

## **Use of fluorescent *in-situ* hybridisation to investigate possible involvement of *Clostridium* spp. during softening and fermentation of cassava**

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**Abstract.** This study was designed to investigate the possible role of *Clostridium* spp. in softening of cassava, using a novel molecular method “fluorescent in-situ hybridisation” (FISH). The technique can be applied directly to fermented cassava, and is used in parallel with conventional cultural isolation techniques. The use of FISH has been proposed to validate the results of cultural techniques and to determine the presence of components of the microflora that were not previously recovered. Peeled and unpeeled cassava pieces were incubated at 40°C, 37°C, 30°C and 25°C for 5 days. Compiled results of current and previous *in vitro* model cassava fermentations, demonstrated large variations in the extent of softening during fermentation. In the most recent study, however, the fermentation did lead to cassava softening. Although low numbers of presumptive *Clostridium* spp. were recovered using conventional cultural methodology, results obtained using the FISH technique failed to demonstrate the presence of this bacterium. It was concluded that, while there is some evidence for the presence of *Clostridium* spp. in cassava fermentations, further work on the methodology of FISH assays is required. Possible reasons for the failure of the technique to detect Clostridia are discussed.