**Microbial ecology of cheese samples**. The evolution of the microbial components during the cheese production process was evaluated by viable counts on their different selective media: M17 for Strepotrophus, MRS (pH 5.4) for Lactobacilli, and YGC for Yeasts [Fig. 1]. Samples belonging to most crucial ripening stages were subjected to a metagenetics analysis based on the 16S rDNA gene (Fig. 2a, for prokaryotes), and on the ITS portion of 18S rDNA (Fig. 2b, for eukaryotes).

![Image of microbial ecology](https://example.com/image.png)

**Figure 2.** Metagenetics, represented at species level, as calculated from 16S rDNA gene (a) and ITS-B (b) profiling. Only taxa with relative abundance > 1% are represented.

**Microbial ecology of cheese samples**. The evolution of the microbial components during the cheese production process was evaluated by viable counts on their different selective media: M17 for Streptococcus, MRS (pH 5.4) for Lactobacilli, and YGC for Yeasts [Fig. 1]. Samples belonging to most crucial ripening stages were subjected to a metagenetics analysis based on the 16S rDNA gene (Fig. 2a, for prokaryotes), and on the ITS portion of 18S rDNA (Fig. 2b, for eukaryotes).

**Figure 3.** Melting curves from qPCR quantifications in Gorgonzola samples using the Ar1 primer set: samples collected from day 1 to day 30. Blot from day 37 to the end of ripening. The arrow indicates the melting curves obtained using pure P. roqueforti DNA as template.

**Increasing qPCR sensitivity and final mycelium quantification.** 5 ng of exogenous pure P. roqueforti DNA were used to extract stool samples in order to quantify low amount of P. roqueforti DNA [Fig. 4a] before Day 37. This allowed the quantification of the fungal mycelium during the whole ripening time [Fig. 4b].

**Volatile Organic Acids (VOAs)**

**Profile.** A remarkable change in the VOAs profile was detected in the same period. In particular, the transition between the lactate acid bacteria-related phase (from day 1 to day 20) to the P. roqueforti-related phase (from day 37 to day 75) is linked to the increase in the relative abundance of some important taste and odor descriptors [Fig. 7]. It is worth of mention the change in relative abundance of hexanoic and heptanoic, which are associated to typical "cheese" flavour, and of octanoic and butanoic acid, which are typically associated to "inadequate" and "unripe" odours, and fundamental for the aromatic properties of several blue cheeses [4].

**Figure 6.** Mass Spectrometry plot describing the peptidic profile of Gorgonzola samples.

**Figure 7.** Box Plot representing the relative abundance (Arbitrary Units) of flavour-related Volatile Organic Compounds in the two key phases of ripening. Quantitative data obtained by UPLC-MS analysis on pure samples. 1) non-mature samples (from day 1 to day 20); 2) mature samples (from day 37 to day 75).

**Conclusions.** In this first deep study on the microbiological evolution and interactions inside Gorgonzola cheese during ripening, many interesting data have been produced. The plate count data mostly provided expected results, and they showed a large increase of yeast numbers before the incubation of cheese wheels into the cabinet, where the yeast activity is expected to produce the highest level of carbon dioxide necessary for the formation of the "caves."] After the preparation of cheese wheels, the internal temperature of each wheel remains relatively high (28-30 °C) for several hours, thus allowing the growth of the yeast that was metamorphosed by the galactose released by S. thermophila and L. delbrueckii lactic fermentation. The metagenetics data based on the 16S rDNA gene profiling revealed the presence of a large abundance of Cellulosimicrobium cellulans DNA, especially on the rinds according to previous observations [1]; otherwise, we showed how the presence of high relative amount of C. cellulosruptum DNA was found in the pasteurized milk, indicating the probable contamination of the pasteurizing plant with this microorganism. In this context the presence of C. cellulosruptum DNA was associated to the increase in the relative abundance of some important taste and odor descriptors [Fig. 7]. It is worth of mention the change in relative abundance of hexanoic and heptanoic, which are associated to typical "cheese" flavour, and of octanoic and butanoic acid, which are typically associated to "inadequate" and "unripe" odours, and fundamental for the aromatic properties of several blue cheeses [4].

**References.**


