The language of cheese-ripening cultures

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For many years, micro-organisms were considered individual organisms that primarily search for nutrients and multiply. Today we know that micro-organisms, which in nature often create complex communities, interact with each other. Cheese ripening is highly dependent on microbial interactions (Irlinger and Mounier 2009). However, only a few microbial interactions important for cheese ripening have been described, and the mechanisms underlying those are not well understood. The positive effects of microbial interactions not only assist in inhibition of undesired micro-organisms including spoilage micro-organisms and food-borne pathogens, but also serve in the development of desired micro-organisms, including starter cultures.

An interesting aspect of microbial interactions is cell-cell communication, often referred to as quorum sensing (for extended reviews, see Ryan and Dow 2008; Ng and Bassler 2009; Raina et al. 2009). The general features of quorum sensing involve production, secretion and detection of signalling molecules. When micro-organisms grow, they produce signalling molecules which are secreted to the extracellular environment. The extracellular concentration of signalling molecules increases proportionally to cell density. When a critical threshold level of signalling molecules corresponding to a certain cell number is achieved, receptors bind the signalling molecules and trigger signal transduction cascades that result in synchronised changes in gene expression. Thus, quorum sensing allows a community of unicellular organisms to turn on group behaviours in a manner similar to multicellular organisms. For intraspecies communication (within the same species), Gram-negative bacteria produce acyl homoserine lactones (AHLs) as signalling molecules. AHLs are composed of homoserine lactone (HSL) rings carrying acyl chains of various numbers of carbon and modifications. In contrast, Gram-positive bacteria produce modified oligopeptides as signalling molecules for interspecies communication. However, most of the time, micro-organisms do not live by themselves but in a mixture of different species. So in parallel, both Gram-negative and Gram-positive bacteria produce the group of signalling molecules referred to as autoinducer-2 (AI-2), which they use for interspecies communication (between different species). This allows bacteria to sense not only how many of its own, but also how many of others are present in the environment. In general, it is anticipated that more than 10% of the genes in the genome are controlled by quorum sensing (Schuster et al. 2003; Wagner et al. 2003). Processes such as bioluminescence, sporulation, virulence, conjugation, biofilm formation and bacteriocin production are among those reported to be under quorum sensing control. Until now, quorum sensing systems have primarily been investigated in pathogenic bacteria, as blockage of these systems might be an alternative to antibiotics. However, as quorum sensing is expected to be a general phenomenon in micro-organisms, it is likely to be of importance in micro-organisms found in foods.

An example of a food product, where quorum sensing could be of importance, is surface ripened cheeses. Surface ripened cheeses including limburger, tilsiter, romadour, brick and the danish danbo are characterised by development of a viscous, red-orange smear consisting of both yeasts and bacteria on the surface during ripening (Brennan et al. 2004). Yeasts, primarily Debaryomyces hansenii, are present during the initial period of ripening, and initiate the ripening process by raising pH at the cheese surface which allows the growth of less acid tolerant bacterial flora, consisting of primarily of Gram-positive coryneforms and staphylococci. The smear on the surface plays an important role in the final aroma and texture of the cheeses, and is often responsible for the unique characteristics of cheeses.

Traditionally, yeasts and bacteria are introduced to the cheese surface by a slurry containing smear from previously produced cheeses. However, slurries may also introduce undesirable micro-organisms, such as Listeria monocytogenes. Therefore, the use of well-defined ripening cultures has been paid attention with the purpose of ensuring the best quality control (Bockelmann et al. 2005). Several starter cultures have been chosen due to properties such as aroma formation and anti-microbial activity.
However, studies have shown that these starter cultures do not necessarily establish well in the cheesemaking environment and are outcompeted by the indigenous flora in the dairy (Petersen et al. 2002; Mounier et al. 2005). This shows that even though a single culture shows good properties on a laboratory scale, it does not among other cultures. Several factors could be of importance in the lack of establishment of starter cultures, such as adhesion properties and NaCl tolerance (Gori et al. 2005; Mortensen et al. 2005). However, other yet unknown factors such as quorum sensing may also be involved.

**Autoinducer-2 (AI-2) activity in dairy-relevant bacteria**

Among bacterial signalling molecules, the group called autoinducer-2 (AI-2) plays a unique role as it consists of the only presently known signalling molecules produced by both Gram-negative and Gram-positive bacteria, and presumably allows bacteria to respond to both endogenously produced AI-2 as well as AI-2 produced by other bacterial species in the vicinity. AI-2s are all derived from the same precursor, 4,5-dihydroxy-2,3-pentanedione (DPD), a by-product of the activated methyl cycle in a reaction catalysed by the LuxS enzyme (Schauer et al. 2001). Through cyclisation, hydration and borate ester formation, if enough borate is present, DPD rearranges spontaneously into a mixture of compounds, of which some function as signalling molecules. Bacterial species recognise chemically distinct forms of AI-2. The LuxP receptor protein of *Vibrio harveyi* binds (2S,4S)-2-methyl-2,3,3,4-tetrahydroxytetrafuran-borate (S-form) (Chen et al. 2002), whereas the receptor protein LsrB of *Salmonella enterica* serovar Typhimurium binds (2R,4S)-2-methyl-2,3,3,4-tetrahydroxytetrahydrofuran-borate (R-form) (Chen et al. 2002). For the moment, the LuxS-AI-2 quorum sensing system has not been investigated as well as for bacteria. The dimorphic yeast *Candida albicans* has especially been investigated with respect to alcohol-based quorum sensing. The sesquiterpene farnesol has been found to be a signalling molecule inhibiting both the yeast-to-hyphal shift and biofilm formation of *C. albicans* (Horny et al. 2001; Ramage et al. 2002). Furthermore, Chen et al. (2004) showed that the aromatic alcohol tyrosol, due to its promotion of hyphal development, shortened the lag phase time of *C. albicans*.

Furthermore, the aromatic alcohols phenylethanol and tryptophol have been found to be signalling molecules in *Saccharomyces cerevisiae*, where they stimulate pseudohyphal growth (Chen and Fink 2006). Furthermore, phenylethanol was found to stimulate invasive growth. Addition of tryptophol resulted in an even greater invasive growth. Furthermore, Chen et al. (2004) showed that the aromatic alcohol tyrosol, due to its promotion of hyphal development, shortened the lag phase time of *C. albicans*.
cerevisiae and Kluyveromyces lactis among others, have been shown to communicate with each other using pulses of ammonia (Palkova et al. 1997). The ammonia pulses result in growth inhibition of the parts of the colonies facing other colonies, allowing for overall coordinated growth of colonies away from one another and toward potential fresh nutrient sources instead. Similarly, we reported ammonia as a signalling molecule involved in the co-ordination of the growth on agar plates for three strains of D. hansenii (Gori et al. 2007). Thus, ammonia pulses appear to be an example of interspecies communication among yeasts. Oriented ammonia production of D. hansenii was both determined on a model substrate previously used for the detection of ammonia as a signalling molecule as well as a cheese substrate mimicking the cheese surface. Furthermore, on the model substrate, increases of ammonia production for double colonies compared to single colonies were observed for all three strains investigated, whereas on the cheese agar only minor increases in ammonia production for double colonies compared to single colonies were observed. The lack of increases in ammonia production on cheese agar might be correlated with the ability of the yeast strains to release amino acids from the cheese agar, as ammonia production involved in signalling has previously been shown to be highly dependent on free amino acids (Zikanova et al. 2002).

**Perspectives**

Increasing research effort is being made in understanding the knowledge of interactions between micro-organisms in microbial communities. The promising results on the potential use of quorum sensing for inhibition of pathogenic bacteria in a clinical context may have the potential to be transferred to the food industry. Increased knowledge of how starter cultures, as well as spoilage and pathogenic micro-organisms, in foods such as dairy products use quorum sensing to control growth may be of particular importance to obtain both better quality and safety. Although spoilage and pathogenic micro-organism growth might be prevented by blockage of their quorum sensing systems, starter cultures might be stimulated by the addition of the so-called pro-quorum sensing compounds, resulting in increased microbial metabolism which could be used to ensure the establishment and growth of starter cultures and thus stimulate important technological properties such as aroma formation and anti-microbial activity.

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**References**


