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School of Biosciences

Division of Food Sciences

CHARACTERIZATION OF THE NON-STARTER BACTERIAL FLORA OF STILTON CHEESE

Diriisa Mugampoza, BSc., MSc.

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Abstract

This study characterised the bacterial flora of a commercially produced Stilton cheese in an effort to determine the contribution of non-starter lactic acid bacteria (NSLAB) to its aroma profile. A total of 123 microbial strains previously isolated from different sites (outer crust, blue veins and white core) of the cheese sample obtained at the end of ripening (~8 weeks) were recovered in MRS and BHI broths and preliminarily identified using conventional microbiological methods in order to establish population diversity and to screen out yeasts and moulds. Organisms identified with partial 16S rDNA sequence analysis were *Lactobacillus plantarum, Lactobacillus brevis, Enterococcus faecalis, Staphylococcus aureus, Acinetobacter baumanii* and *Psychrobacter* spp., with the genus *Lactobacillus* being the dominant (75%) group detected in all the sampled sites. Cluster analysis of pulse-field gel electrophoresis patterns associated the *Lactobacillus* isolates according to their site of isolation.

Lb. plantarum isolates, two from each of the cheese sites, were evaluated for tolerance to heat stress and to different levels of salt, acid and relative humidity (RH) in order to ascertain whether the stress conditions associated with the isolation site could select the phenotype of microbial species recovered. The $D_{72°C}$ values revealed that isolates obtained from the outer crust were more heat sensitive suggesting they may have colonised the cheese post-pasteurisation. All the isolates were sensitive at pH range 3-4 but could grow at pH range 4.5-5. Similarly, isolates could grow at 3.5-5% (w/v) sodium chloride but were suppressed at 10%. Lactobacilli from the outer crust were the most halo-tolerant growing at 8% sodium chloride. For all strains, survival was low at 33-54% RH when cells were suspended in sterile de-ionised water but survived better at 33% RH in maximum recovery diluent (MRD) suggesting cellular protection by MRD.

Lb. plantarum isolates from each site (outer crust=7; blue veins=19; white core=24) were tested for antimicrobial activity against *Listeria monocytogenes, Escherichia coli, Pseudomonas aeruginosa, Staph. aureus, Salmonella* Typhimurium, *Clostridium sporogenes, Lb. pentosus* and *Lactococcus lactis* using the plate agar overlay and paper disc diffusion assays. All the 59 *Lactobacillus* isolates were tested for plantaricin EF genes using PCR. The nature of antimicrobial activity was examined using cell-free supernatants treated to neutralise acids and/or hydrogen peroxide. Treatment with proteinase K was used to ascertain whether activity was due to bacteriocin (putative plantaricin) production. On solid medium,

the isolates had antimicrobial activity against Gram-negative and Gram-positive bacteria, each isolate showing activity against more than one species. *Lb. pentosus, Ps. aeruginosa, E. coli* and *L. monocytogenes* were the most sensitive whereas *Cl. sporogenes* was the most resistant spp. Activity against these organisms was mainly attributed to acid, and to a less extent, hydrogen peroxide and plantaricin production. Whereas *Lb. plantarum* isolates had a high prevalence of plantaricin EF genes, there was weak evidence for plantaricin production in liquid medium assays. Plantaricin production was only demonstrable among *Lb. plantarum* isolates from the veins and core against *Lb. pentosus*, implying the phenomenon was largely dependent on the genotype/strain of *Lb. plantarum* and was only active against closely related lactic acid bacteria.

Subsequently, the effect of growth and survival dynamics of the different genotypes of the organism on the volatile aroma profiles of milk was examined. Individual isolates, one from each of the cheese sites, were co-cultured with acid-producing *Lc. lactis* (APL) and non acid-producing *Lc. lactis* (NAPL) in UHT milk under simulated cheese ripening conditions. During early fermentation (0-48 h, 30^{o}C), the isolate obtained from the blue veins stimulated more growth of *Lactococcus* strains in mixed culture when compared to single cultures and to *Lactobacillus* isolates obtained from other sites in mixed culture. The volatile profiles of all *Lb. plantarum* strains grown alone were not significantly different (p>0.05). The type and levels of volatiles detected in mixed culture depended on the genotype/strain of *Lb. plantarum* inoculated as well as the acidification capability of *Lc. lactis* with which it was co-cultured. Co-culture of *Lactobacillus* isolates with APL resulted in increased aldehyde and alcohol production, whereas with NAPL only acetoin synthesis was enhanced. Salt addition had minimal effect on the volatile profiles. During further incubation (12 weeks, 18^{o}C), growth of *Lb. plantarum* strains was better in salted samples inoculated with NAPL. The NAPL strain remained stable at 7 $\log_{10}$ CFU/ml throughout, while the APL rapidly declined from 9 to less than 5 $\log_{10}$ CFU/ml. The highest level of alcohols, organic acids and acetoin was detected from samples inoculated with the pure culture of the *Lactobacillus* isolate obtained from the blue veins. Co-culture of the isolate with APL enhanced acid and alcohol production, whereas its co-inoculation with NAPL increased acetoin synthesis. As *Lb. plantarum* is an incidental organism in cheese, its presence is unpredictable; it was therefore concluded that occurrence of different genotypes of the organism could be a major contributory factor to the variations in the cheese quality characteristics from batch to batch.