**_Lactobacillus parabuchneri_ produces histamine in refrigerated cheese at a temperature-dependent rate**

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Running title: Histamine production under refrigeration

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## Abstract

High histamine concentrations in food can cause histamine poisoning. In spite of the cold chain, cheeses with high concentrations of histamine are on the market. In this work, we studied whether *Lactobacillus parabuchneri,* the microorganism mainly responsible for histamine accumulation in cheese, is able to grow and produce histamine at the usual temperature range of refrigerators. Further, we analyzed whether refrigeration is really effective to prevent the accumulation of histamine in different types of cheeses supplemented with histidine and contaminated with *L. parabuchneri.* Our results showed *L. parabuchneri* to be able to grow and produce histamine at refrigeration temperatures. Moreover, *L. parabuchneri* produced toxic histamine concentrations in refrigerated cheeses from only 14 days. The results obtained in this work show that in the presence of *L. parabuchneri*, refrigeration delays but does not prevent the accumulation of toxic histamine levels in cheese.

## Keywords

Biogenic amines; histamine; *Lactobacillus parabuchneri*; cheese; refrigeration.

## 1. Introduction

Histamine is a biogenic amine (BA) that can be accumulated in fermented or spoiled foods by the microbial decarboxylation of histidine. Although histamine has important physiological functions in humans, the ingestion of foods or beverages containing it in large quantities can lead to histamine poisoning, the symptoms of which include headache, urticaria, rashes and dizziness (Ladero et al., 2010). Histamine is the BA most frequently involved in food poisoning (European Food Safety Authority (EFSA), 2011). Moreover, histamine has recently been shown to be cytotoxic (Linares et al., 2016), and this cytotoxicity is reported synergistic with that of tyramine (del Rio et al., 2017), another BA found frequently at high concentrations in cheese.

Histamine poisoning is commonly associated with the consumption of spoiled fish or fish products. At present, the histamine content in food is only regulated for fish and fish products (limited to 200-400 mg/kg by European Union Commission Directives 2073/2005 and 1019/2013, and to 50 mg/kg by the Food and Drug Administration USA [Food & Drug Administration (FDA), 2001]). However, fermented meats, vegetables, dairy products and alcoholic beverages may accumulate large amounts of histamine (Alvarez & Moreno-Arribas, 2014), in many cases exceeding its cytotoxicity threshold (440 mg/kg) (Linares et al., 2016). In some types of cheese (e.g. blue chesses and long-ripened cheeses), histamine can even exceed 1000 mg/kg (Fernández et al., 2007), and has been involved in cases of histamine poisoning (EFSA, 2011; Silla Santos, 1996).

The accumulation of histamine occurs because of the microbial decarboxylation of histidine, a survival mechanism used in acidic environments (Trip et al., 2012). The histamine-producing microorganisms in fermented products mainly belong to lactic acid bacteria (LAB) that carry the histidine decarboxylase gene cluster, either in the bacterial chromosome or in a plasmid (Diaz et al., 2015; Lucas et al., 2005). These bacteria may be present in the vat milk, be part of the starter cultures, or contaminate the food during manufacturing (Linares et al., 2012). The presence of histamine-producing LAB in cheese is difficult to avoid, especially in those made from low quality raw milk or manufactured in cheese dairy factories contaminated with such microorganisms (Ascone et al., 2017). However, some can survive long term pasteurization (65°C for 30 min) allowing them to appear in cheeses made from milk treated in this way (Ladero et al., 2011). It is therefore recommended that cheese be stored at low temperature after ripening to reduce the potential accumulation of histamine (Linares et al., 2012).

*Lactobacillus parabuchneri* was recently reported to be largely responsible for the accumulation of histamine in many types of cheeses (Diaz et al., 2016a; Diaz et al., 2016c; O'Sullivan et al., 2015). It is important to note that some histamine-producing *L. parabuchneri* strains isolated from cheese were previously wrong classified as *Lactobacillus buchneri* (Sumner et al., 1990; Sumner et al., 1985), and remain so-called in many publications and databases despite their reclassification (Diaz et al., 2016a; Fröhlich-Wyder et al., 2013). The species is habitually present in cheese, can produce large amounts of histamine, and can adhere to stainless steel, a characteristic that increases its potential to contaminate food during processing (Berthoud et al., 2017; Diaz et al., 2016a; Diaz et al, 2016b). However, the physiology of histamine production in *L. parabuchneri* at refrigeration temperatures has never been investigated. In the present study we investigated the growth and the histamine forming capacity of three *L. parabuchneri* strains (isolated from cheese) at refrigeration temperatures (4 - 8°C) in histidine supplemented MRS and cheese samples.

## 2. Materials and Methods

### *2.1. Bacterial strain and growth conditions*

The *L. parabuchneri* strains used in this work were IPLA 11122, IPLA 11117 and IPLA 11150. All were originally isolated from different types of cheese, are genetically different (their genome sequences are available at GenBank, accession no. NZ_LXIA00000000.1, NZ_LYDQ00000000.1 and NZ_LXUG00000000.1 respectively), and all are known to produce histamine (Diaz et al., 2016a; Diaz et al., 2016b). They were routinely grown in MRS (pH 6.0) (Oxoid, Basingstoke, England) at 37ºC.

In a preliminary test of the capacity of *L. parabuchneri* to grow at low temperature, *L. parabuchneri* IPLA 11122 was cultured in MRS supplemented with 5 mM L-histidine (MRS + His) (Sigma-Aldrich, Madrid, Spain) at 4, 8, 10, 12 and 14ºC for 79 days. Growth was monitored using a spectrophotometer (Eppendorf, Hamburg, Germany), measuring the optical density at 600 nm ($OD_{600}$).

The effect of temperature on growth and histamine production was examined in *L. parabuchneri* IPLA 11122, IPLA 11117 and IPLA 11150 cells grown in MRS broth (control) or MRS + His at 4ºC and 8ºC. Samples for analysis were taken every 7 days over 71 days. Growth at both temperatures was determined by plate counting (cfu/mL) on MRS agar. Histamine production by these cultures was analyzed as described below. Three biological replicates were used in each experiment.

As a positive control of growth and histamine production, cells were grown in MRS broth or MRS + His at 37ºC (the optimal growth temperature of *L. parabuchneri* [unpublished]), with samples taken for analysis at 24 and 48 h.

*2.2. Histamine production in cheese*

Seven commercially available cheeses with low histamine contents (<25 mg/kg) and with a creamy or grated texture to facilitate the homogenization after inoculation with *L. parabuchneri*, were selected: cheese 1, cream cheese made from blue cheese; cheese 2, grated Mozzarella cheese; cheese 3, grated Emmental cheese; cheese 4, cream cheese made from melted cheese; cheese 5, cream cheese; cheese 6, cream cheese made from Camembert cheese; and cheese 7, another cream cheese. Cheeses (100 g) were supplemented with histidine (final concentration of 20 mM), inoculated with 1 ml of a phosphate-buffered saline solution containing $10^8$ cfu/mL of strain *L. parabuchneri* IPLA 11122 (the strain that produced the largest amount of histamine at 4ºC) to achieve a final inoculum of $10^6$ cfu/g, homogenized and incubated aerobically at either 4ºC or 8ºC. To prevent any microbial contamination, cheeses were inoculated under sterile conditions and kept into sterile screw cap pots. Histidine and histamine were determined before incubation, and histamine production monitored at 14, 24, 42, 56 and 70 days. To provide positive controls, a portion of the inoculated samples was incubated at room temperature for 5 days. Uninoculated samples were kept at 4ºC for more than 70 days and used as negative controls.

### *2.3. Histidine and histamine quantification by ultra-high performance liquid chromatography*

Culture supernatants were obtained by centrifugation (2000 x *g* for 10 min at 25ºC) and cheese extracts obtained following the method of Herrero-Fresno et al., (2012). Histidine and histamine in culture supernatants and cheese extracts were quantified by ultra-high performance liquid chromatography (UHPLC) using a Waters H-Class ACQUITY UHPLC apparatus controlled by Empower 2.0 software, and employing a UV-detection method based on derivatization with diethyl ethoxymethylene malonate (Redruello et al., 2013).

### *2.4. Statistical analysis*

Results are presented as the means ± standard deviation of three biological replicates. Means were compared using ANOVA with post-hoc Bonferroni correction. Significance was set at *p*<0.05. All statistical calculations were made using SPSS v.15.0 software (SPSS Inc., IL, USA).

## 3. Results

### *3.1.* Growth of L. parabuchneri *at refrigeration temperatures*

*L. parabuchneri* IPLA 11122 was incubated for 79 days in MRS supplemented with 5 mM histidine at 4, 8, 10, 12 and 14ºC. Surprisingly, it was able to grow with $OD_{600}$ absorbance values of >4 being reached at all the temperatures assayed (Fig. 1A). Typical growth curves were recorded at 8, 10, 12 and 14ºC, with the exponential growth phase starting about 7 days after inoculation. As expected, the higher the temperature the steeper the slope. At 4ºC, growth was considerably slower, with a long lag phase lasting 28 days and a less steep exponential phase. However, a high final $OD_{600}$ value of 4.1 was still reached (Fig. 1A). Longer incubation times at 4ºC did not induce greater growth (data not shown). Maximum growth at the control temperature (37ºC) was reached after 48 h of incubation ($OD_{600}$=5.9).

In view of these results, IPLA 11122, IPLA 11117 and IPLA 11150 were incubated for 71 days at 4ºC and 8ºC on MRS with and without 5 mM histidine to examine the possible influence of histamine biosynthesis on growth (Figure 2). The absence of histidine did not affect the growth of the strains, as determined by plate counting (results no shown). All the strains grew at 8ºC. The maximum counts were obtained after 57 days, and were very similar for all three strains (IPLA 11122 = 10.03 ± 0.2 log cfu/mL; IPLA 11117 = 9.94 ± 0.1 log cfu/mL; IPLA 11150 = 9.8 ± 0.11 log cfu/mL). No significant differences were seen between the maximum counts obtained at 8ºC or 37ºC (maximum counts at 37ºC: IPLA 11122 = 9.88 ± 0.0 log cfu/mL; IPLA 11117= 9.91 ± 0.19 log cfu/mL; IPLA 11150 = 10.05 ± 0.18 log cfu/mL).

When incubated at 4ºC, the three strains showed different behaviours. IPLA 11122 (Fig. 2A) showed the greatest growth, with maximum counts obtained after 57 days (9.56 ± 0.06 log cfu/mL). IPLA 11117 reached its maximum count after 71 days (7.96 ± 0.82 log cfu/mL) (Fig. 2B). In contrast, IPLA 11150 did not grow at 4ºC; the counts even decreased from log 5.61 ± 0.11 to 4.23 ± 0.17 after 71 days (Fig. 2C). Nevertheless, the strain remained viable until the end of the incubation period.

### *3.2. Histamine formation by* L. parabuchneri *at refrigeration temperatures*

Histamine production of the investigated *L. parabuchneri* strains was monitored over the growth curve. In a first experiment, histamine production by IPLA 11122 was followed at 4, 8, 10, 12 and 14ºC (Fig. 1B). No significant differences were seen between cultures grown at 12ºC and 14ºC, with the highest histamine concentration (4.23 ± 0.08 mM) reached after 28 days. Histamine production was slower in cultures grown at 8ºC and 10ºC than at 12ºC or 14ºC, but the maximum histamine concentration (4.17 ± 0.11 mM) was also reached after 28 days of incubation. Histamine production in the cultures stored at 4ºC, however, was considerably slower. The maximum concentration of histamine was reached (4.17 ± 0.04 mM) after 42 days.

In a second experiment, histamine production by strains IPLA 11122, IPLA 11117 and IPLA 11150 at 4ºC and 8ºC was investigated (Fig. 2). No statistical differences were seen between the three strains after 22 days at 8ºC (the highest histamine concentrations were 4.06 mM ± 0.05 for IPLA 11122, 4.00 ± 0.05 mM for IPLA 11117, and 4.05 ± 0.03 for IPLA 11150) (Fig. 2A, 2B and 2C). Longer incubation times did not significantly increase histamine accumulation. Cultures grown at 37ºC reached after 48 days of incubation 3.94 ± 0.09 mM for IPLA 11122, 3.97 ± 0.12 mM for IPLA 11117 and 3.96 ± 0.12 mM for IPLA 11150 (data not shown).

The three strains also produced histamine when grown at 4ºC, but histamine formation differed considerably. IPLA 11122 (Fig. 2A) produced as much histamine after 43 days of incubation (3.96 ± 0.03 mM) as did the 37ºC control after 2 days, and IPLA 11117 (Fig. 2B) accumulated 2.48 ± 0.93 mM of histamine until the end of the experiment. Although IPLA 11150 did not show growth at 4ºC, it was able to produce 0.33 ± 0.03 mM of histamine during the 57 days of incubation (Fig. 2C). Thus, IPLA 11117 and IPLA 11150 produce less histamine when grown at 4ºC than at 37ºC.

### *3.3. Production of histamine in cheese by* L. parabuchneri *IPLA 11122 at different refrigeration temperatures*

*L. parabuchneri* IPLA 11122, the strain that produced the largest amount of histamine at 4ºC, was selected for further experiments. Seven commercial cheeses were supplemented with histidine and after inoculation with strain IPLA 11122, the production of histamine was monitored by UHPLC analysis after 14, 24, 42, 56 and 70 days at 4ºC and 8ºC. A representative UHPLC chromatogram of the cheeses analyzed is showed in Figure 3. Uninoculated cheeses were kept at 4ºC until the end of the experiment as negative controls. As a positive control, inoculated cheeses were incubated at 37ºC for 5 days. The histamine content of the cheeses at the beginning of the experiment ranged from 19 to 22 mg/kg. After 70 days of incubation, all cheeses accumulated histamine at both 4ºC and 8ºC. At 4ºC, the concentration ranged from 118 to 1030 mg/kg, while at 8ºC figures of 175-1838 mg/kg were recorded (Fig. 4). Histamine accumulation in the negative controls was <50 mg/kg. For cheese 7, which showed the strongest formation of histamine, the legal maximum value of 200 mg/kg for fish was reached between day 24 and 42 at 4°C, and less than 14 days at 8°C.

## 4. Discussion

To our knowledge, this is the first time that the accumulation of histamine in cheese at refrigeration temperatures, and the ability of histamine-producing bacteria (isolated from cheese) to grow and produce histamine under such conditions, have been studied. Recently, strains of *L. parabuchneri* have been repeatedly isolated from cheeses with high histamine contents (Berthoud et al., 2017; Diaz et al., 2016a; Diaz et al., 2016b), and culture-independent studies shown that strains of this species are mainly responsible for the accumulation of histamine in cheese (Berthoud et al., 2017; Diaz et al., 2016c; O'Sullivan et al., 2015). It is therefore of interest to know whether *L. parabuchneri* is capable of producing histamine at low storage temperatures. To date, histamine production at such temperatures has only been reported for *Morganella morganii* (Kim et al., 2002) and *Photobacterium iliopiscarium* (Takahashi et al., 2015) (Gram-negative bacteria isolated from fish).

As expected, the growth of the three strains in histidine supplemented MRS broth and the production of histamine declined with temperature (Fig. 1 and Fig. 2). However, it is remarkable that, although the ability of *L. parabuchneri* to grow and produce histamine at refrigeration temperatures was strain dependant, all the examined strains produced histamine even at 4ºC, including IPLA 11150, which was unable to grow under these conditions. Remarkably, an increase in incubation temperature from 4°C to 8ºC increased histamine production by a factor of up to 37 within the first 20 days.

Similar results were obtained in histidine supplemented cheese samples inoculated with *L. parabuchneri* IPLA 11122. Histamine accumulated at both temperatures tested, though it was greater at 8ºC. The differences in histamine accumulation observed among the cheeses stored at either 4ºC or 8ºC could be due to a differential growth of the histamine-producing strain, probably as consequence of the different additives present in each type of cheese. The results obtained clearly show that refrigeration delays histamine formation but is not a sufficient measure to prevent the accumulation of high amounts of histamine in cheese. The temperature reduction from 8°C to 4ºC (the usual temperature range of domestic refrigerators) contributed significantly to reducing histamine formation and extending the time to exceed the maximum histamine level of 200 mg/kg applied to fish.

## 5. Conclusions

In conclusion, the present study shows that individual strains of *L. parabuchneri* grow and produce histamine at refrigeration temperatures. Besides, cheeses contaminated with *L. parabuchneri* and supplemented with histidine accumulated histamine at 8ºC and even 4ºC, although more slowly. The 200 mg/kg legal limit for fish was reached in some of the cheeses from only 14 days. This time could be even lower in other types of cheeses whose characteristics were more favourable for the accumulation of BA. Although it is important to keep the conservation temperature as low as possible, refrigeration delays but does not prevent the accumulation of toxic concentrations of histamine in cheese. Therefore, it is essential to avoid the presence of histamine producing *L. parabuchneri* in dairy environments.

## 6. Figure legends

**Figure 1.** Growth and histamine production of *L. parabuchneri* IPLA 11122 at different refrigeration temperatures. Cells were grown for 79 days in MRS supplemented with 5 mM histidine at different refrigeration temperatures (4, 8, 10, 12 and 14ºC). A) Bacterial growth was determined by measuring the absorbance of the culture at 600 nm ($OD_{600}$). B) Histamine was determined by

UHPLC. Values are given as the means of three independent experiments ± the standard deviation.

**Figure 2.** Growth and histamine production of three *L. parabuchneri* strains in MRS supplemented with 5 mM histidine at 4°C and 8°C. Bacterial growth (cfu/mL) and histamine formation (mg/kg) was monitored over a period of 71 days. A) *L. parabuchneri* IPLA 11122, B) *L. parabuchneri* IPLA 11117 and C) *L. parabuchneri* IPLA 11150. The graphs show the mean values and standard deviations obtained from three independent experiments.

**Figure 3**. Representative UHPLC chromatogram of a cheese sample (cheese 7) supplemented with 20 mM of histidine and $10^6$ cfu/mL of *L. parabuchneri* IPLA 11122 after 56 days of incubation at 4ºC. Peaks corresponding to histidine and histamine are indicated.

**Figure 4.** Histamine accumulation (mg/kg) in cheese at different refrigeration temperatures. Cheeses were supplemented with 20 mM histidine, inoculated with *L. parabuchneri* IPLA 11122 and incubated at either 4ºC (white bars) or 8ºC (black bars). A) cheese 1, B) cheese 2, C) cheese 3, D) cheese 4, E) cheese 5, F) cheese 6 and G) cheese 7 (see text for types).

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## 8. References

Alvarez, M. A., & Moreno-Arribas, M. V. (2014). The problem of biogenic amines in fermented foods and the use of potential biogenic amine-degrading microorganisms as a solution. *Trends in Food Science & Technology,* **39**, 146-155.

Ascone, P., Maurer, J., Haldemann, J., Irmler, S., Berthoud, H., Portmann, R., Fröhlich-Wyder, M.-T., & Wechsler, D. (2017). Prevalence and diversity of histamine-forming *Lactobacillus parabuchneri* strains in raw milk and cheese – A case study. *International Dairy Journal*, **70**, 26-33.

Berthoud, H., Wüthrich, D., Bruggmann, R., Wechsler, D., Fröhlich-Wyder, M.-T., & Irmler, S. (2017). Development of new methods for the quantitative detection and typing of *Lactobacillus parabuchneri* in dairy products. *International Dairy Journal*, **70**, 65-71.

del Rio, B., Redruello, B., Linares, D. M., Ladero, V., Fernandez, M., Martin, M. C., Ruas-Madiedo, P., & Alvarez, M. A. (2017). The dietary biogenic amines tyramine and histamine show synergistic toxicity towards intestinal cells in culture. *Food Chemistry,* **218**, 249-255.

Diaz, M., del Rio, B., Ladero, V., Redruello, B., Fernández, M., Martin, M. C., & Alvarez, M.A. (2015). Isolation and typification of histamine-producing *Lactobacillus vaginalis* strains from cheese. *International Journal of Food Microbiology*. **23**, 215:117-123.

Diaz, M., del Rio, B., Sanchez-Llana, E., Ladero, V., Redruello, B., Fernández, M., Martin, M. C., & Alvarez, M. A. (2016a). Histamine-producing *Lactobacillus parabuchneri* strains isolated from grated cheese can form biofilms on stainless steel. *Food Microbiology,* **59***,* 85-91.

Diaz, M., Ladero, V., del Rio, B., Redruello, B., Fernandez, M., Martin, M. C., & Alvarez, M. A. (2016b). Biofilm-forming capacity in biogenic amine-producing bacteria isolated from dairy products. *Frontiers in Microbiology,* **7**:591

Diaz, M., Ladero, V., Redruello, B., Sanchez-Llana, E., del Rio, B., Fernandez, M., Martin, M. C., & Alvarez, M. A. (2016c). A PCR-DGGE method for the identification of histamine-producing bacteria in cheese. *Food Control,* **63**, 216-223.

European Food Safety Authority (EFSA) (2011). Scientific opinion on risk based control of biogenic amine formation in fermented foods. *EFSA Panel on Biological Hazards (BIOHAZ). EFSA Journal,* **9**, 2393–2486.

Fernández, M., del Rio, B., Linares, D., Martín, M. C., & Alvarez, M. A. (2006). Real-time polymerase chain reaction for quantitative detection of histamine-producing bacteria: use in cheese production. *Journal of Dairy Science,* **89**, 3763-3769.

Fernández, M., Linares, D. M., del Rio, B., Ladero, V., & Alvarez, M. A. (2007). HPLC quantification of biogenic amines in cheeses: correlation with

PCR-detection of tyramine-producing microorganisms. *Journal of Dairy Research,* **74**, 276-282.

Food & Drug Administration (FDA) (2001). Scombro toxin (histamine) formation. In Fish and fishery products hazards and controls guide. Washington, DC: P. H. S. Department of Health and Human Services, Food and Drug Administration, Center for Food Safety and Applied Nutrition, Office of Seafood.

Fröhlich-Wyder, M.T., Guggisberg, D., Badertscher, R., Wechsler, D., Wittwer, A., & Irmler, S. (2013). The effect of *Lactobacillus buchneri* and *Lactobacillus parabuchneri* on the eye formation of semi-hard cheese. *International Dairy Journal,* **33**, 120-128.

Herrero-Fresno, A., Martinez, N., Sanchez-Llana, E., Diaz, M., Fernandez, M., Cruz Martin, M., Ladero, V., Alvarez, M.A. (2012). *Lactobacillus casei* strains isolated from cheese reduce biogenic amine accumulation in an experimental model. *International Journal of Food Microbiology,* **157**, 297-304.

Kim, S. H., Price, R. J., Morrissey, M. T., Field, K. G., Wei, C. I., & An, H. (2002). Histamine Production by *Morganella morganii* in Mackerel, Albacore, Mahi-mahi, and Salmon at Various Storage Temperatures. *Journal of Food Science,* **67**, 1522-1528.

Ladero, V., Calles-Enriquez, M., Fernandez, M., & A. Alvarez, M. (2010). Toxicological Effects of Dietary Biogenic Amines. *Current Nutrition & Food Science,* **6**, 145-156.

Ladero, V., Sánchez-Llana, E., Fernández, M., & Alvarez, M. A. (2011). Survival of biogenic amine-producing dairy LAB strains at pasteurisation conditions. *International Journal of Food Science & Technology*, **46**, 516-521.

Linares, D. M., del Rio, B., Ladero, V., Martinez, N., Fernandez, M., Martin, M. C., & Alvarez, M. A. (2012). Factors influencing biogenic amines accumulation in dairy products. *Frontiers in Microbiology,* **3**, 180.

Linares, D. M., del Rio, B., Redruello, B., Ladero, V., Martin, M. C., Fernandez, M., Ruas-Madiedo, P., & Alvarez, M. A. (2016). Comparative analysis of the *in vitro* cytotoxicity of the dietary biogenic amines tyramine and histamine. *Food Chemistry,* **197***, Part A*, 658-663.

Lucas, P. M., Wolken, W. A., Claisse, O., Lolkema, J.S., & Lonvaud-Funel, A. (2005). Histamine-producing pathway encoded on an unstable plasmid in *Lactobacillus hilgardii* 0006. *Applied and Environmental Microbiology*, **71**, 1417-1424.

O'Sullivan, D. J., Fallico, V., O'Sullivan, O., McSweeney, P. L., Sheehan, J. J., Cotter, P. D., & Giblin, L. (2015). High-throughput DNA sequencing to survey bacterial histidine and tyrosine decarboxylases in raw milk cheeses. *BMC Microbiology,* **15**, 266.

Redruello, B., Ladero, V., Cuesta, I., Alvarez-Buylla, J. R., Martin, M. C., Fernandez, M., & Alvarez, M. A. (2013). A fast, reliable, ultra high performance liquid chromatography method for the simultaneous determination of amino acids, biogenic amines and ammonium ions in cheese, using diethyl ethoxymethylenemalonate as a derivatising agent. *Food Chemistry,* **139**, 1029-1035.

Silla Santos, M. H. (1996). Biogenic amines: their importance in foods. *International Journal of Food Microbiology,* **29**, 213-231.

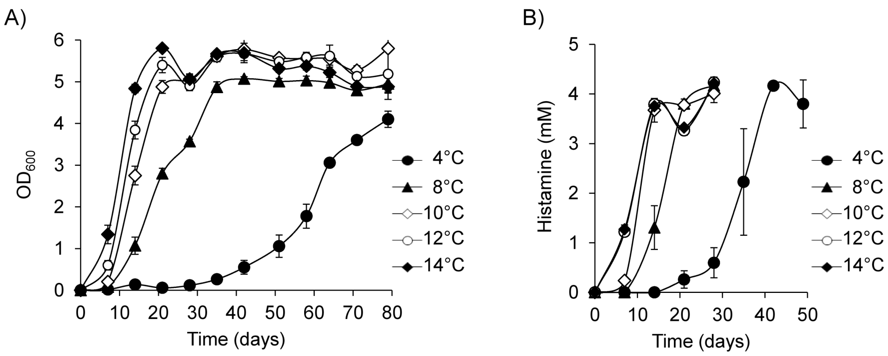
Srey, S., Jahid, I. K., & Ha, S.-D. (2013). Biofilm formation in food industries: a food safety concern. *Food Control,* **31**, 572-585.

Sumner, S. S., Roche, F., & Taylor, S. L. (1990). Factors controlling histamine production in Swiss cheese inoculated with *Lactobacillus buchneri*. *Journal of Dairy Science*, **73**(11), 3050-3058.

Sumner, S. S., Speckhard, M. W., Somers, E. B., & Taylor, S. L. (1985). Isolation of histamine-producing *Lactobacillus buchneri* from Swiss cheese implicated in a food poisoning outbreak. *Applied and Environmental Microbiology*, **50**, 1094-1096.

Takahashi, H., Ogai, M., Miya, S., Kuda, T., & Kimura, B. (2015). Effects of environmental factors on histamine production in the psychrophilic histamine-producing bacterium *Photobacterium iliopiscarium*. *Food Control,* **52**, 39-42.

Trip, H., Mulder, N.L., Lolkema, J.S. (2012). Improved acid stress survival of *Lactococcus lactis* expressing the histidine decarboxylation pathway of *Streptococcus thermophilus* CHCC1524. *Journal of Biological Chemistry*. **287**, 11195-11204.


A)
OD600
Time (days)
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B)
Histamine (mM)
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14°C

Figure 1

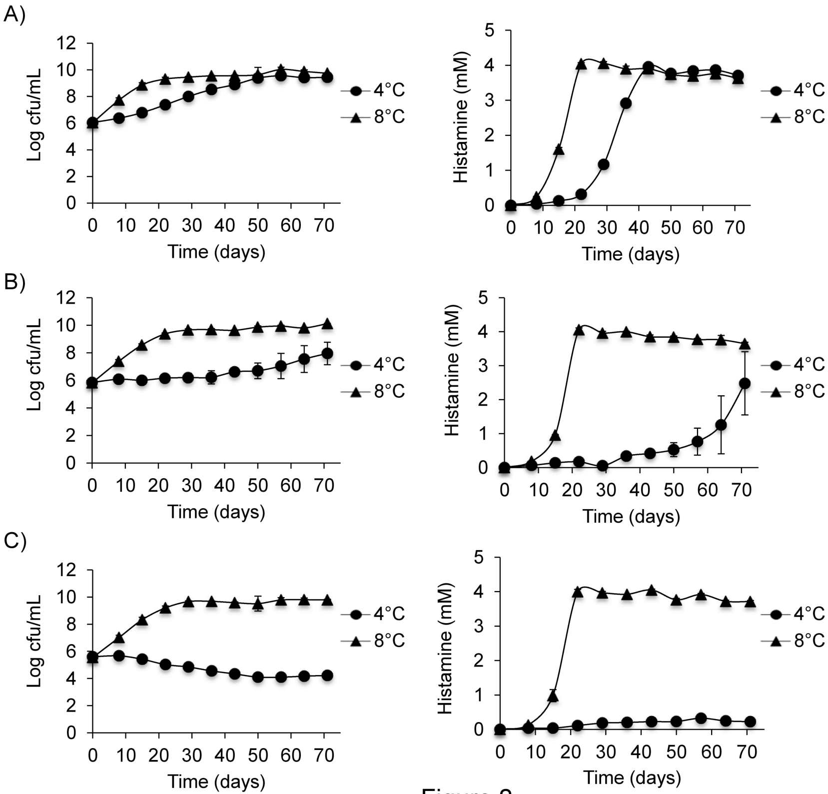
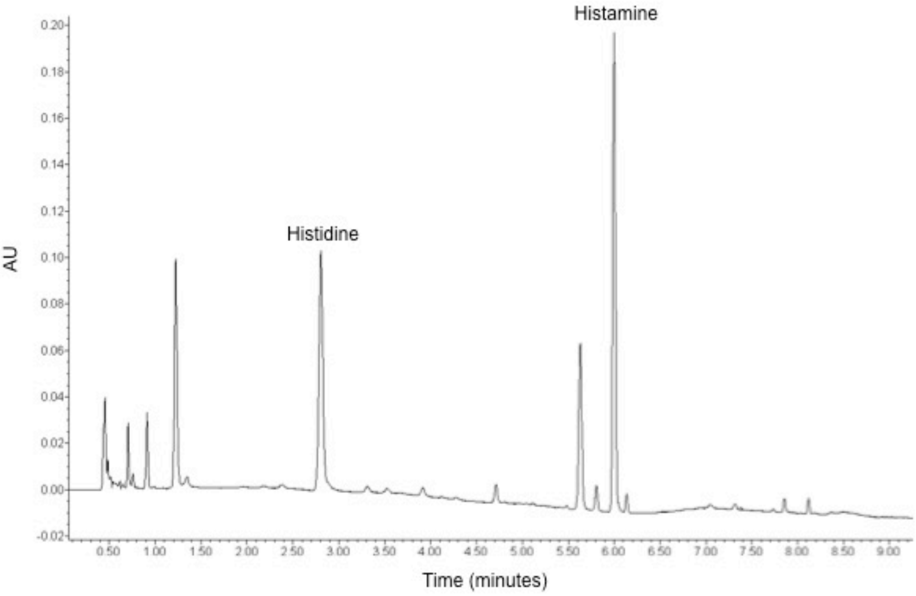



Figure 2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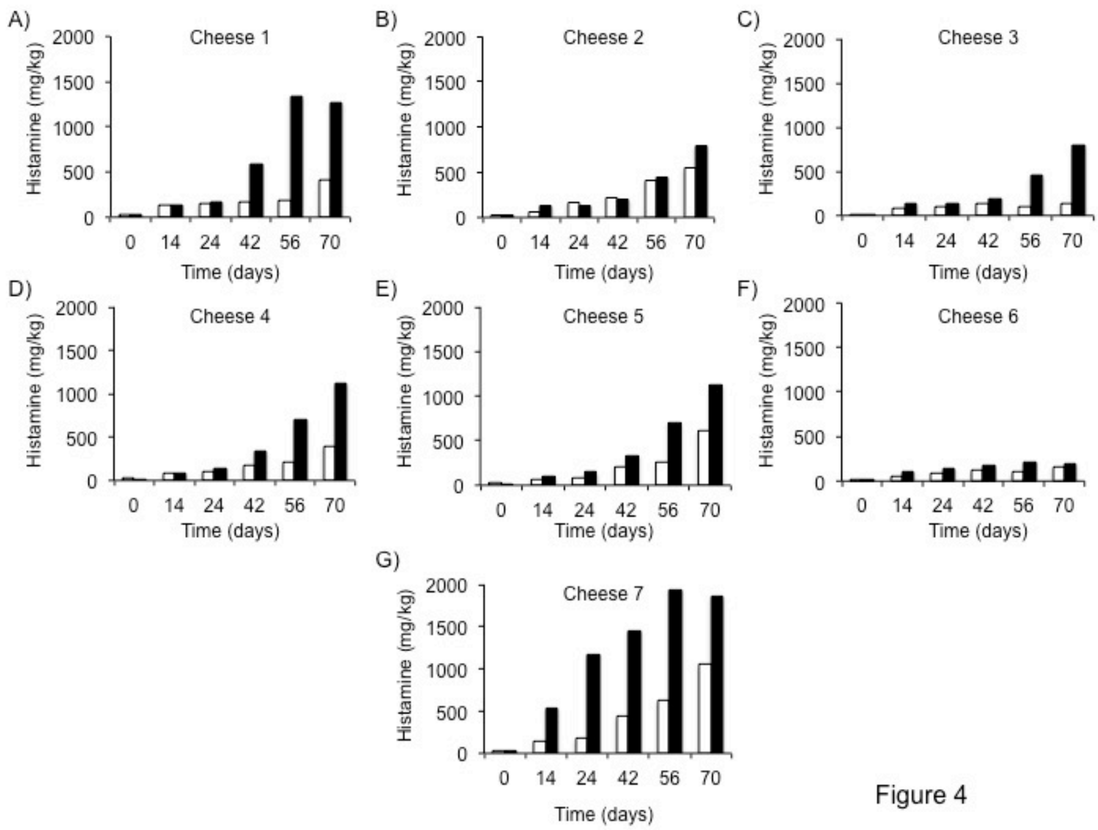
Time (minutes)

Figure 3

462



A)
Cheese 1
B)
Cheese 2
C)
Cheese 3
D)
Cheese 4
E)
Cheese 5
F)
Cheese 6
G)
Cheese 7
Histamine (mg/kg)
2000
1500
1000
500
0
0 14 24 42 56 70
Time (days)


Figure 4