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Bioethanol strains of *Saccharomyces cerevisiae* characterised by microsatellite and stress resistance

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ABSTRACT

Strains of *Saccharomyces cerevisiae* may display characteristics that are typical of rough-type colonies, made up of cells clustered in pseudohyphal structures and comprised of daughter buds that do not separate from the mother cell post-mitosis. These strains are known to occur frequently in fermentation tanks with significant lower ethanol yield when compared to fermentations carried out by smooth strains of *S. cerevisiae* that are composed of dispersed cells. In an attempt to delineate genetic and phenotypic differences underlying the two phenotypes, this study analysed 10 microsatellite loci of 22 *S. cerevisiae* strains as well as stress resistance towards high concentrations of ethanol and glucose, low pH and cell sedimentation rates. The results obtained from the phenotypic tests by Principal-Component Analysis revealed that unlike the smooth colonies, the rough colonies of *S. cerevisiae* exhibit an enhanced resistance to stressful conditions resulting from the presence of excessive glucose and ethanol and high sedimentation rate. The microsatellite analysis was not successful to distinguish between the colony phenotypes as phenotypic assays. The relevant industrial strain PE-2 was observed in close genetic proximity to rough-colony although it does not display this colony morphology. A unique genetic pattern specific to a particular phenotype remains elusive.

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Introduction

The Brazilian industrial fermentation process for fuel alcohol production has certain atypical characteristics that allow for the entry and growth of wild yeast strains. The conditions are so conducive to wild-yeast growth that occasionally their development is found to compete with that of the selected starter yeast strain. One of the major reasons for this is that the methodology followed by the Brazilian ethanolic fermentation industry does not rigorously implement sterile conditions; other prominent reasons include yeast recycling along with sugarcane harvesting.[1] As a result of the above mentioned reasons, contamination by wild strains of *Saccharomyces* has a very frequent occurrence in the bioethanol industry. At times it has been observed that the growth patterns of the indigenous strains are so robust that they dominate the fermentation process to the extent of replacing the starter yeast strain. The status or influence of the contaminant strains in the fermentation process is dependent upon characteristics such as the fermentative performance, cell sedimentation rate, filamentation as well as biofilm development.[2] Indigenous strains with rough colony morphology are frequently observed during the ethanolic fermentation process and are associated with pseudohyphal growth and high sedimentation rate; these strains result in problems that are similar to those observed for flocculent strains.[3,4] As a word of caution, it is to be noted that the cell aggregation caused as a result of pseudohyphae should not be confused with flocculation.

Chain formation in yeast is observed when the younger bud fails to separate from the mother cell[5]; under such circumstances the newer cell remains attached to the parent post-mitosis leading to the formation of 'snowflake yeasts'.[6]

A study conducted by Reis et al.,[4] comparing rough-colony strains with their smooth-colony counterparts, demonstrated that the rough-colony strains have significantly lower and slower fermentative kinetics when monitored in a batch system over a 48-h period under conditions where sugarcane juice was used as the substrate. High residual sugar concentration has been documented to be a factor that is closely associated with the presence of wild *S. cerevisiae* strains in the fermentation process.[3,4]

Environmental conditions are known to be key factors capable of influencing and affecting differences in colony and cell morphology.[7,8] In addition, signalling cascades such as the MAPK, TORC, SNF1 and RIM101 pathways, are also known to be involved in influencing morphological changes.[8] However, in the latter case, the resultant morphological changes are usually of a transitory nature.[9,10]

Curiously, in spite of the presence of clear demonstrable differences in colony morphology and cell arrangement between smooth-colony and rough-colony strains, the restriction analysis of mitochondrial DNA and PGFE (chromosome karyotyping) both failed to uncover any underlying genetic differences. The differences in morphology were concluded to be a consequence of environmental conditions that influence and cause differential gene expression.[11] Kuthan et al.[12] reported that *Ty*-coding genes and subtelomeric genes that are induced by stress conditions interfere with the colony morphology of yeasts. A report by Cavalieri et al.[13] that analysed metabolic patterns indicated that there were significant differences in the gene expression profiles of the colony variants (filigreed, rough and smooth) especially with respect to ammonia and amino acid transporters.

In that direction, a study by Ratcliff et al.[6] that compared a unicellular strain of *S. cerevisiae* and an evolved strain of snowflake yeast showed that 1035 genes were significantly differentially expressed between the two. The authors noted that seven of the ten most downregulated genes were regulated by the transcription factor *ACE2* in conditions wherein both *ACE2* alleles were identical in the diploid state of the yeast. A study by Rodrigues[14] on spontaneous derivatives of JAY270/PE-2 presenting an altered colony morphology (roughened surfaces, irregular edges, cell sedimentation resembling flocculation in liquid media) revealed that loss of heterozygosity of the gene *ACE2* (as a result of frameshift mutation) was responsible for the development of the rough-colony phenotype. PE-2 is one of the most important industrial yeast strains used in the Brazilian distilleries.[3] *ACE2* heterozygosity should be investigated in the yeast strains displaying rough-colony morphology frequently isolated from the ethanolic fermentation to assess the real origin of this phenotype.

In spite of clear differences in colony morphology and cell arrangement, in depth analysis into genetic differences between smooth and rough-colony strains have failed to reveal the presence of any underlying variations at a DNA level so far. The PCR microsatellite methodology has been extensively used for *S. cerevisiae* strain identification especially when assessing the wine fermentation populations[15–17]; more recently this technology has been used for assessing the biodiversity of native bioethanol yeast strains.[18] This technique has been revealed to be sensitive and robust enough to detect the extensive genetic diversity of the indigenous strains of *S. cerevisiae* in Brazilian ethanol-producing units.[18]

Microsatellites or SSRs (Simple Sequence Repeats) are short segments of DNA that are repeated in tandem and are known to be co-dominantly inherited and dispersed throughout the genome.[19] The sixteen chromosomes of *S. cerevisiae* genome are known to be very rich in the presence of microsatellites as well as numerous polymorphic alleles.[20] Perez et al.[19] evaluated the genetic variability of 51 isolates of *S. cerevisiae* using the microsatellite methodology. With the use of six microsatellites they uncovered a total of 57 alleles and generated 44 genotypes.

Despite the result of loss of heterozygosity of *ACE2* to be the probable origin of rough-colony morphology in *S. cerevisiae*,[6,14] previous studies here reported were more conclusive regarding to the differences in gene expression than to the genetic differentiation at DNA level between different *S. cerevisiae* phenotypes. In view of the remarkably high discriminatory power of the microsatellite marker-based assessment, this technique was applied in our study in an attempt to evaluate the genetic variability amongst strains of *S. cerevisiae* isolated from industrial ethanol units. The ultimate objective of the study was to discover a genetic pattern that could be used to differentiate between the two colony phenotypes (rough and smooth). Additionally, the phenotypic characteristics such as resistance to stress and cell sedimentation were also surveyed. It was hypothesised that the association between molecular traits and phenotypic features could help

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