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# Culture-Independent Analysis of Bacterial Ecosystems in Milk and Cheese Samples Using Denaturing High Performance Liquid Chromatography

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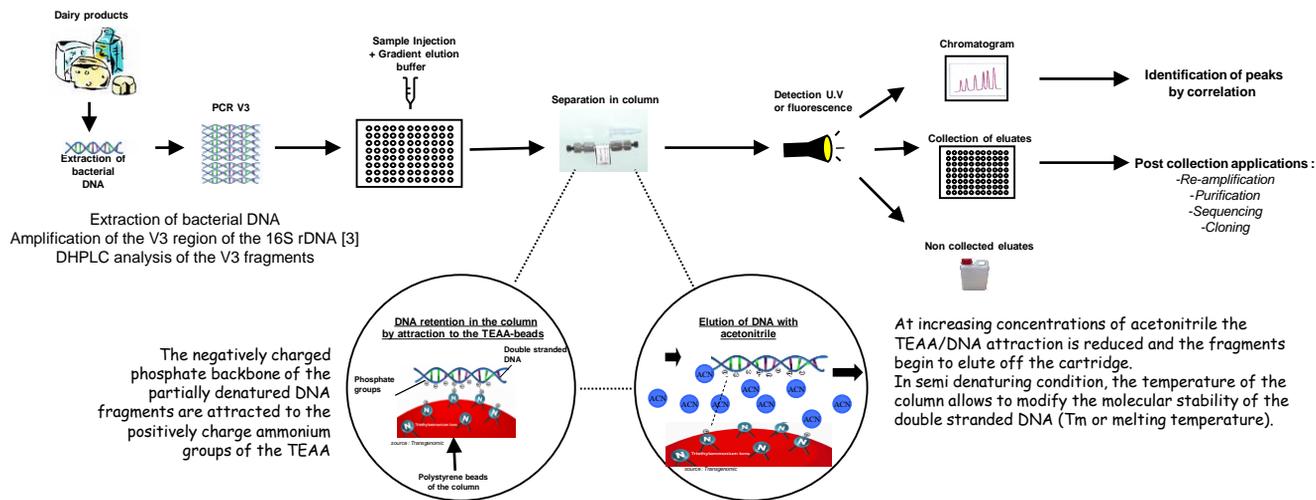
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DHPLC has been commonly used since the 1990s in medical research to detect DNA mutations and polymorphisms [1]. More recently, DHPLC protocols were adapted for the separation of bacterial PCR-amplified fragments [2].

We tested the feasibility of applying DHPLC (WAVE system; Transgenomic Ltd) to the field of the microbial ecology of dairy products for: i) identification and separation of pure strains in mixed cultures (lactic acid bacteria, staphylococci), ii) analysis of bacterial biodiversity in dairy products, iii) diagnosis of mastitis (by analysis of milk samples), and iv) following the dynamics of bacterial populations from milk to ripened cheese. We present here some of the results obtained in the various applications of DHPLC.

## Principle of DHPLC

DHPLC is an ion-pair reverse-phase chromatography. The stationary phase is composed of polystyrene beads grafted to an alkyl group. The mobile phase contains triethylammonium acetate (TEAA) and acetonitrile.



## Applications and Results

We tested the DHPLC for various applications in microbial ecology of dairy products.

First, we injected into the DHPLC system V3 amplicons of reference strains of lactic acid bacteria (Fig.1) and staphylococci (Fig.2).

Resulting peak profiles showed characteristic signals for each reference strain. We used these signals to identify later on bacterial species in dairy products.

We compared the potential of DHPLC with TTGE by analyzing Mediterranean cheese samples with the two molecular methods (Fig.3). Indeed, DHPLC offers a greater detection sensitivity using fluorescence.

We also used DHPLC technique to monitor the dynamics of bacterial populations from milk to ripened cheese (Fig.4).

A set of samples from ewe milk was analyzed by DHPLC to identify potentially infected milks (UV detection) (Fig. 5).

Infected samples were compared to the staphylococci database (Fig. 2) to identify the contaminating species (Fig. 6).

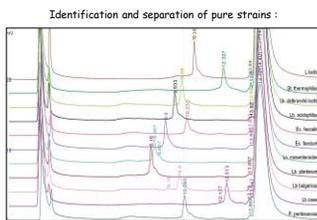


Fig 1 : Strains of lactic acid bacteria

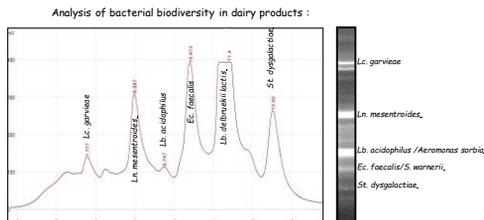


Fig 3. DHPLC chromatogram and TTGE band of Mediterranean cheese sample.

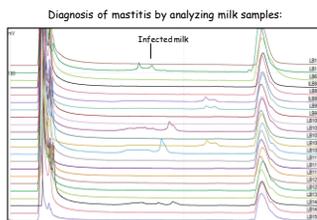


Fig 5 : Diagnosis of infected ewe milk.

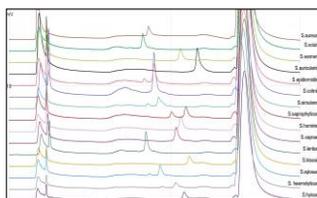


Fig 2 : Strains of staphylococci

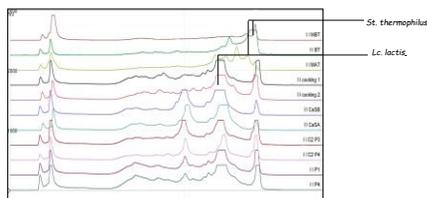


Fig 4. Monitoring the dynamics of bacterial populations from milk to ripened cheese.

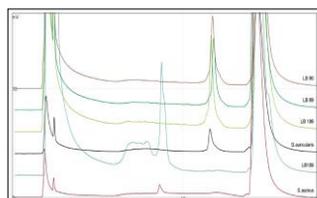


Fig. 6 : Identification of staphylococcal species in infected milk.

In conclusion, our results show the interest of the DHPLC in the field of microbial ecology of food products to complement the current repertory of methods for analyzing complex ecosystems. We have tested this technique for possible applications in dairy bacterial ecology and have shown that DHPLC technique is valuable. Moreover DHPLC is fully automated and offers very promising perspectives in the field of bacteriological diagnosis.

### References :

- [1] Le Maréchal, C., Audrézet, M.P., Quéré, I., Roguères, O., Langonné, S., Férec, C., 2001. Complete and rapid scanning of the cystic fibrosis transmembrane conductance regulator (CFTR) gene by denaturing high-performance liquid chromatography (D-HPLC): major implications for genetic counselling. Hum. Genet. 108, 290-298.
- [2] Goldenberg, O., Herrmann, S., Marjoram, G., Noyer-Weidner, M., Hong, G., Bereswill, S., Göbel, U.B., 2007. Molecular monitoring of the intestinal flora by denaturing high performance liquid chromatography. J. Microbiol. Methods 68, 94-105.
- [3] Ogier, J.C., Lafarge, V., Girard, V., Rault, A., Maladen, V., Gruss, A., Leveau, J.Y., Delacroix-Buchet, A., 2004. Molecular fingerprinting of dairy microbial ecosystems by use of temporal temperature and denaturing gradient gel electrophoresis. Appl. Environ. Microbiol. 70, 5628-5643.

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