Title Occurrence and diversity of free-living protozoa on butterhead lettuce

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Citation International Journal of Food Microbiology, Volume 147, Issue 2, 27 May 2011, Pages 105-111

Keywords Free-living protozoa (FLP); Lettuce; Light microscopy; Denaturing gradient gel electrophoresis (DGGE); Diversity; Most probable number (MPN)

Abstract

The occurrence and diversity of free-living protozoa (FLP) on butterhead lettuce (*Lactuca sativa* L.) was investigated using four different sampling techniques (washing, swabbing, homogenization, and excising). FLP were recovered from all leaf samples (n = 64), and cultures were FLP-positive after 1 week. Identification of FLP was performed by light microscopy and sequencing of denaturing gradient gel electrophoresis (DGGE)-separated 18S rRNA gene fragments. *Bodo saltans*, *Spumella* (-like) spp. and Cercozoa were the most common heterotrophic nanoflagellates. Amoebae belonged mainly to the Vannellida and Tubulinida. *Colpoda steinii* and *Cyclidium glaucoma* were the most common ciliates. The total number of FLP on middle leaves estimated by the Most Probable Number method ranged from 9.3×10^2 MPN/g to 2.4×10^5 MPN/g leaf, with flagellates (92 MPN/g to 2.4×10^5 MPN/g) being more abundant than amoebae (< 3 MPN/g to 9.3×10^3 MPN/g) and ciliates (< 3 MPN/g to 9.3×10^2 MPN/g). Washing or rinsing leaves followed by spin-drying in a household salad spinner reduced the protozoan number with maximum one log unit. Our survey shows that FLP on lettuce leaves are a common and diverse but largely unexplored group of microorganisms.