# Characterization of Serpa cheese microbiome by Next Generation Sequencing (NGS) as assurance of specificity

SCIENTIFIC CONFERENCE ON RAW MILK



M.T. Santos<sup>1</sup>, P. Serol<sup>1</sup>, C. Lampreia<sup>1</sup>, O. Amaral<sup>1</sup>, A. Macedo<sup>1,2</sup>, C. Egas<sup>3,4</sup>, S. Ruiz-Moyano<sup>5,6</sup>, M.J. Benito<sup>5,6</sup>, M.G. Córdoba<sup>5,6</sup>, N. Alvarenga<sup>2,7,8</sup>

<sup>1</sup>ESA, IPBeja, Portugal; <sup>2</sup>LEAF, ISA, U. Lisboa, Portugal; <sup>3</sup>A.S.U., Biocant, Portugal; <sup>3</sup>C.N.C.B., U. Coimbra, Portugal; <sup>5</sup>EIA, U. Extremadura, Spain; <sup>6</sup>INURA, U. Extremadura, Spain; <sup>7</sup>UTI-INIAV, Portugal; <sup>8</sup>GeoBioTec, U. Nova de Lisboa, Portugal

nuno.alvarenga@iniav.pt

# Objectives

The aim of this work was to assess the specificity and biodiversity of autochthonous microbial consortium associated with Serpa cheese by High Throughput DNA Sequencing (HTS), thus contributing to the preservation of the specificity of this Portuguese dairy heritage.

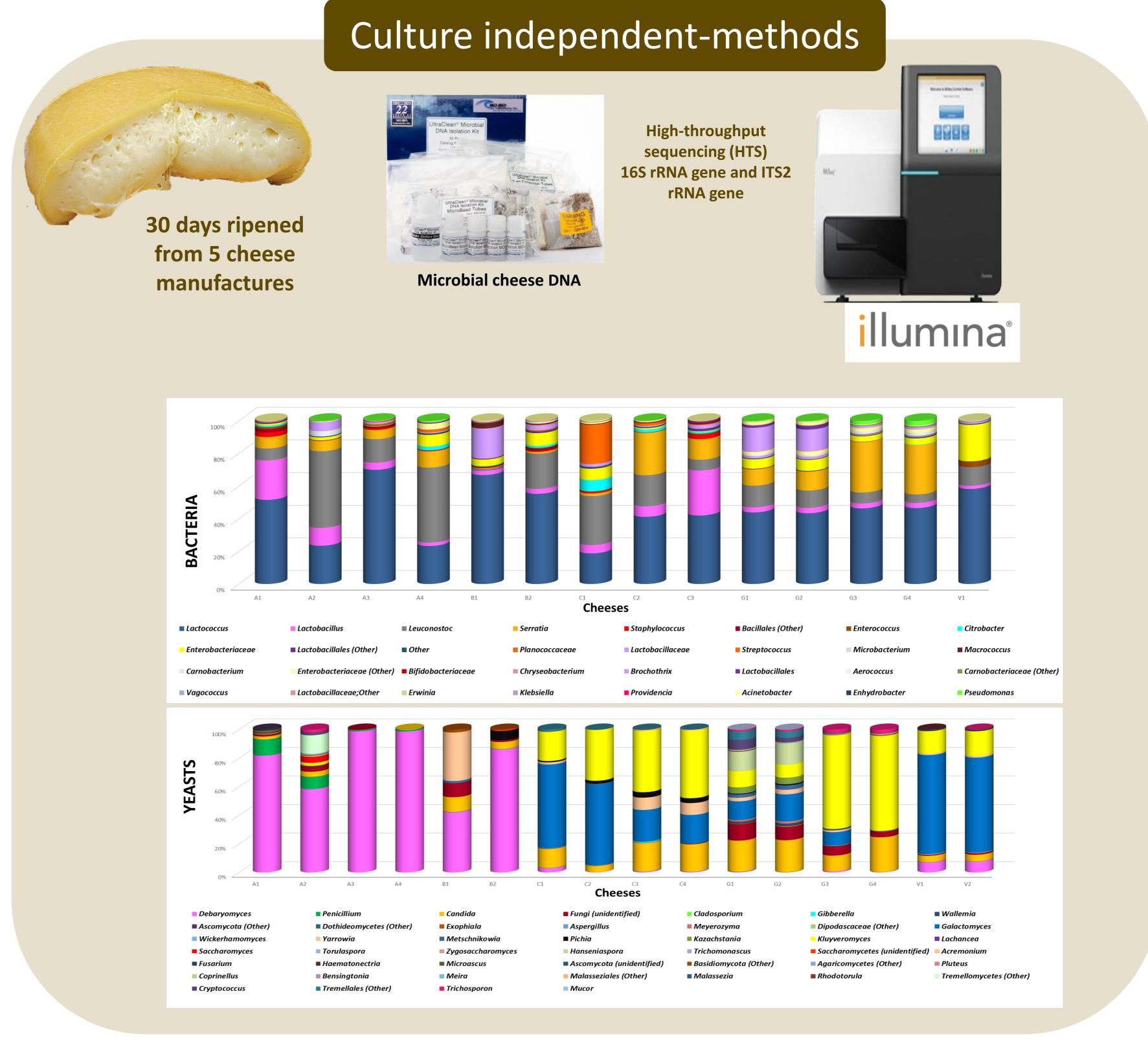
## Introduction

Serpa is a Protected Designation of Origin cheese, as provided for in Regulation (EEC) 2081/92 of the European Commission, as such, it must be manufactured in the defined geographic area. Serpa traditional manufacturing process maintained through the ages in region, requires the use of raw ewe's milk and vegetable coagulant based on dried flowers of *Cynara cardunculus* L., without any commercial starter, which emphasizes the role of the selected autochthonous microbiota. Its proliferation and qualitative composition will play a key role in its specificity. This microflora comes mainly from raw milk, but also from the whole surrounding environment.

## Conclusions

This approach confirms the diversity of the Serpa cheese microbiota and also that microflora mainly corresponded to lactic bacteria and to lesser extent, enterobacteria, estafilococos and yeasts. No pathogenic bacteria were identified in the different samples. The results obtained by HTS identify *Lactococcus* genus contributing to approximately 40% to 60% of the population, followed by *Leuconostoc* and *Lactobacillus*. The culture-independent methods established the prevalence of *Debaryomyces spp*. and *Kluyveromyces spp*. but also of *Galactomyces spp*.

### Culture dependent-methods **Genomic DNA** PCR-rRNA 16S illeula / Datcii 30 days ripened rRNA from 5 cheese manufactures Sequence Mesophilic aerobic bacteria PCA agar **Gene bank data** Lactic acid bacteria MRS pH 5,7 product base **BLAST algorithm** Staphylococci Baird Parker agar •Enterobacteria VRBG agar •Fungi RBC agar LAB identification CE TOACTGACTO GCTGCGTC GETCG GCTCG GCGCGC GA GCGGGCATCA GCTCAC TCAA AG GC GGTAA TA CGGT TA 140 GCT GA 350 350 400 400 410 ■ Lactobacillus casei halaMamanahman halamahman halamahman halamahman halamah ■ Enterococos hirae Lactobacillus brevis % of isolates identified YEASTS identification Pichia fermentans Candida pararugosa Candida zeylanoides ■ Candida cabralensis Yarrowia lipolytica Moniliella suaveolens % of isolates identified



**Acknowledgements:** SerpaFlora – Valorização da flora autóctone do queijo Serpa, PDR 2020–101–031018, FEDER/PDR 2020 Program; FCT – Fundação para a Ciência e Tecnologia for the financial support to the research units LEAF/ISA-UL

















