On the Microbiological Profile of Traditional Portuguese Maize Bread

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INTRODUCTION

The earliest method of obtaining reliable leavens was to keep a piece of fermented dough to be used as additive in the fermentation of the next dough. Such piece is called the sour ferment, and tradicionally is designated by "**isco**" in some regions of Minho.

From an economic point of view, bread made of maize (know as "**broa**"in several Nothern region) has a great importance in Portugal due the significant number of small farmers who produce this traditional product. This type of bread also plays an important social function via helping fixation of people in rural areas and preventing desertification towards urban areas. Finally, this product has a great potential to address issues by modern consumers with respect to natural and balanced foods.

The promising future of broa will require greater and more constant quality. This quality can only be legally enforced after certification, which might eventually be granted via definition of *Appéllation d'Origine Protegée* (AOP) regions. To this goal, several microbiological analyses of samples made available by traditional producers in two different periods and from different geographical locations, were performed. At present, we are investigating more throroughly the microbiological characteristics of the raw materials (sourdough, and maize and rye flours) used to produce this bread.

MATERIAL AND METHODS

In order to check for the existence of a wide diversity of microorganisms, a large number of culture media and incubation conditions were selected. ***Table 1*** shows the different conditions and types of microbiological analysis done.

Flour of maize and rye, as well as sourdough samples (10 g) from "Cabeceiras de Basto" was suspended in 90 ml of sterile 2% (w/v) sodium citrate and homogeneized in sterile beakers for 12 min. Serial decimal dilutions were then made on 0.1% (w/v) sterile peptone water.The samples were plated in duplicate.

Cicloheximide (150 mg/l) was added to all selective media to prevent growth of yeasts. Redution of 2,3,5-tripheniltetrazolium (TTC) by *Enterococcus* spp is useful to differentiate the colonies. On the other hand, 100 mg/ml of neomycin sulphate added to the culture medium brings about a better selectivity. *Clostridium* spp require anaerobic extreme conditions, so they were incubated on the RCM medium used CO_2 + H_2O or N_2.

Total viable counts were performed after inoculation and incubation on such media. Purified strains were first subject to several tests (Gram, catalase, oxidase, homo/heterofermentative test). Strains were further characterized via appropriate APIs galleries. ***Figure 1*** represents the schematic procedure used with the APIs.

Table 1 - Experimental Conditions

Culture media and antibiotics	Microorganisms	Conditions
PCA	Total viable counts (general and thermophilic viable counts) Spore counts (mesophilic and thermophilic spore counts)	T = 30 and 55 ºC; 24 h Spread plate under aerobic conditions T = 30 and 55 ºC; 24 h Spread plate under aerobic conditions
VRBDA	Enterobacteriaceae [1]	T = 30 ºC; 24 h T = 37 ºC; 24 h Pour-plate method
YEDCA 2 vials/l X209	Yeasts and Molds	T = 30 ºC; 24 - 48 h Spread plate under aerobic conditions
BCM 100 ml/l X073 2 vials/l X074	*Bacillus cereus*	T = 37 ºC; 24 –48 h Spread plate under aerobic conditions
MRS	*Lactobacillus* (*Pediococcus* and *Leuconostoc)*	T = 30 ºC; 48 h Spread plate under anaerobic conditions
MSA,	*Staphylococcus cereus*[2] (*Micrococcus*)	T = 30 ºC; 24 - 48 h T = 37 ºC; 48 h Spread plate under aerobic conditions
RCM	*Clostridium*	T = 30 ºC; 48 h Spread plate under anaerobic conditions
M17 agar	*Streptococcus* (*Lactococcus*)	T = 30 ºC; 48 h Spread plate under anaerobic conditions
KF Streptococcus agar	*Streptococcus* (*Enterococcus*)	T = 37 ºC; 24 h Spread plate under anaerobic conditions
MSE	*Leuconostoc*[3]	T = 30 ºC; 24 h Spread plate under aerobic conditions

[1] – *Enterobacter* species grow rapidilly on the usual enteric media. In general, strains from environmental sources grow better at 20 – 30 ºC rather than 37 ºC, whereas strains from clinical sources grow better at 37 ºC. *Escherichia* has an optimum temperature at 37 ºC.

[2] - Species from heavily contamined sources (*e. g.* feces) should also be inoculated on a selective medium such as MSA. The use os 2 temperatures has the objective to analyse the differences in the growing of *Micrococcus.*

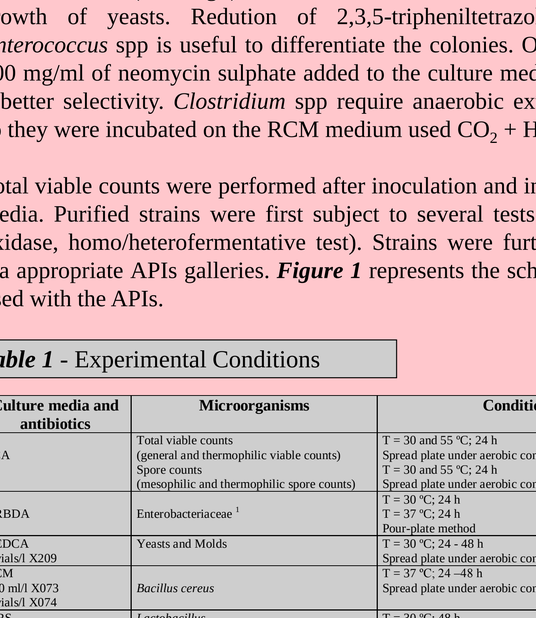

Figure 1 - Procedure used for identification of the isolates strains.

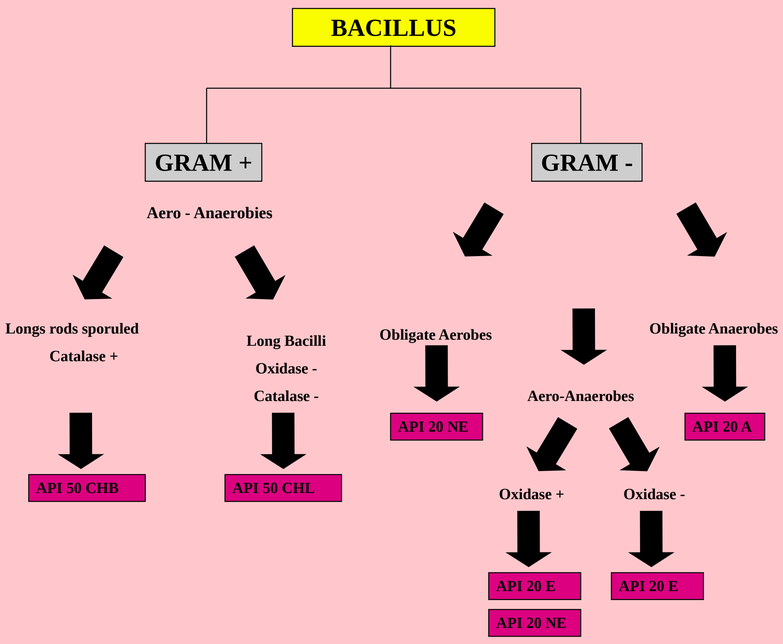

Figure 1 (cont.) - Procedure used for identification of the isolates strains.

EXPERIMENTAL RESULTS

Figure 2 shows the percentage of isolates for each culture media used. The total number of isolates was 419. ***Figures 3*** to ***10*** represent the main genera of the strains isolated in each medium.

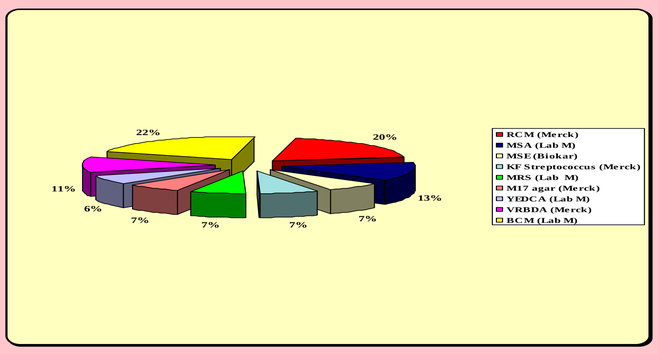

Figure 2 - Percentage of isolates for each culture media used.

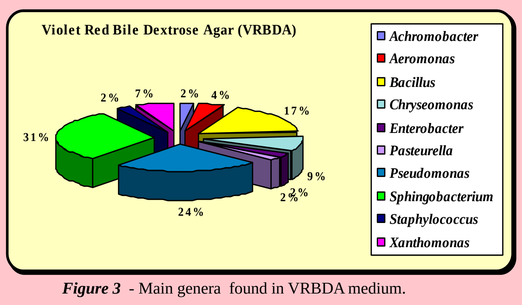

Figure 3 - Main genera found in VRBDA medium.

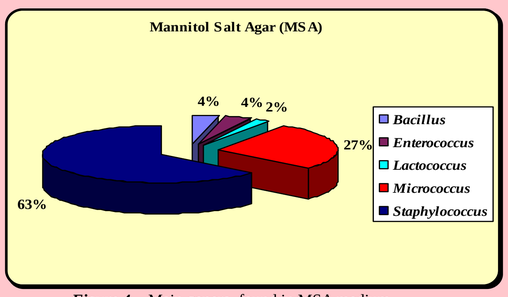

Figure 4 - Main genera found in MSA medium.

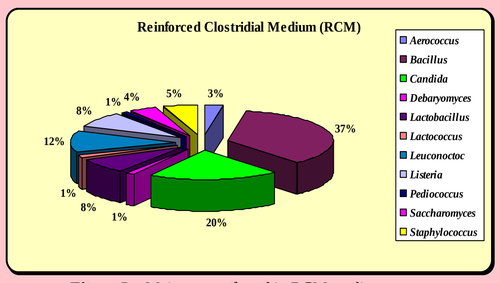

Figure 5 - Main genera found in RCM medium.

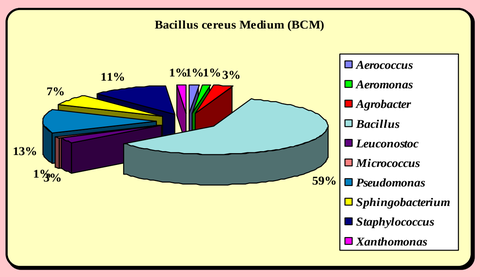

Figure 6 - Main genera found in RCM medium.

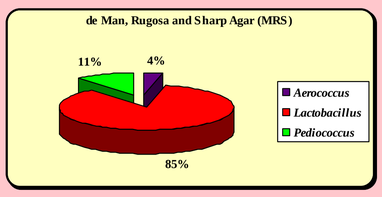

Figure 7 - Main genera found in MRS medium.

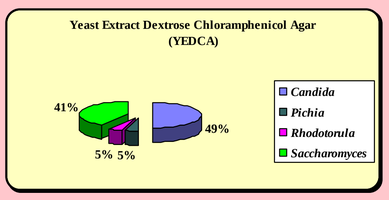

Figure 8 - Main genera found in YEDCA medium.

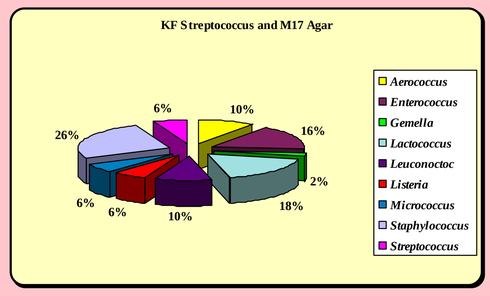

Figure 9 - Main genera found in KF and M17 medium.

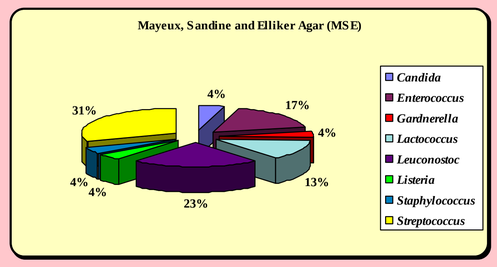

Figure 10 - Main genera found in MSE medium.

.***Figures 11*** to ***13*** shows the main strains isolated from sourdough, maize and rye.

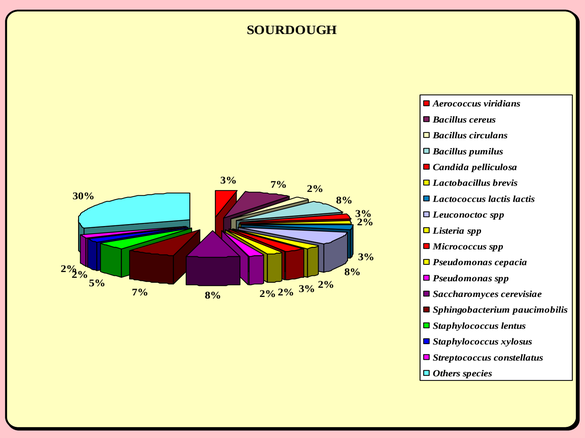

Figure 11 - Different strains founded in the sourdough.

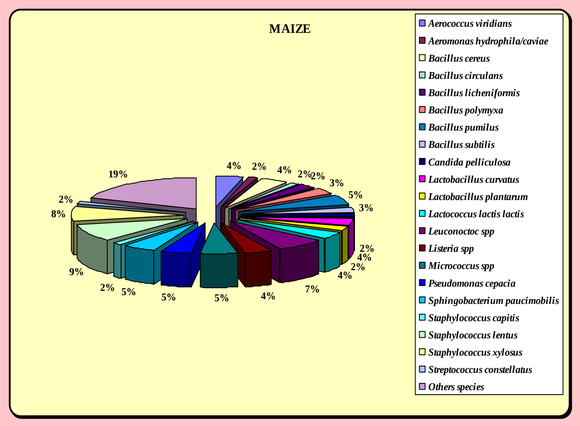

Figure 12 - Different strains founded in the maize flour.

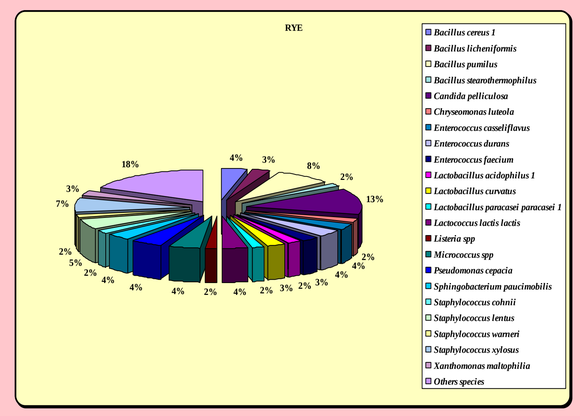

Figure 13 - Different strains founded in the rye flour.

ACKNOWLEDGEMENTS

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