

Contributions to the Understanding of the Microbial Ecology in a Portuguese Traditional Sourdough Bread



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INTRODUCTION

BROA is a traditional type of sourdough bread, which is manufactured at the farm level in Northern Portugal in the absence of a starter culture, following ancient manufacture protocols. The dough is produced from maize and rye flours, and inoculation thereof is via a given amount of previously fermented dough, kept covered from batch to batch.

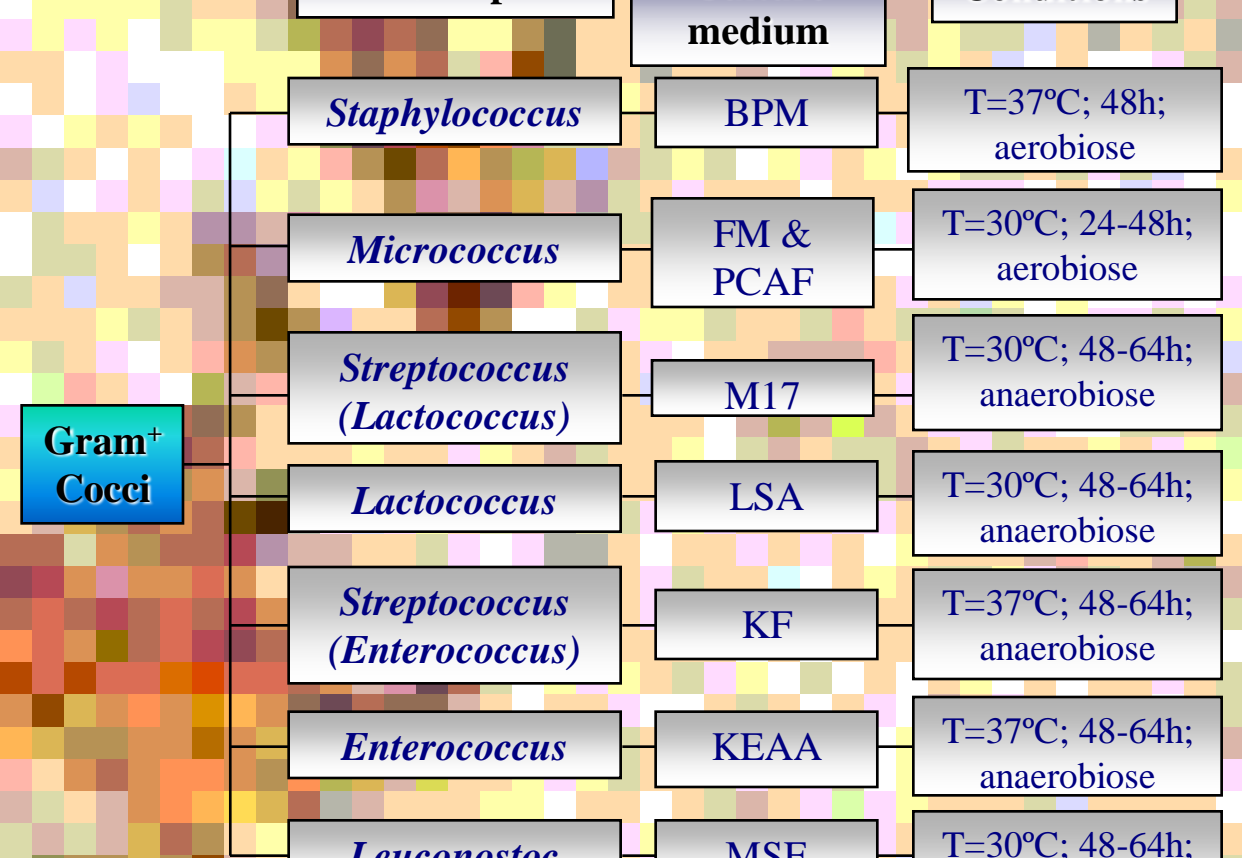
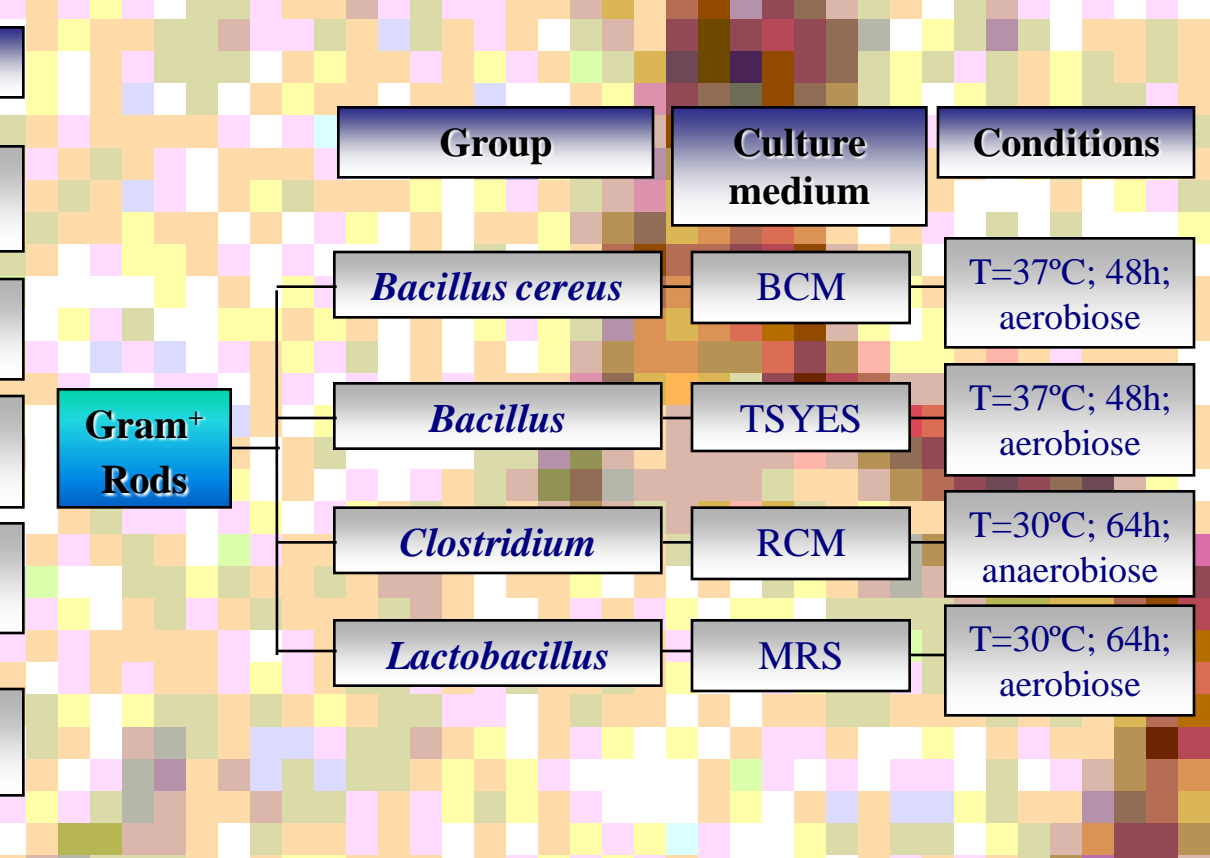
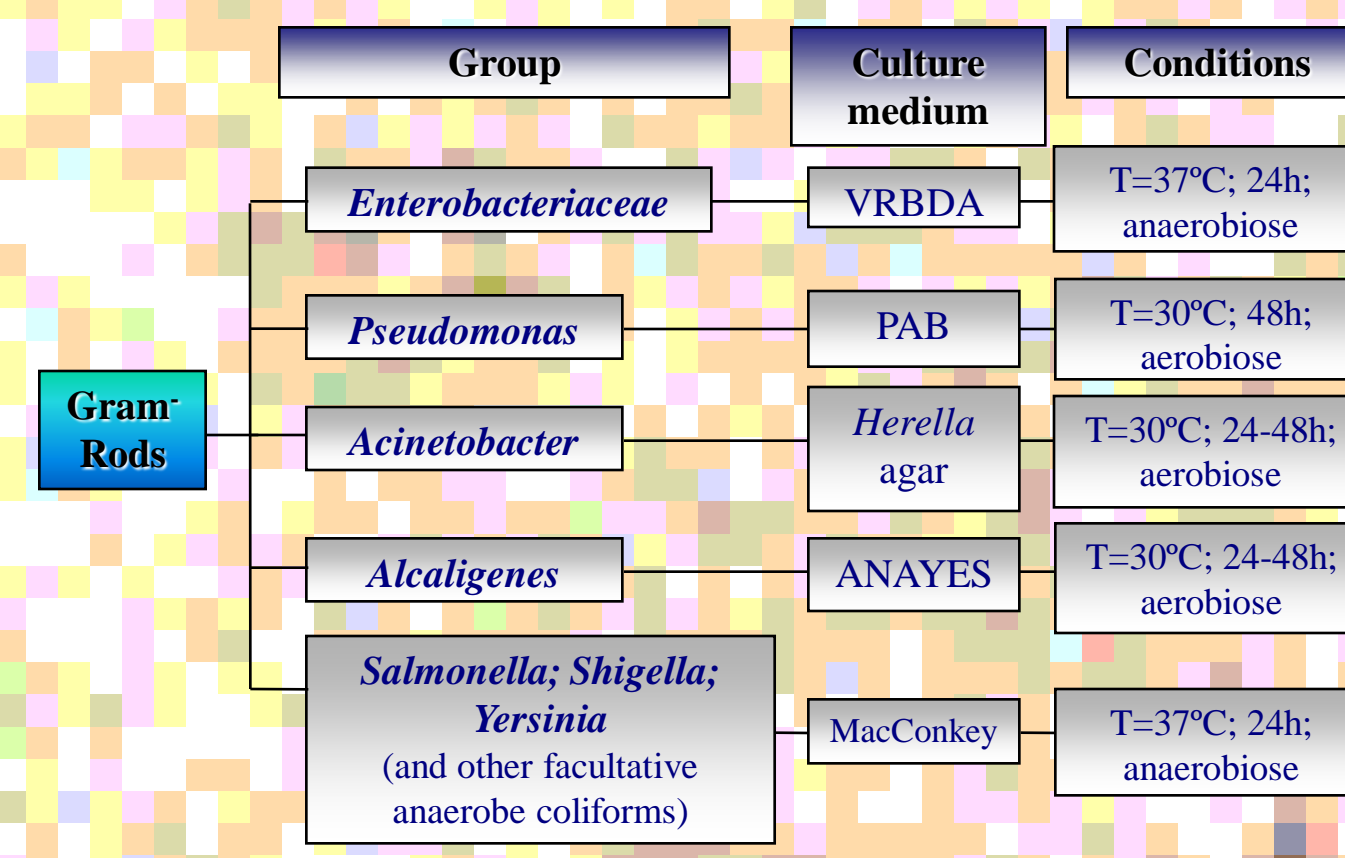
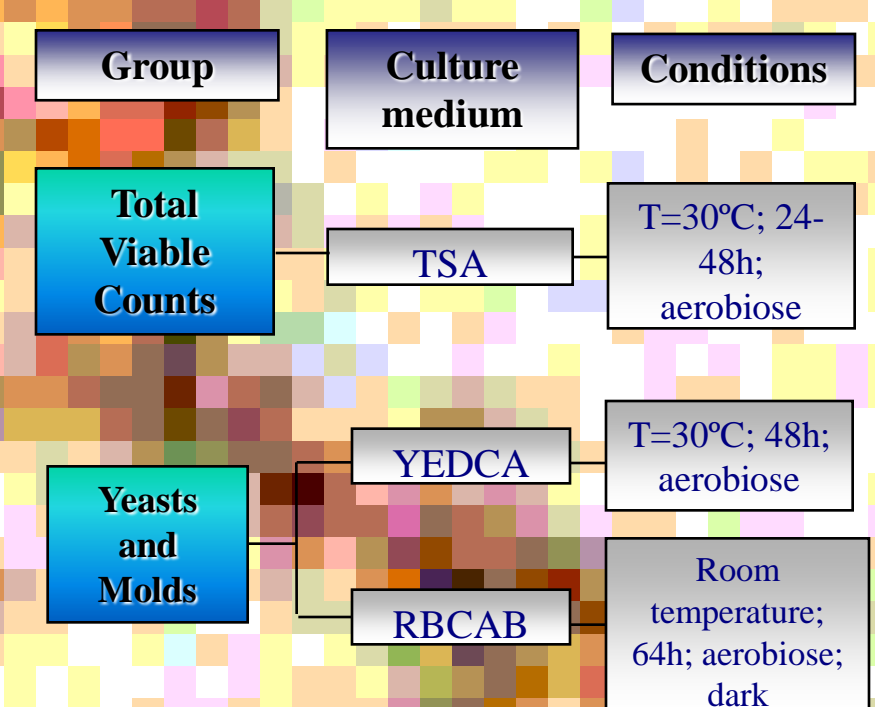
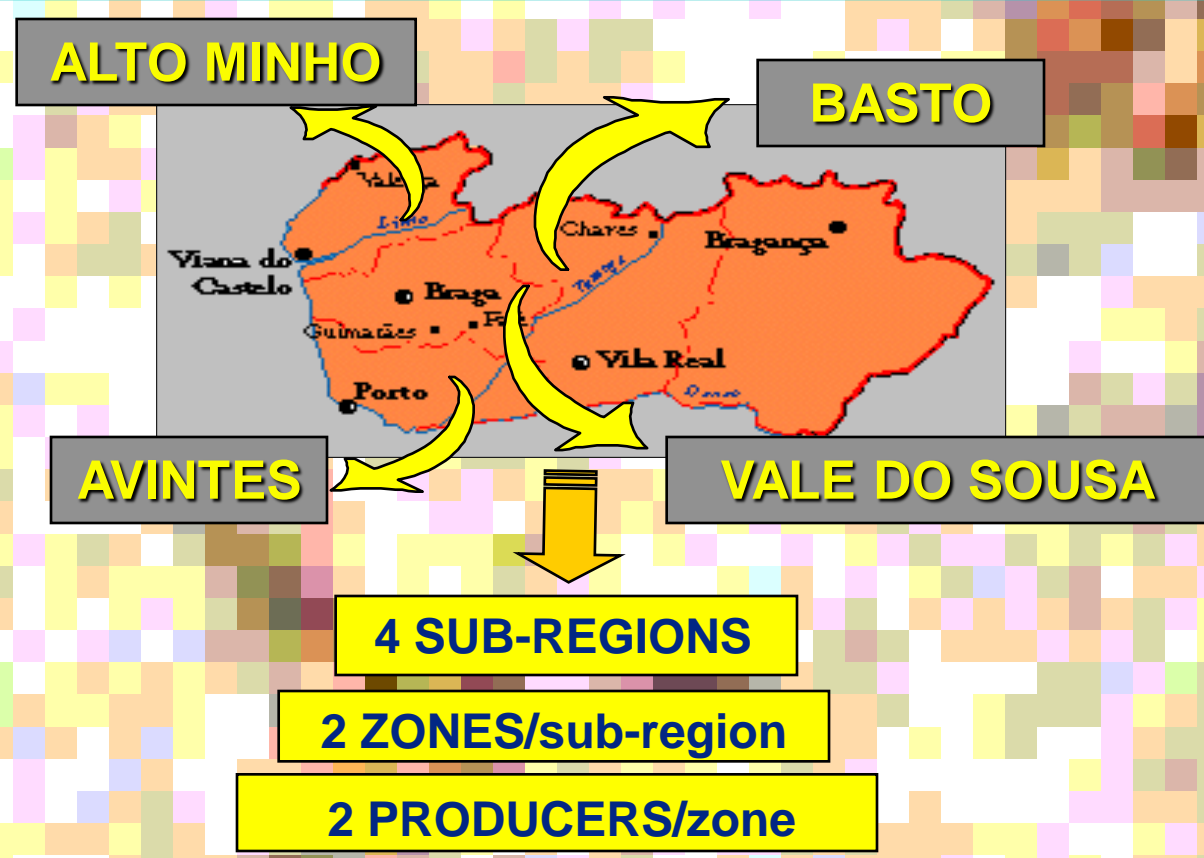
The manufacture of *BROA* has important impacts, from both economic and social standpoints, but a long way is yet to be tracked before such speciality food can be officially certified. To this goal, a thorough microbiological study of sourdough was made, which has shown that it contains a diverse, unique and rather complex wild microflora. Total viable counts for a wide group of microorganisms were obtained after inoculation of sourdough samples, supplied by several traditional producers in Northern Portugal, on 19 selective culture media, which were in turn incubated under a total of 22 distinct conditions: such microorganisms encompassed yeasts, molds, Gram⁻ rods, Gram⁺ rods (endospore-forming and nonsporing), Gram⁺ cocci (catalase⁺ and catalase⁻), and general mesophilic and thermophilic viable forms (vegetative and spores).

The main conclusion was that samples of sourdough cannot be distinctly separated by region, in terms of microbiological quantitative and qualitative profile; however relationships could at times be pointed out.

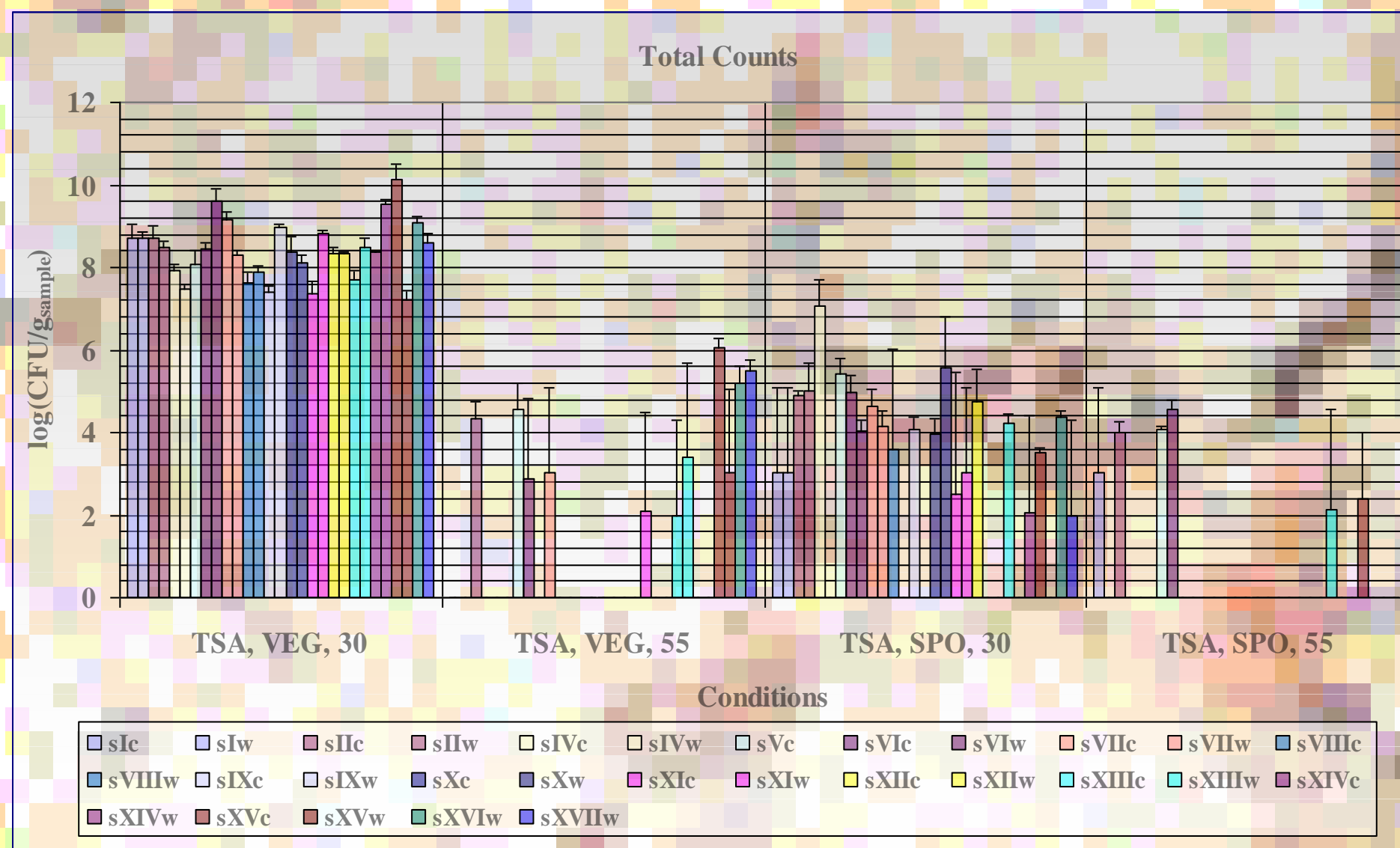
EXPERIMENTAL METHODS

Samples of sourdough (*s*) were taken from producers in different geographical points (*I,II,III,IV,V*) and in two different periods, the cold (*c*) and warm (*w*) season:

In order to check for the existence of a wide diversity of microorganisms, a large number of culture media and incubation conditions were selected, according to the schematic procedure showed below:

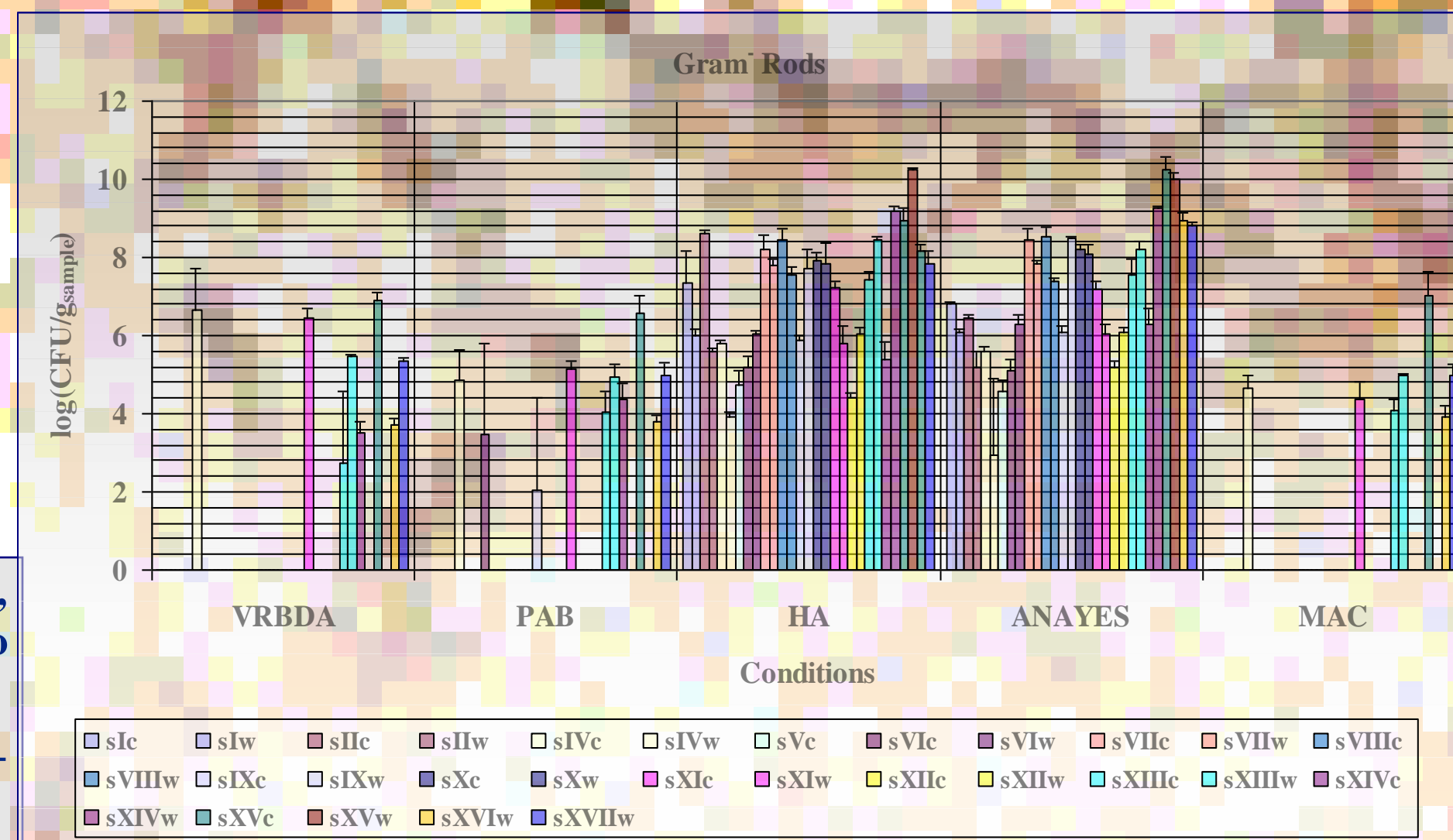


RESULTS and DISCUSSION



- Good correlations were found among all sporulated forms - TSA (30 and 55°C), BCM and TSYES), except RCM, Spores

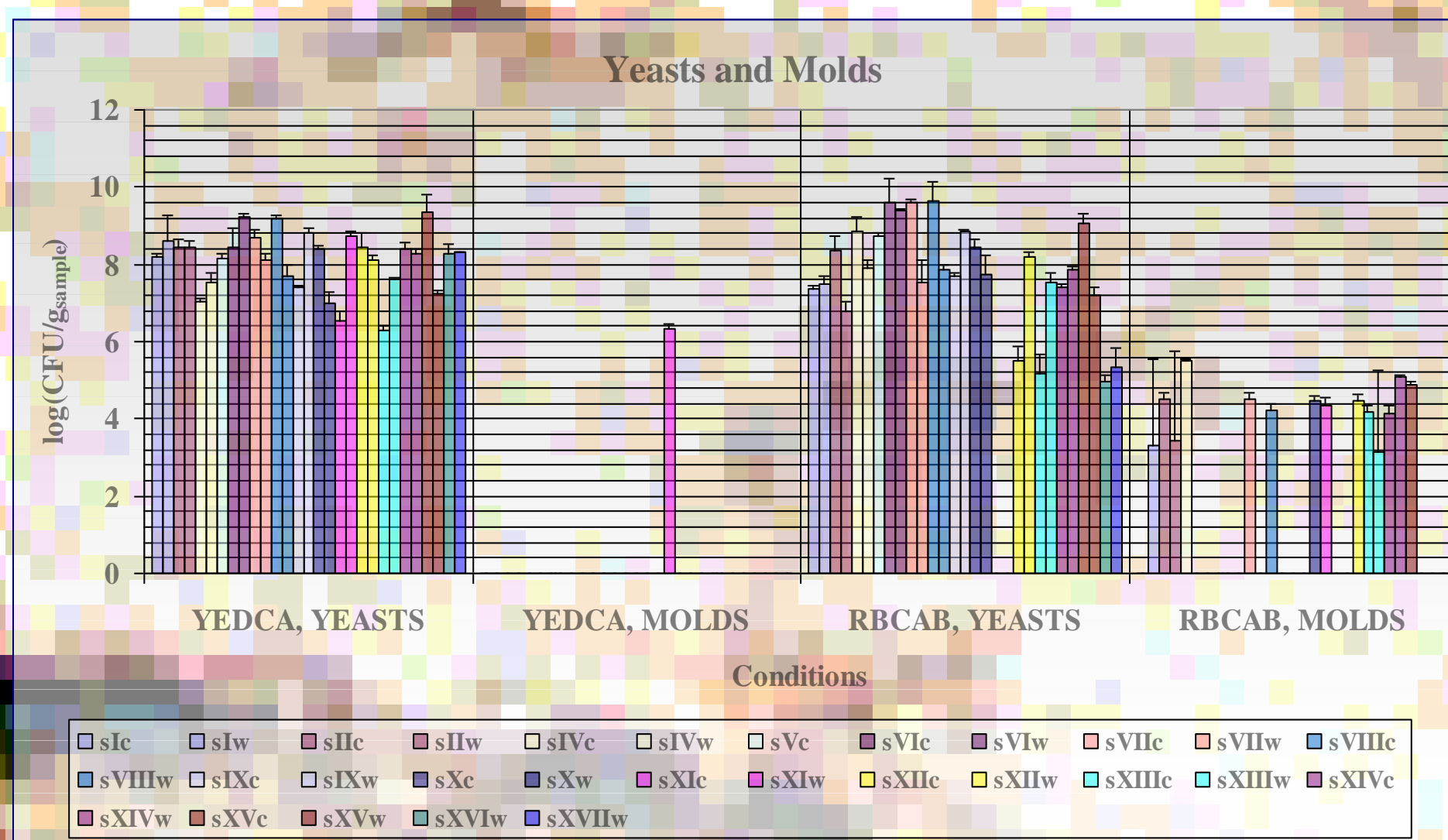
- High correlation is observed between VRBDA, PAB and MacConker media (>93%), and also between HA and ANAYES (93%)
- Some high correlations exist involving Gram⁻ rods and Gram⁺ cocci



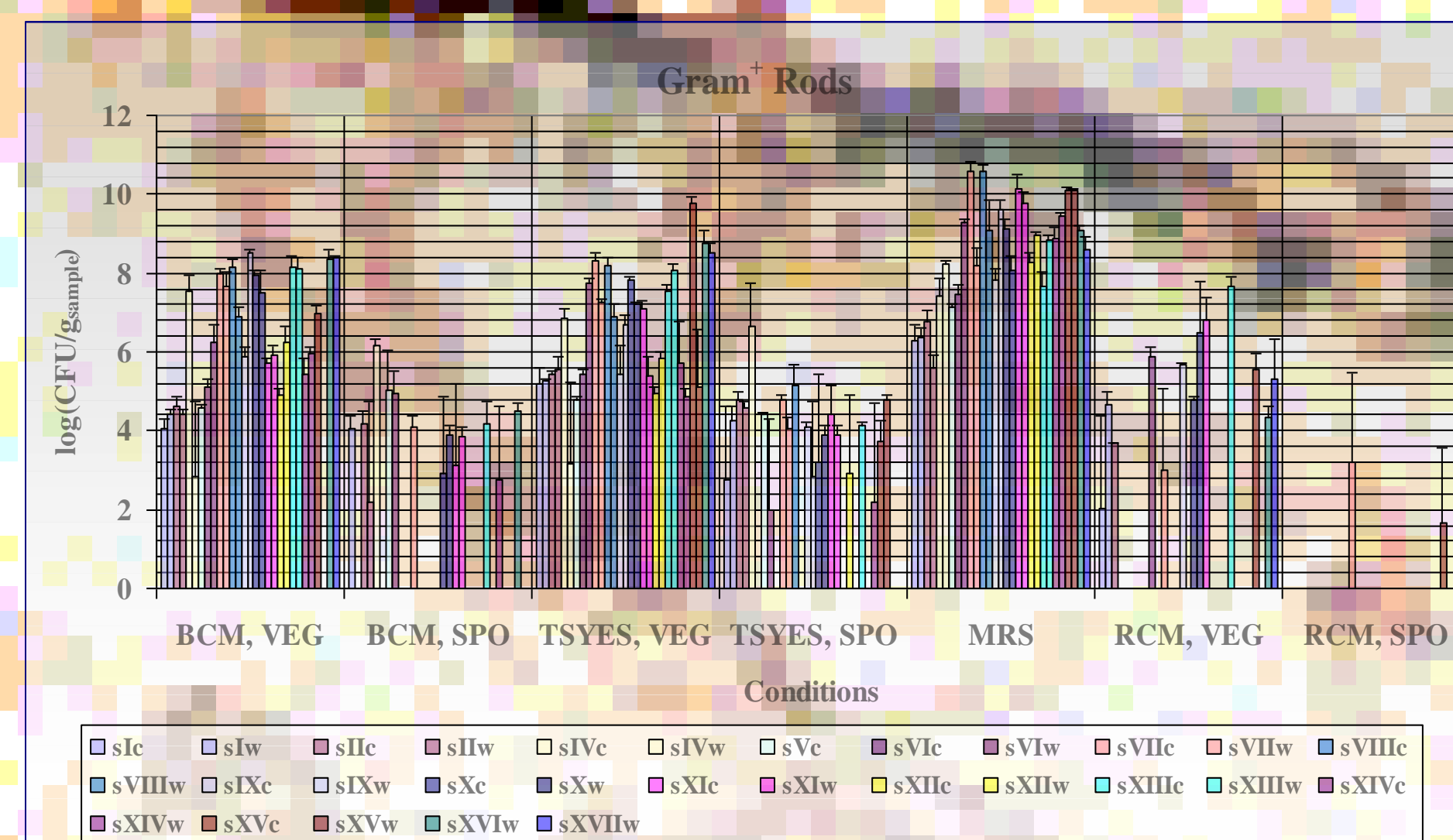
- There is a low correlation between the Gram⁺ catalase⁻ cocci *Staphylococcus* (BPM) and the Gram⁺ catalase⁻ cocci (remain media)

- FM and PCAF, both for *Micrococcus*, exhibit a high correlation (70%); however, there is also a high correlation with BCM, Veg, and ANAYES, which can indicate a low selectivity for these media

- MSE has good correlations with other media for Gram⁺ cocci, and either TSYES and HA. It is somewhat unusual to find *Bacillaceae* growth in media for Micrococcaceae

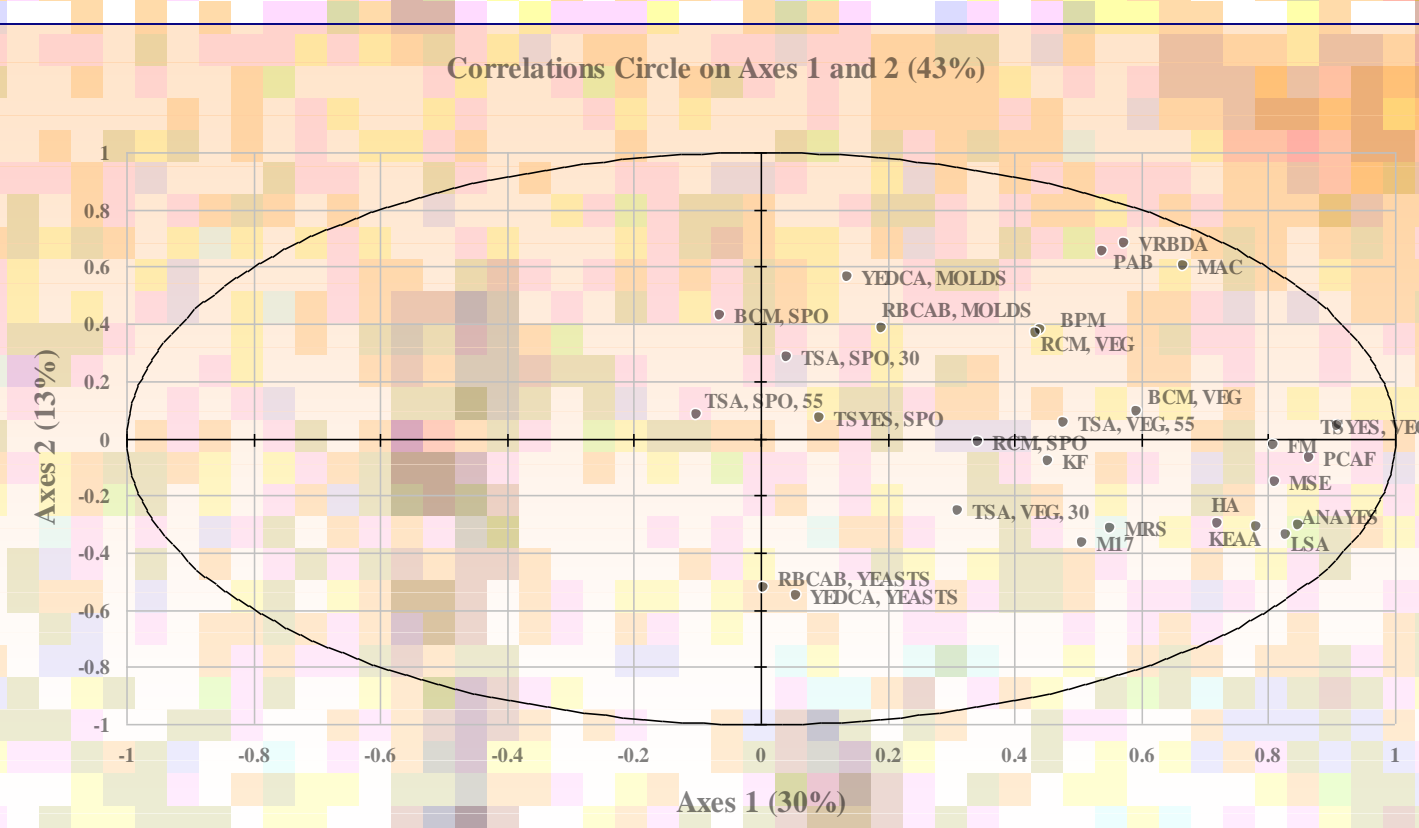
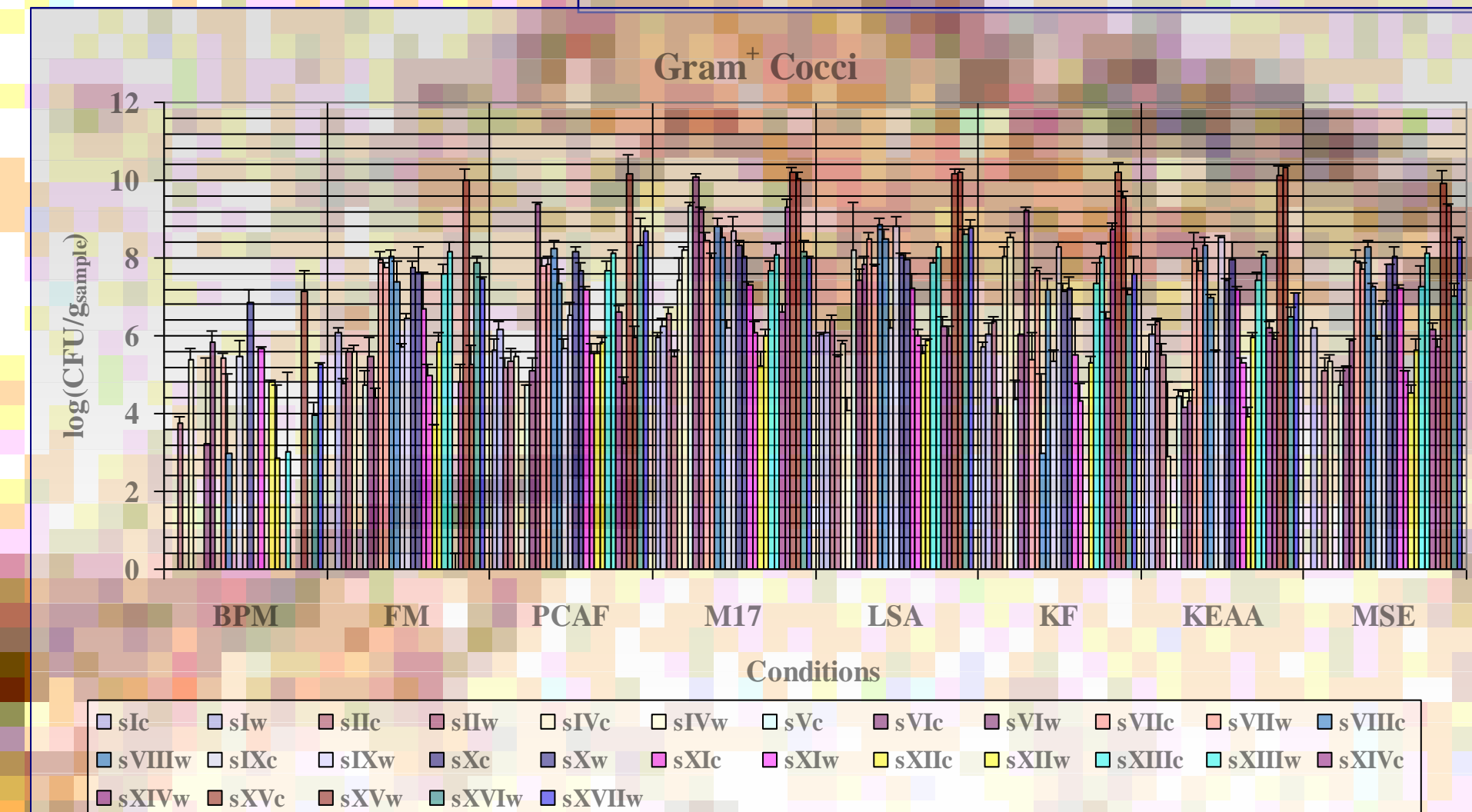


- Yeast counts on YEDCA, and RBCAB are well correlated. Nevertheless the first medium is more selective to yeasts and the other to molds
- Mold counts in these two media do not correlate well

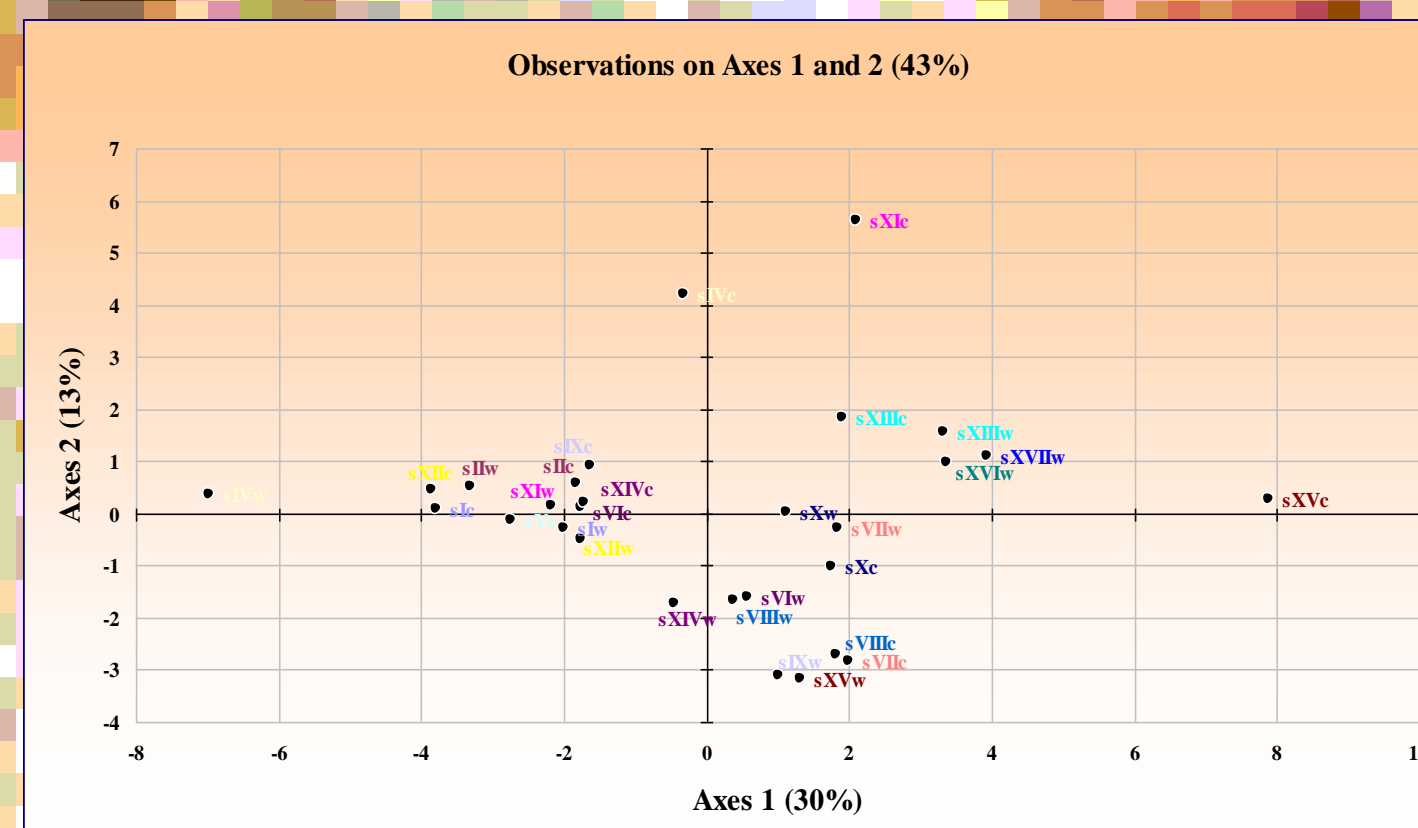


- A good correlation was found between BCM, Veg and TSYES, Veg (76%), and a poor correlation with *Lactobacillus* (MRS) and *Clostridium* (RCM, Veg). Actually, BCM and TSYES are both selective to *Bacillus* genera, but BCM is more specific to *Bacillus cereus*

- There are high correlations between BCM, Veg, and some culture media for Gram⁺ cocci; it is common to find synergisms between some strains belonging to Gram⁺ cocci and Gram⁺ rods



- Variables from each cluster (with high positive or negative correlations) do not allow a good distinction of the objects studied, in terms of log(CFU/g_{sample})
- Variables with low correlation could define the axes that discriminate among the objects studied, for instance: TSA, Veg, 30 with VRBDA (8%) or with RBCAB, Molds (5%); TSA, Veg, 55°C with RBCAB, Yeasts (-3%) or with BCM, Veg (8%); TSA, SPO, 55 with VRBDA (0.3%), YEDCA, Molds (-9%), RCM, SPO (2%), KF (-3%), HA (-4%), or with Mac (4%). However, the best 2 axis are obtained with the Principal Components (PC)
- TSYES, Veg, PCAF, ANAYES, LSA, MSE, FM, KEAA, HA are the Principal Components with more positive contribution for PC1 (c. >72%), and VRBDA and PAB for PC2 (68 and 65%, respectively)



- Sourdoughs I and II (from *Paredes de Coura*) lie close together; the same happens with sourdoughs V and VI (*Melgaço*), but not with IV (also from *Melgaço*). Except for sample sIVw, all sourdoughs (coming from *Alto Minho* region (producers I to VI) are positioned on semi-negative axis of PC1

- In the *Basto* region (producers VII to X), sourdoughs from *Cabeceiras de Basto* (producers VII and VIII) are very close, in the semi-positive axis of PC1; samples from *Celorico de Basto* (producers IX and X) are also together, but somehow separated from sIX.c. Samples from the *Basto* region have, consequently, common characters

- In the *Vale do Sousa* region (producers XI to XIV), sourdoughs from *Lousada* (XI and XII) are close, except sXIC. From *Penafiel* (XIII and XIV), sourdough XIII is distant from the others belonging to that region

- Samples from *Avintes*, in different seasons (sXVc and sXVw), have very different microbial parameters
- Industrial samples (XVI and XVII) are very similar

It can be concluded that samples from each region can not be easily separated, in terms of microbiological parameters; however, in many cases, a relationship can be found

- First Principal Component (PC1), explains 30% of the variance in the initial matrix, whereas PC2 explains 13%
- PC1 and PC2 together describe 43% (cumulative percentage) of the total variance in the initial matrix. High order PCs therefore influence less than 57% of the model
- First 9 PC's explain 86% of the variance: it was possible to reduce the 22 initial variables to only 9, with a minimal loss of information
- First 9 PC's can be defined from the correlation circle plotted on axes 1 and 2 (or loading plot). For instance (by Correlation coefficient (c. c.), and decreasing order):
= TSYES, Veg with PCAF, FM, BCM, Veg, LSA, MSE, ANAYES, Mac, KEAA, HA, VRBDA and PAB (c. c. >49%);
= BPM with TSYES, Veg, Mac, VRBDA, PCAF, RCM, Veg, PAB, TSA, SPO, RCM, SPO, FM and BCM, SPO (c. c. >33%);
= RCM, SPO with TSA, SPO, TSA, SPO, TSA, SPO, TSA, SPO (c. c. >38%);
= RBCAB, Yeasts and YEDCA, Yeasts with c. c. >34%

- Looking at the plot of observations on axes 1 and 2 (or score plot), it can be concluded that there is a considerable variability for each producer in different seasons ("c" and "w")
- Sample sIVw has, in general, the lowest counts in the majority of the culture media. In the correlation circle, they are positioned in the semi-positive axis of PC1
- The isolated position of sXIC is because it is the only sample with YEDCA, Molds values
- Sample sXVc has the highest TSYES, Veg counts

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