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Diversity of *Lactobacillus* Species of Stilton Cheese Relates to Site of Isolation

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This study has characterized the dominant non-starter *Lactobacillus* species isolated from different sites in a Stilton cheese to establish its diversity, stress-tolerance, anti-microbial activity and potential contribution to quality of cheese. Fifty-nine *Lactobacillus* isolates were cultured from the outer crust, blue veins and white core of the cheese and were speciated phenotypically and by 16S rDNA sequence analysis. *Lactobacillus plantarum* was the dominant species detected with only two isolates identified as *Lactobacillus brevis*. Strains were typed by pulse-field gel electrophoresis (PFGE) using the enzyme *NotI* to examine their genomic diversity. Cluster analysis of PFGE patterns produced five major clusters which associated isolates with their sites of isolation within the cheese. One *L. plantarum* isolate from each cheese site was selected and evaluated for salt, acid, relative humidity, and heat tolerance to determine whether stress conditions within the isolation site selected their phenotype. $D_{72^{\circ}\text{C}}$ values were 6, 13, and 17 s for strains from the crust, veins and core, respectively, suggesting strains on the crust may not have been able to survive pasteurization and therefore had been added post-pasteurization. All strains recovered from heat injury within 24–48 h at 4°C. pH values of 3, 3.5, and 4 suppressed growth but strains showed a varying ability to grow at pH 4.5 and 5; isolates from the core (which has the lowest pH) were the most acid-tolerant. All strains grew at 3.5 and 5% salt but were suppressed at 10%; those from the crust (which has a lower water activity) were the most halo-tolerant, growing at 8% salt whereas strains from the core were sensitive to this salt concentration. All 57 *L. plantarum* isolates were examined for antimicrobial activity and variable activity against *Lactobacillus pentosus* and other genera was demonstrated; plantaricin EF genes were present in 65% of strains. It was concluded that there are varied phenotypes and genotypes of *Lactobacillus* in a Stilton cheese according to site of isolation. Occurrence of different *L. plantarum* genotypes could contribute to variation in the cheese quality from batch to batch and provides criteria for selecting isolates as potential adjunct cultures.

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