Comparision of the Native Microflora of Portuguese "BROA" and that of Similar Sourdough Breads

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INTRODUCTION

Ground cereals mixed with water produce a dough which, after some time and owing to microorganisms present therein, may eventually become a sourdough which is characterized by a typical acid taste and an increased volume due to formation of gas. Both characteristics result from fermentation by complex endogenous microflora, and this type of fermentation was probably one of the first microbial processes employed by Man for food preservations purposes. According to some authors, the microflora in sourdough is a set of compatible strains of yeasts and lactococci (or cocci), synergistic interaction of which is likely to be an important factor towards production of unique flavours and textures. One bread that is still produced following such an ancient manufacturing procedure, at the farm level, in the Minho Region is (widely) known as **BROA**.

Manufacture of **BROA** plays important roles, from both economic and social standpoints, but a long way is yet to be tracked before such food specialty can be officially certified. To this goal, sequential steps of microbial quantification and identification have been taken, namely using samples from said maize bread manufactured in Cabeceiras de Basto.

Almost 420 isolates were tested, via appropriate BioMérieux API™ kits; most of them had been already recorded in traditional sourdough breads from other countries, although their profiles were somewhat unique in our case.

MATERIAL AND METHODS

Total viable counts in samples of sourdough, maize and rye flour were obtained after inoculation on 10 different solid selective media poured onto *Petri* dishes. The identification of 419 isolates was via appropriate BioMérieux API™ galleries, after several preliminary biochemical and morphological tests; such identification has shown that the total counts on each medium do not correspond exactly to the expected group of microorganisms for that culture medium. In order to check for the existence of a wide diversity of microorganisms, a large number of culture media and incubation conditions were selected (see ***Table 1***).

Flour of maize and rye, as well as sourdough samples (10 g) from *Cabeceiras de Basto*, were suspended in 90 ml of sterile 2% (w/v) sodium citrate, homogeneized in sterile beakers for 12 min and kept without agitation for an extra 8 min. Serial decimal dilutions were then made on 0.1% (w/v) sterile peptone water. The samples were plated in duplicate.

Total viable counts were performed after inoculation and incubation on such media. Purified strains were first subject to several tests (Gram and spore staining, motility, catalase, oxidase, homo/heterofermentative and aerobic/anaerobic fermentation tests). Strains were further characterized via appropriate API galleries (see ***Figure 1***).

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	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 aureus* (*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*	T = 30 ºC; 24 h Spread plate under aerobic conditions

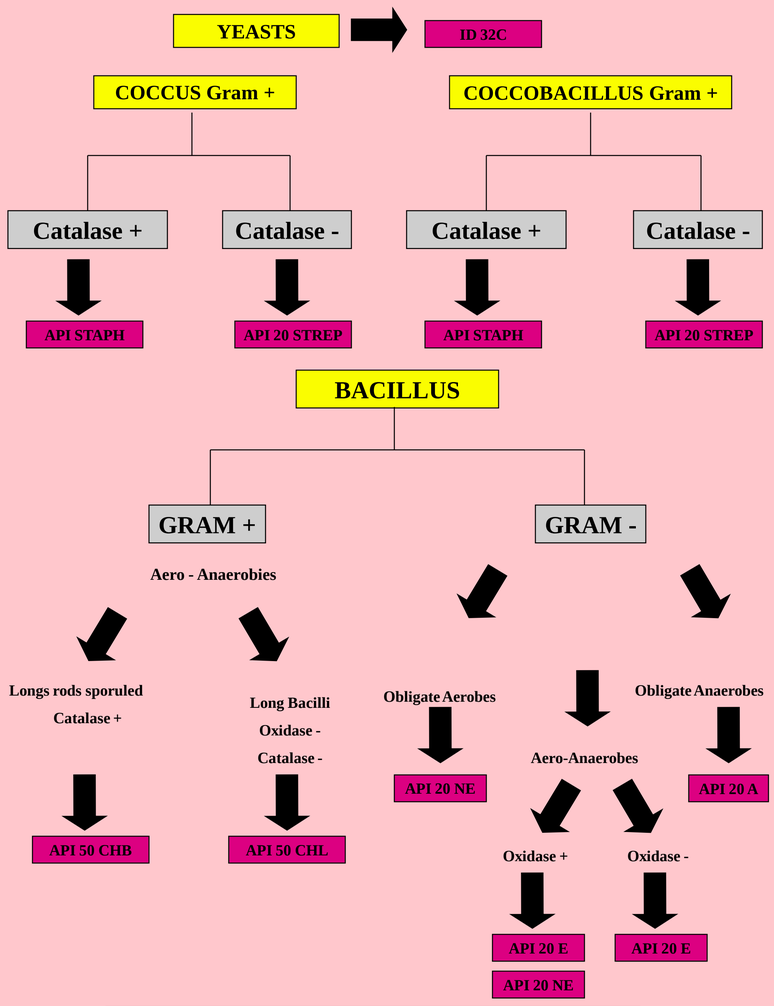

Figure 1 - **Procedure used for identification of the isolates strains.**

EXPERIMENTAL RESULTS AND CONCLUSIONS

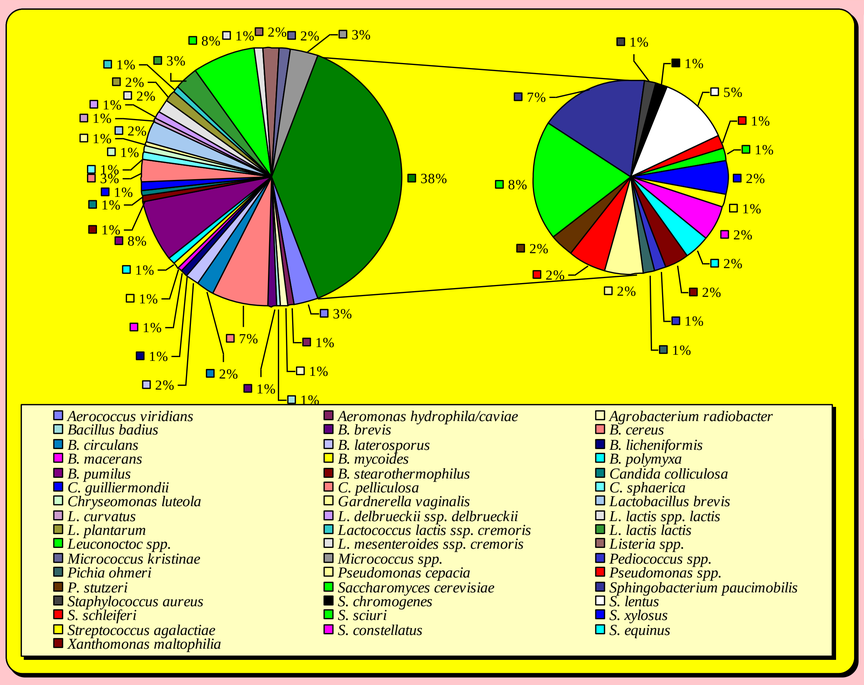

Figure 2 - **Species and corresponding frequency of appearance in SOURDOUGH.**

Sourdoughs of similar breads originating in other countries contain complex microflora, where yeasts and lactic acid bacteria predominate, and which, owing to synergistic interactions, produce distinct acidic tastes and unique flavors [1 - 12]; these microorganisms and LAB are favored by the environmental conditions prevailing during storage, *i.e.* low temperatures and high relative humidities.

According to these authors [1 - 12], the microflora in sourdough is a set of compatible strains of yeasts and LAB, synergistic interaction of which is likely to be an important factor towards production of unique flavours and textures.

A major consideration concerning sourdough fermentation is its effect upon texture of the final bread, as a consequence of the carbohydrate metabolism of LAB, which is affected by flour composition or interactions with yeasts [7].

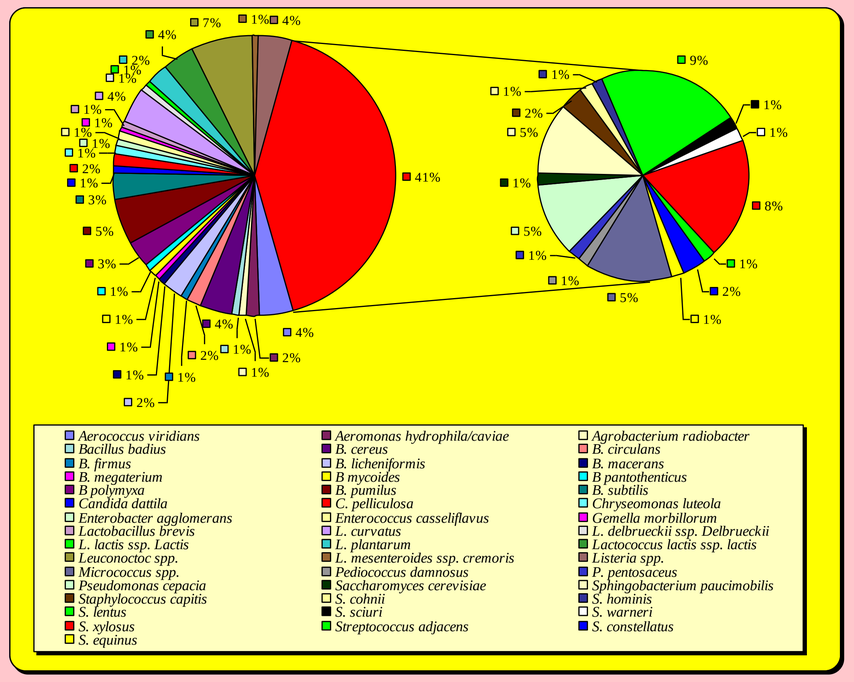

Figure 3 - **Species and corresponding frequency of appearance in MAIZE.**

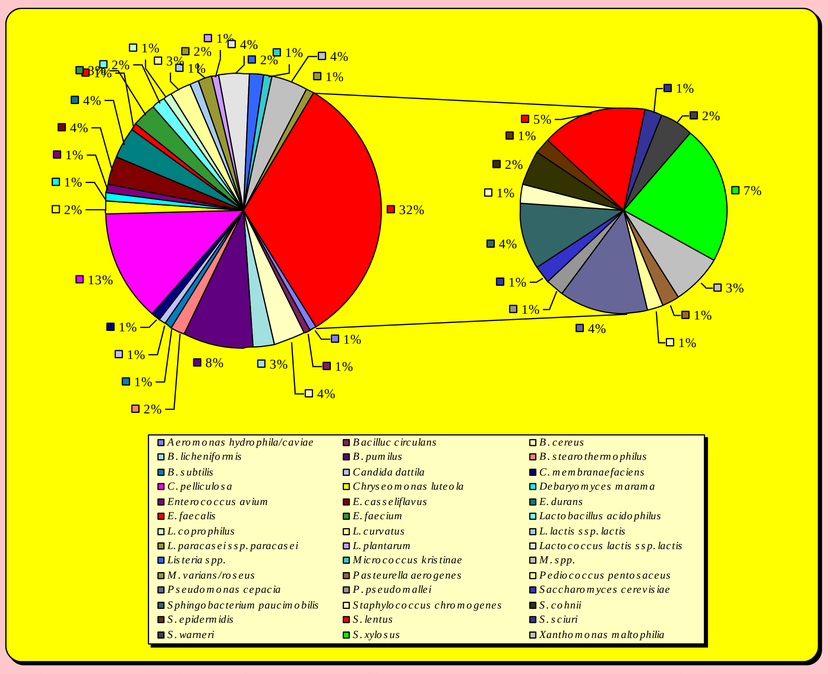

Figure 4 - **Species and corresponding frequency of appearance in RYE.**

LAB and yeasts contributed by sourdough account for several volatile compounds produced during sourdough fermentation [5]: homofermentative LAB are responsible for development of a final bread with good grain and elastic crumb, whereas heterofermentative LAB improve taste and contribute to the leavening process.

It should be emphasized that sourdough leavening is mostly determined by CO_2 produced as a result of the fermentative activity of yeasts present: although the gas developed in the sourdough contributes to open up texture, the lactic and acetic acids produced by LAB play a determinant role in taste [2, 8].

Although this work has attempted to characterize the microecology prevailing in the main precursors of *Broa* (maize and rye flours, and sourdough), the characteristics of this matrix create a unique environment in which specialized, wild strains of microorganisms can grow.

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ACKNOWLEDGMENTS

The authors are grateful to several members of the Regional Directorate of Agriculture of Entre Douro e Minho (DRAEDM) and several local farmers for cooperation within the experimental program described. Financial support for author J. M. R. was provided by a Ph.D. fellowship within the program for the Creation of National Infrastructures in Applied Science and Investigation (CIÊNCIA), administered by the National Board for Scientific and Technological Investigation (JNICT, Portugal). Partial financial support was received from PAMAF – IED (Ministry of Agriculture, Portugal) through a research grant entitled "Pão de milho: caracterização do processo tradicional de produção e melhoramento tecnológico".