented milk extracts, referred to as cell-free wheys (CFW) were kept at 4 °C until used.

Two unfermented controls were prepared. In the first series, milk was supplemented with 0.75% (w/v) glucono-δ-lactone (GDL) (Sigma®, Steinheim, Germany), instead of the lactic culture, which acidified the medium to pH 5.2, a level considered desirable in Cheddar cheese manufacture. The progressive acidification properties of GDL justified this choice. Adding lactic acid would have enable milk coagulation, but not with a Cheddar cheese acidification profile. GDL enabled us to simulate the fermentation, with the rennet coagulation step, without the cells. This first control medium, whey supplemented with GDL, is coded WG. In the second series, 0.25% (w/v) of a casein hydrolysate (Casamino acids, Difco, St. Louis, MO, USA) was added with the GDL as well. This second control medium, WG supplemented with casamino acids, is coded WGCA. In both WG and WGCA rennet was added, as in the experimental treatments. These acid rennet-coagulated milk probes were then subsequently centrifuged, neutralised and filtered as described for the CFW.

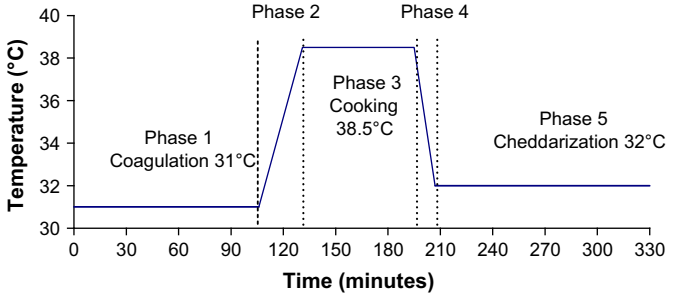

Fig. 1. Temperature profile during the milk fermentation step carried out for the preparation of the cell-free extract.

2.3. Automated spectrophotometry assays

For the automated spectrophotometry (AS) assays, the inocula were obtained from M17-grown cultures centrifuged (5000 × *g* for 30 min) and resuspended in a 0.2 M potassium phosphate (BDH Inc, Toronto, ON, Canada) to an OD of 0.6 at 600 nm using a Beckman 7400 spectrophotometer (Coulter, Fullerton, CA, USA).

For each of the test strains, 200 μL of the various CFW were distributed, in duplicate, into the wells of a microplate (Honeycomb™, Labsystems, Helsinki, Finland) and were inoculated with 20 μL of the standardized (OD_{600} = 0.6) cell suspension. Microplates were then placed into a Bioscreen C™ unit (Labsystems) and incubated at 30 °C for 24 h. The Bioscreen system was set to take OD readings (600 nm) of each well every 15 min after the plate had been shaken for 10 s at the "high" level. Four independent assays were carried out, and the data reported are the average of these replicates. The initial OD consequent on inoculation was subtracted from the subsequent OD values during incubation. Thus, OD_{max} data actually constitute increases in OD during the incubation.

Time-OD curves were typical of bacteria growth curves, with lag, exponential and stationary growth phases. Mathematical analysis of the curves enabled the determination of the maximum growth rate (μ_{max}), which typically occurs in the early exponential growth phase when the pH values and nutrient levels are high, as well as of the highest biomass level attained (OD_{max}).

2.4. Fermentation assays

Four lactococci were selected to test the validity of the predictive AS data in combination with *S. thermophilus*. Each culture was growth in M17 and populations ranging from 1.5 to 2.3 × 10^9 cfu mL^{-1} were obtained. Milk (Pûr Filtre) was inoculated and incubated in the Cheddar simulation process (Fig. 1) as carried out for the preparation of the CFWs. Four paired cultures (*S. thermophilus* R0083 together with one of the four *Lactococcus* strains) were tested. Inoculation levels were adjusted so that the same number of cells (1.4–1.5 × 10^7 cfu mL^{-1}) were added. Pure cultures were also prepared as controls, each inoculated with 1.5 × 10^7 cfu mL^{-1} of the tested strain. Samples were taken at the end of the incubation and analysed for viable counts. Three independent assays were carried out, and the data presented are the average of these replicates.

2.5. Enumerations

For the analysis of the inocula, the cell suspension was serially diluted in sterile 0.1% peptone (Difco). In the first dilution tube, the cell chains were broken by applying a 30 s homogenisation step using sterile disposable Omni-tips generator probes on an Omni TH (Omni International, Marietta, GA, USA) unit operated at maximum speed. Subsequent dilutions were carried under standard methods (Swanson, Busta, Peterson, & Johnson, 1992).

With the curds obtained in the fermentation assays, 10 g of curd was mixed with 90 mL of 2% sodium citrate (FIL, 1996) and blended in

a Stomacher 400 unit (Seward Ltd., Worthing, UK) for 45 s. Subsequent dilutions were done in the 0.1% peptone water with the Omni TH high-shear homogenisation step carried out as in the first dilution.

In mixed cultures, lactococcal counts were obtained by plating on M17-Xgal (see below) following an incubation of 48 h at 20 °C, while *S. thermophilus* colonies were counted following incubation of M17 plates at 45 °C for 48 h. Preliminary assays showed that no lactococci colonies appeared on plates incubated at 45 °C. Lactococci gave large white colonies on M17-Xgal, which confirmed species identification since *S. thermophilus* gives pin point blue colonies on this medium. The X-gal medium was prepared as follows. A solution of X-Gal (5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside; Biosynth AG, Staad, Switzerland) was prepared by adding 0.5 g of X-Gal to 5 mL of N, N,-dimethylformalide (Fisher Scientifics International Inc., Nepean, ON, Canada) and was sterilized by filtration (22 μm Millex® GP; Millipore). It was kept at 4 °C in an opaque container. Sterile M17 agar was then supplemented by 0.01% (v/v) of the X-Gal solution.

2.6. Statistical analyses

ANOVA were carried out on SPSS 12.01 for Windows (SPSS Inc, Chicago, IL, USA.) using the Student–Newman–Keuls test ($P = 0.05$). *t* tests were carried out with InStat 3.1 software (GraphPad, San Diego, CA, USA).

3. Results and discussion

This study was designed to simulate Cheddar cheese manufacture. When comparing such an approach with traditional milk fermentations carried out at a constant temperature until coagulation, three important parameters differ: 1) addition of rennet, 2) cheddarization temperature profile and 3) fermentation limited to 5.5 h. These conditions introduce, respectively, 1) proteolysis of milk proteins, 2) temperatures that can provoke uncoupling between growth and acidification of the lactococci (Breheny, Kanasaki, Hillier, & Jago, 1975) and 3) limited growth time.

3.1. Growth rates using spectrophotometry

The μ_{max} values of the lactococci were slightly affected by their prior fermentation. Indeed, only two of the twelve *L. lactis* strains showed statistically significant ($P < 0.05$) lower μ_{max} values when comparing data from WG and their own CFW (Table 1). Although the ANOVA only detected 2 such cases, slightly higher μ_{max} data were systematically noted in the WG (Table 1). Thus a paired *t* test was carried out with the 10 other strains, and it revealed that, on the average, the μ_{max} in WG was 20% higher than in the corresponding "own CFW" and that this difference was highly significant ($P = 0.006$). This was presumably because of the depletion of growth factors. In comparing WG and CFW data it must be kept in mind as well that GDL was used to acidify the WG, rather than lactic acid.

When the lactococci grew in the "CFW of R0083", the μ_{max} values were only 5% lower than in the corresponding WG, and this difference was not statistically significant in the paired *t* test analysis ($P = 0.87$). Since the ANOVA did not detect any strain which significantly grew better in the "CFW of R0083" than in the WG (Table 1), there does not appear to be an instance where *S. thermophilus* strongly stimulated the growth rate of a *Lactococcus* culture. Rather, these analyses suggest that some lactococci and streptococci might not compete for the same nutrients. Thus, when growth of the *Lactococcus* strains was ascertained by comparing "CFW of R0083" with "own CFW" rather than with WG data of the lactococci, two strains (ULAAC I11 and ULAAC C14) actually grew significantly ($P < 0.05$) faster in the R0083 CFW (Table 1). A paired *t* test carried out with the 10 other strains revealed that μ_{max} data were on the average significantly higher ($P = 0.002$) by 0.06 h^{-1} in the R0083 CFW. Therefore, the maximum growth rate of the lactococci in their own CFW was on the average 13% lower than those noted in *S. thermophilus* R0083 CFW. These data suggest that the presence of *S. thermophilus* in a Cheddar starter might not be as detrimental on the growth rate of the lactococci during cheese manufacture as would pairing with a similar *Lactococcus* culture.

Data also showed that the addition of casamino acids (WGCA) did not enhance μ_{max} values (Table 1). Although amino acids are recognised as being growth supplements for lactic cultures when added in whey (Champagne, St-Gelais, & Audet, 1996), these data confirm a previous study showing that casamino acids are not the ideal source of amino acids for lactococci (Juillard et al., 1995; St-Gelais, Roy, Hache, Desjardins, & Gauthier, 1993).

Statistical analysis did not detect significant ($P < 0.05$) effect of the strain of lactococci on μ_{max} values of *S. thermophilus* R0083 in the CFW of the lactococci (Table 2).

3.2. Biomass levels using spectrophotometry

A much different picture than that observed with the μ_{max} emerged with respect to maximum biomass (OD_{max}). A regression analysis between OD_{max} and μ_{max} values of *S. thermophilus* R0083 in the lactococci CFW did not show a high correlation ($R^2 = 0.47$). Therefore, parameters of medium composition that affect μ_{max} differ from those which affect OD_{max}. The OD_{max} value is linked to the ability of the medium to generate extensive growth, not necessarily rapid growth. The form of the nutrients would influence the OD_{max} in

Table 1
Growth of the various *Lactococcus lactis* strains in the cell-free whey (CFW) of *Streptococcus thermophilus* R0083, in unfermented WG (whey from milk acidified by glucono-delta-lactone) and WGCA (WG supplemented with casamino acids), as well as in their own CFW.[d]

Medium	*Lactococcus lactis* strain											
	ULAAC I16	ULAAC I15	ULAAC I11	ULAAC I09	ULAAC I02	ULAAC B15	ULAAC A08	ULAAC A26	ULAAC C14	ULAAC C02	ULAAC C08	RBL10
μ_{max} (h^{-1})												
CFW of R0083	0.40[a]	0.36[a]	0.61[a]	0.42[a]	0.37[a]	0.37[a]	0.67[a]	0.60[a]	0.36[a]	0.35[a]	0.42[a]	0.55[a]
Own CFW	0.36[a]	0.39[a]	0.27[b]	0.34[a]	0.30[a]	0.29[a]	0.49[a]	0.52[a]	0.18[b]	0.27[a]	0.38[a]	0.42[a]
WG	0.44[a]	0.43[a]	0.66[a]	0.39[a]	0.45[a]	0.38[a]	0.63[a]	0.53[a]	0.32a	0.39[a]	0.43[a]	0.46[a]
WGCA	0.36[a]	0.33[a]	0.58[a]	0.33[a]	0.36[a]	0.43[a]	0.68[a]	0.55[a]	0.39[a]	0.43[a]	0.40[a]	0.57[a]
OD_{max} (600 nm)												
CFW of R0083	0.02[a]	0.12[a]	0.30[a,b]	0.09[b]	0.06[c]	0.02[c]	0.22[a,b]	0.26[a]	0.03[b]	0.02[b]	0.02[b]	0.37[b]
Own CFW	0.04[a]	0.30[a]	0.07[c]	0.02[c]	0.03[c]	0.03[c]	0.20[a,b]	0.11[b]	0.07[b]	0.03[b]	0.04[b]	0.38[b]
WG	0.04[a]	0.05[b]	0.16[b,c]	0.03[c]	0.03[c]	0.03[c]	0.07[b]	0.09[b]	0.03[b]	0.03[b]	0.03[b]	0.15[a]
WGCA	0.13[a]	0.16[a]	0.41[a]	0.21[a]	0.25[a]	0.19[a]	0.37[a]	0.40[a]	0.34[a]	0.24[a]	0.18[a]	0.40[b]

[a,b,c] For a given variable (μ_{max} or OD_{max}), and in a given column, values that are followed by the same superscript letter are not judged to be significantly different ($P > 0.05$).
[d] Values given represent the average of 4 separate assays.

Table 2
Growth of *Streptococcus thermophilus* R0083 in the cell-free wheys (CFW) of various *Lactococcus lactis* strains.[c]

L. lactis CFW of strain	Growth of *S. thermophilus* R0083[d]	
	OD_{max}	μ_{max}
RBL10	0.23[a,b]	0.50[a]
ULAAC C02	0.26[b]	0.46[a]
ULAAC C08	0.29[b]	0.49[a]
ULAAC C14	0.27[b]	0.43[a]
ULAAC A08	0.11[a,b]	0.44[a]
ULAAC A26	0.16[a,b]	0.49[a]
ULAAC B15	0.23[a,b]	0.47[a]
ULAAC I02	0.19[a,b]	0.49[a]
ULAAC I09	0.11[a,b]	0.47[a]
ULAAC I11	0.07[a]	0.53[a]
ULAAC I15	0.23[a,b]	0.44[a]
ULAAC I16	0.24[a,b]	0.44[a]

[a,b] In a given column, values which are followed by the same superscript letter are not judged to be significantly different ($P > 0.05$).
[c] Values represent the average of 4 separate assays.
[d] Growth of *S. thermophilus* R0083 in its own CFW gave OD_{max} and μ_{max} readings of 0.13 and 0.38 h^{-1} respectively.

a different fashion than the μ_{max} values. As an example, free peptides and amino acids would enable faster growth (μ_{max}) than proteins. But it is ultimately the highest concentration of the essential nitrogenous growth factor that would influence OD_{max} most.

There were significant differences in biomass levels of *S. thermophilus* grown in the CFW of the lactococci (Table 2). It is known that bacteriocin-based negative interactions between lactococci and streptococci occur (Avila, Garde, Medina, & Nunez, 2007). Bacteriocin production by the lactococci was not seen in this study, since none of the OD_{max} values of *S. thermophilus* grown in the CFW of the lactococci that were lower than 0.13 (*S. thermophilus* R0083 OD_{max} in its own CFW) were judged to be statistically different. However, potential beneficial interactions between lactococci and *S. thermophilus* R0083 were identified by AS. Indeed, OD_{max} values of *S. thermophilus* R0083 in the CFWs of ULAAC C02, ULAAC C08 and ULAAC C14 were significantly higher than in the ULAAC I11 CFW (Table 2). As mentioned previously, this might be the reflection of different nutrient assimilation patterns rather than symbiosis.

Growth of the lactococci in WG resulted in rather small OD_{max} values, suggesting low levels of free nutrients. Supplementation with casamino acids significantly improved biomass levels for at least 10 lactococci strains (Table 1). Thus, the benefits of amino acids were greater on biomass levels (OD_{max}) than on maximum growth rates (μ_{max}).

Growth of the lactococci in their own pre-fermented medium gave OD_{max} values which were not different than those in WG except for two strains (Table 1). These data suggest that the growth factors were not highly depleted by prior growth in milk and/or that some growth factors were liberated in the whey during the initial fermentation.

Pre-fermentation by *S. thermophilus* R0083 was not detrimental to the biomass levels of lactococci. Three cultures actually had significantly higher OD_{max} values in CFW of R0083 than in their own CFW (Table 1).

Although no strong inhibitions were noted between the lactococci tested and *S. thermophilus* R0083, all these OD_{max} and μ_{max} data suggest that some mixed *Streptococcus–Lactococcus* cultures might favour the lactococci or the streptococci. Therefore, assays were carried out to assess the validity of AS methodology to predict growth of the mixed cultures during actual milk fermentations simulating the Cheddar cheese manufacture conditions.

Four *Lactococcus* strains were selected based on their growth patterns in the R0088 CFW. Strain ULACC C14 was selected because AS data showed that prior growth of R0083 was not favourable to its growth. Strain ULAAC I11, on the contrary, showed improved growth in the CFW of R0083. RBL10 and ULAAC C02 appeared unaffected by prior growth of R0083 and provided the potentially "neutral" pairings.

3.3. Growth of paired cultures during milk fermentation

After 5.5 h of fermentation under the simulated Cheddar temperature profile (Fig. 1), increases in viable counts in the pure lactococci cultures varied between 0.67 and 1.61 log cfu mL^{-1} (Table 3). Since care was taken to inoculate at the same cfu levels, these data showed that some cultures were much better adapted to growth in milk than others. This confirms previous observations (Stadhouders, Jansen, & Hup, 1969), and is typically related to the ability of the lactococci to utilise lactose or to carry out proteolysis (Desmazeaud, 1983). It must be kept in mind that the 38 °C cooking temperature might also affect growth of the lactococci (Breheny et al., 1975).

Regarding mixed cultures, *L. lactis* RBL10 and ULAAC I11 showed cfu increases similar to that of *S. thermophilus* R0083 and the strain ratio in the final product was similar to that at milk inoculation (Table 3). This was not the case with *L. lactis* ULAAC C02 and ULAAC C14 which demonstrated lower increases in viable counts than the streptococci in the fermented milk (Table 3). However, data from pure cultures also showed a lower cfu increase of these strains during the Cheddar-simulated fermentation. Therefore, since very different increases in viable counts with the lactococci were noted in pure cultures it was judged best to assess the effect of the presence of *S. thermophilus* R0083 on the growth of the lactococci by comparing increases in lactococci cfu between pure and mixed cultures. Data show that, in a simulated Cheddar cheese fermentation, subtle interactions between *S. thermophilus* and *L. lactis* strains did occur. Increases of up to 20% in viable counts of strains *L. lactis* RBL10 and ULAAC I11 were observed when paired with *S. thermophilus* R0083, and paired *t* tests showed that these increases were statistically significant ($P < 0.05$). The AS data also indicated higher OD_{max} values with these strains, when "CFW of R0058" and WG were compared, and in one instance (RBL10) it was also statistically significant (Table 2). These data suggested that the AS data could be effective in predicting the cfu counts in the mixed cultures, and a regression analysis was carried out. When the relationship between the effect of pre-fermentation by *S. thermophilus* on OD_{max} and that of mixed cultivation on cfu variations in the development of the lactococci was examined, an R^2 of 0.83 was

Table 3
Increase in viable counts after 5.5 h of milk fermentation under simulated Cheddar cheeses processing conditions in pure and mixed cultures.

Mixed or pure culture	Strain	Increase in cell count (log_{10} cfu mL^{-1})[a]
Pure	*S. thermophilus* R0083	1.61
	L. lactis RBL10	1.67
	L. lactis ULAAC I11	1.22
	L. lactis ULAAC C14	0.72
	L. lactis ULAAC C02	0.67
Mixed	*S. thermophilus* R0083	1.59
	L. lactis RBL10	1.73
	S. thermophilus R0083	1.54
	L. lactis ULAAC I11	1.54
	S. thermophilus R0083	1.63
	L. lactis ULAAC C14	0.61
	S. thermophilus R0083	1.61
	L. lactis ULAAC C02	0.78

[a] Cultures were inoculated at 1×10^7 cfu mL^{-1} (i.e., log_{10} cfu $mL^{-1} = 7.0$); values are the average of 4 separate assays.

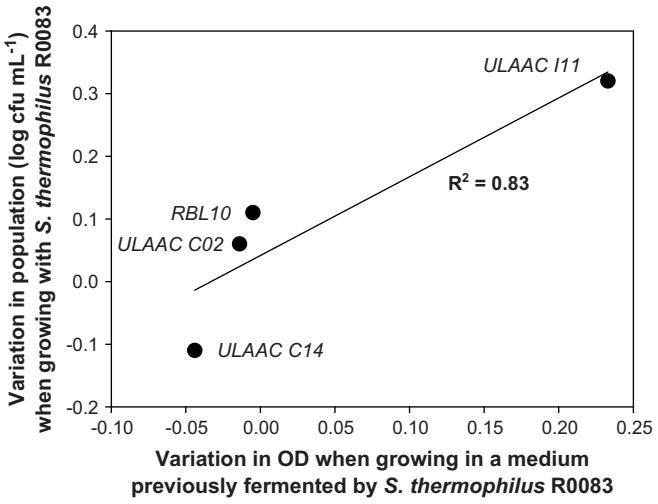

Fig. 2. Relationship between *Lactococcus–Streptococcus* strains interactions noted in the automated spectrophotometry assays and those noted in the milk fermentation assays.

obtained (Fig. 2). These data showed that the AS data were useful in predicting the growth of the lactococci expressed as cfu when paired with *S. thermophilus* R0083.

Under the conditions used in this study, the growth of *S. thermophilus* was less affected by the presence of the lactococci, since increases in number of streptococci in paired cultures varied by less than 0.1 log cfu mL^{-1} compared with that of the pure *S. thermophilus* culture (Table 3). Accordingly, statistical analysis did not find any significant effect of the addition of the lactococci on the viable counts of the streptococci. However, when the same type of regression analysis performed for the lactococci (Fig. 2) was carried out on *S. thermophilus* data, the R^2 value was of 0.93 (data not shown). Thus, AS data were effective in predicting the growth of *S. thermophilus* R0083 in the presence of the various lactococci as well.

AS had been previously shown to enable the detection of bacteriocins (Skyttä & Mattila-Sandholm, 1991) that have marked effects on the evolution of populations in starters. However, there are sometimes more subtle interactions between mesophilic (Babel, 1977; Bellengier et al., 1997; Hugenholtz & Veldkamp, 1985) and thermophilic cultures (Moon & Reinbold, 1976) that are difficult and labour-intensive to ascertain using traditional viable count techniques. This study showed that the AS data could be a tool for predicting the growth patterns of lactococci in paired Cheddar cheese cultures.

4. Conclusions

In Cheddar cheesemaking, streptococci can be used for protection against bacteriophages, high acidification rates during cheddarization, low post-acidification during pressing and storage and limited EPS production, while *Lactococcus lactis* ssp. *lactis* biovar. *diacetylactis* are mainly useful in flavour production. When applying a Cheddar cheese starter composed of *S. thermophilus* and *L. lactis*, ratios of streptococci to lactococci will therefore impact on flavour, texture and post-acidification. AS provided some insight into the relationship between lactococci and streptococci in mixed cultures by showing that pre-fermentation could affect both the growth rates and biomass levels. Thus, 1) μ_{max} of the lactococci in *S. thermophilus* R0083 CFW was on the average 13% higher than in their own CFW, 2) μ_{max} values of *S. thermophilus* R0083 in the CFW of the various lactococci were not significantly affected by the strain of lactococci, 3) pre-fermentation by *S. thermophilus* R0083 was not detrimental to the biomass levels lactococci, and three cultures actually had significantly higher OD_{max} values in CFW of R0083 than in their own CFW. AS proved to be a useful tool for predicting growth of both streptococci and lactococci in mixed cultures under Cheddar cheese manufacturing conditions. Further studies are warranted to ascertain if this methodology can serve to select other dairy strains used in multiple cultures, such as in yoghurt.

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