Study of the microbial diversity of doped milk powder by metagenomic analysis: Quantification of alive bacteria by exclusion of dead bacteria

Papa Abdoulaye Fall¹, Sophie Burteau¹, Emilie Detry¹, Carine Nezer¹, Bernard Taminiau², Georges Daube²

¹QUALITY PARTNER s.a.: Rue Hayeuenus, 62 4040 Herstal Belgique; ²Université de Liège, Faculté de Médecine vétérinaire, Fundamental and Applied Research for Animal & Health (FAARAH), Sart-Tilman, bkt. B43b, 4000 Liège, Belgique
afa@quality-partner.be

INTRODUCTION

Metagenomic is a scientific field that allow to describe and explore the microbial diversity of samples from various environment. Study of bacterial diversity and quantification of the taxa making up this diversity was performed on milk powder doped with bacteria being the object of a interlaboratory study organized by the Network of Analyses and Exchanges in Food Microbiology (RAEMA), Paris, France.

MATERIALS & METHODS

- A large bacterial diversity with the following genus: Lactococcus, Lactobacillus, Leuconostoc, Streptococcus, Pseudomonas, Geobacillus, Anoxybacillus, Acinetobacter, Aerococcus, Corynebacterium, Chryseobacterium and the inoculated bacteria.

- An important proportion of identified DNA sequences (55.5 % and 57.7 %) is attributed to Lactococcus lactis subsp. cremoris SK11 in conventional extracted samples and mechanical extracted samples respectively.

- When the exclusion of the DNA of dead bacteria was included before the extraction, this proportion dropped to 26.3 %.

- Subsequently a redistribution of the proportions of detected flora, showed an increase in Enterococcus faecalis proportion to 36.6 % (previously 1.8 %). This strain and Staphylococcus aureus constitute the major inoculated flora.

RESULTS

- Identification
- Bioinformatic analysis
- Library preparation qPCR Kapa
- DNA extraction & specific treatments
- High throughput sequencing Illumina Miseq
- DNA 16S amplification

CONCLUSION & PERSPECTIVES

These results demonstrate that the pre-treatment helps to mask the DNA of dead bacteria to better follow the populations of added bacteria with the exception of Salmonella (inoculated to the low level of 10 UFC / g).

However, the effect of the pre-treatment is variable on the various bacterial species and thus it must be optimized to obtain the exact proportions as by culture standardized culture techniques.

Figure 1: Proportions of bacterial species according to the type of extraction (%)

Figure 2: Proportions of bacterial species with and without exclusion of dead bacteria DNA (%)

ENTEROCOCCUS SP.
CITROBACTER SP.
E. COLI
S. AUREUS
SALMONELLA ANATUM
L. MONOCYTOGENES
C. PERTINGENS

PROPORTIONS AS BY CULTURE
H业余
HOWEVER WITH THE EXJECTION OF
THE EXCLUSION OF THE DNA OF
A REDISTRIBUTION OF THE PROPORTIONS OF
ENTEROCOCCUS
STREPTOCOCCUS
PSEUDOMONAS
ACINETOBACTER
AEROCCUS
CORNEBACTERIUM
CHRYSEOBACTERIUM