Title: Tracing back food spoiling bacteria during enzymatic cleaning with 16S rDNA metagenetic

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Abstract

Introduction: Leuconostoc spp. was described as spoiling bacteria in several food products. These bacteria have the particularity to grow very quickly and to dominate the bacterial flora throughout the shelf life even if the initial concentration is very low. *Leuconostoc* spp. is present in the environment of the food industry and could be harbored in biofilms. The biofilms are very resistant to conventional sanitizing methods and new strategies are proposed to eradicate them such as enzymatic cleaning.

Purpose: This study aims to analyze the microbial flora from the environment of a food process during conventional and enzymatic cleaning to identify sources of spoiling bacteria and their impacts on the food products.

Methods: Conventional and enzymatic cleaning was applied in a food company producing Italian dishes during 3 months. A total of 244 samples were analyzed, including surface samples, cleaning in place systems and food products throughout the shelf life by classical microbiology and 16S rDNA metagenetics. Statistical analysis was carried out with the R project software with different packages.

Results: During conventional cleaning, *Leuconostoc* spp. became the most predominant bacteria at the end of the shelf life (55,0±24,1%). After the implementation of the enzymatic cleaning, the proportion of this bacteria is reduced in the food products at the end of the shelf life (0,62 ±1,23 %) with a significant difference between conventional and enzymatic cleaning ($P<0,05$). *Leuconostoc* spp. was also detected on some equipment during conventional cleaning and was reduced after implementation of enzymatic cleaning ($P<0,05$).

Significance: The 16S rDNA metagenetic analysis is useful tool to identify the source of contamination by spoiling bacteria from the food equipment. Enzymatic cleaning reduces the proportion of spoiling bacteria from installations and improve the microbial quality of the food products.