Mutagenesis and PCR procedures to analyze *Saccharomyces cerevisiae* isolated from Saudi Arabia

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Abstract: *Saccharomyces cerevisiae* is yeast of extensive industrial application. It is used widely for production of alcohol, organic acids and bakery items. Besides, it is also used in genetic research because many cellular processes such, genetic recombination etc. are conserved between the yeasts and larger eukaryotes. Its relatively small genome can be manipulated and analyzed readily. Strains of *S. cerevisiae* are known to produce enzymes. In this work, we report data on the molecular characterization of *S. cerevisiae* strains. The aim was the individuation of molecular techniques yielding strain-specific profiles useful as new markers during the industrial application process. In this study we have attempted to induce mutations using diethyl sulfate (DES) and compare of mutants with standard strains and a local isolate. RAPD-PCR Technique analysis is done to verify the mutations.
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1. Introduction

Biotechnology in the food processing industry makes use of micro-organisms for the preservation of food and for the production of a range of value-added products such as enzymes, flavors, vitamins, and food ingredients. Biotechnological applications in the food-processing sector, therefore, involve the selection and manipulation of micro-organisms with the objective of improving process control, product quality, consistency of yield, and increasing the process efficiency (Berger, 2009).

Biotechnological processes applied to the improvement of microbial cultures for use in food-processing industry include traditional methods of genetic improvement ('traditional biotechnology') such as classical mutagenesis and conjugation. These methods generally focus on improving the quality of micro-organisms and yield of metabolites. Hybridization is also used for the improvement of yeasts involved in baking, brewing and in beverage production. *Saccharomyces cerevisiae* strains have, for example, been studied for improved of fermentation process and biopreservation, and for capability to increase the wholesomeness and sensory qualities (Bauer et al., 2007).

The use of DNA-based diagnostic techniques for strain differentiation can allow for the tailoring of starter cultures to yield products with specific characteristics. For this purpose, random amplified polymorphic DNA (RAPD) techniques have been widely applied. The results of these analyses have led to the development of many improved starter cultures which are currently in use for the commercial production of substances having different characteristics (Valyasevi and Rolle, 2002).

Saccharomyces cerevisiae yeasts are well known from centuries for their importance in the production of fermented foods (cider, bakery products, etc.). However, industrial fermentations are complex ecological and biochemical processes, during which yeast cells are subjected to a number of adverse conditions. Mostly they are exposed to different kinds of stress, such as oxidative, hyperosmotic, ionic, high temperatures, nutrient limitations and starvation (Querol and Rolle, 2002). For instance, during ethanol production due to its toxicity, yeast metabolism and growth are severely damaged (Mehraj Pasha, et al., 2013). Extreme conditions like these lead to reduction in growth survival rate, and therefore tend to reduce fermentation efficiency. Better and faster the yeast strains are able to adapt to changes in the environment, higher is the probability of being the dominant strain during the industrial process.

Molecular basis of the technological properties of industrial yeast strains is still largely unknown. However, it is well documented that the fast growing yeast cells possess vast possibilities for adaptation to the ecological environment by changing the specific expression profiles of their genomes (Perez-Ortin et al., 2002). Furthermore, genetic research on industrial yeast strains has revealed that these microorganisms are capable of rapid response to changing environmental conditions by adaptive genome reorganizations (Mortimer, 2002). The later may undergo different chromosomal changes such as:

spontaneous mutations, mitotic crossing-over, gene conversion, chromosome rearrangements (Fernandez-Espinar et al., 2001; Abhilasha Singh Mathuriya and Sharma VN, 2010; Puig and Perez-Ortin, 2000; AnuradhaR, et al., 2013; Samreena Baqal and Ujwala Jadhav, 2012), as well as formation of interspecific hybrids between different industrial strains of *Saccharomyces* (DeBarros-Lopez et al., 2002).

In this sense the development of rapid and simple methods for yeast strain differentiation and characterization of genetic stability is an essential tool for industrial fermentations. The availability of typing techniques that enable detection of genetic rearrangements at strain level is necessary for both yeast users and producers, in order to ensure that commercial dry yeasts are identical to the original selected strains. Over the years, several methods have been developed for differentiation of yeast strains such as karyotyping, RAPD and PCR fingerprinting, but their reproducibility requires special care and occasionally the interpretation of results can be difficult (Gomes et al., 2000; Schuller et al., 2004).

In addition to wide-ranging roles in beverage and food industry, *Saccharomyces cerevisiae* plays an important role as a model organism in biochemistry, genetics and molecular biology; and in 1996 the complete genome of this yeast species was determined. Lately, evidence has Therefore, exploring the biodiversity of indigenous fermentative strains can be an important contribution towards understanding and selecting strains with specific phenotypes. In recent years, several methodologies of typing based on DNA polymorphisms have been developed which have allowed discrimination among closely related yeast strains.

In this study we propose to induce mutations using chemical mutagens and assess the efficiency of production of some industrial enzymes by the mutants in comparison with standard strains and a local isolate. Subsequently, genotyping with PCR procedures will be done to elucidate the genetic markers associated with superior traits of the mutants.

2. Material and Methods

Two local strains of *Saccharomyces cerevisiae* isolated from Riyadh and *S. cerevisiae-wild type* will be used for inducing mutations. Untreated cultures of the same strains will be retained as controls. Diethyl sulfate (DES) will be used as a mutagenic agent; and doses of 0.5, 1.0, 1.5, 2.0, 2.5, and 3.0 mM will be applied for induction of mutation. Target strains will be grown overnight in yeast extract-peptone-dextrose (YEPD) broth; and different doses of the mutagen will be added to separate aliquots which will be grown at 30°C for 3 days. After the treatment, survival rate in each dose will be evaluated and a large number of pure colonies form the surviving cells will be generated on YEPD agar medium for further selection of the mutant lines. (Justin et al. 2001) and (Kncerova et al. 1984).

PCR procedures: Differentiation between the indigenous S. cerevisiae isolates was performed by RAPD-PCR with primer M13 (5′-GAGGGTGGCGGTTCT- 3′), using the DNA extraction and methods described by (Andrighetto et al. 2000), (Capece and Romano 2009) and (Romano et al. 2008).

3. Results

All mutagens regardless of volume caused mutations in the biosynthetic pathway. This can be seen through growth on the selective media plates. shows how each volume of mutagen affected the yeast. In Figure-1 the mutation and survival rates for each mutagen can be found. these numbers represent the varying effects that each mutagen had on the cells. As can be seen in this table, each volume of mutagen caused a change in the survival of *Saccharomycese cerevisiae*. This experimental set was a complete success. After several trials with the PCR protocol we finally were able to get a clean isolation product. These products are found in Figure-2 below. On the far left is the 1 kb ladder. The next five lanes represent *S.cerevisiae* locally strain, Selection-1, Selection-2, Selection-3 and Selection-4. It is stated that they are located properly on the gel; therefore, we would be able to sequence them if we had time. the position of each band is as follows: 2: ~2050bp, 3: ~1700bp, 4: ~1400bp, 5: ~2100bp, and 6: ~1700bp.

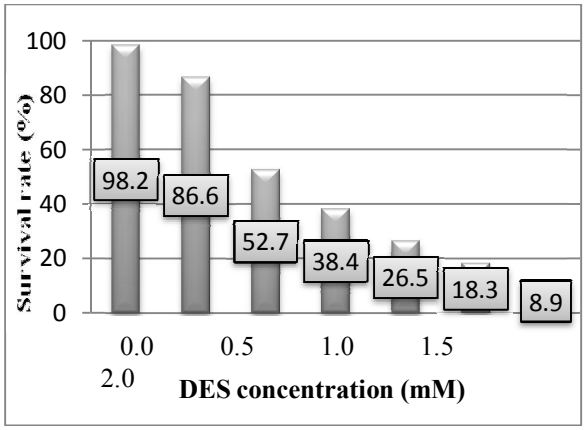

Figure -1. Survival of *S. cerevisiae* cultures treated with different doses of DES.

These numbers were determined by the position of the 1kb ladder located at the far left of the gel (lane1). These sizes correspond to known values.

As a result, we can be certain that we have recovered the proper segment of DNA to sequence. Time constraints prevented us from performing the PCR, but the isolates are frozen and will be available to the department for future sequencing. Mutations in the genome of a fungus are known to drastically alter the phenotypes including production of secondary metabolites (Armstrong et al., 2002).

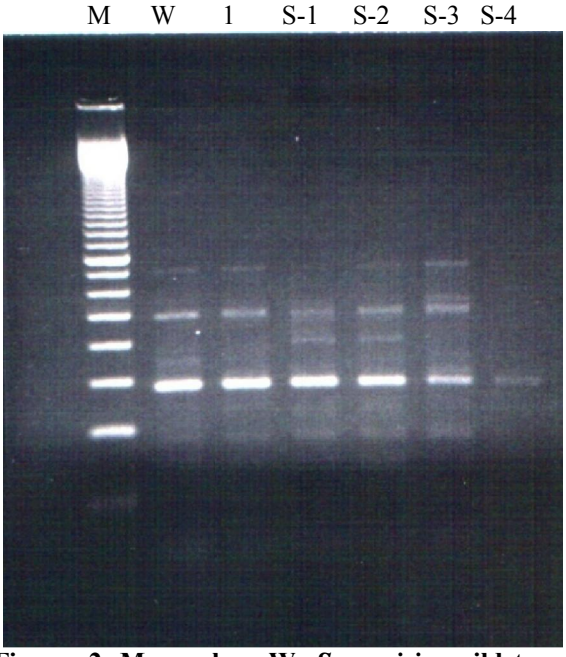

Figure -2. M: marker, W: *S.cerevisiae*-wild type, 1: *S.cerevisiae*-local strain, S-1: mutant1, S-2: mutant2, S-3: mutant3, S-4: mutant4.

4. Discussions

Frequency of mutation, presumed at par with the rate of mutation, has been depicted in Fig-1. Highest and similar frequencies were recorded in 1.0-2.0 mM concentration of DES. At the highest tested dose of 3.0mM which severely affected the survival also (Fig-1), only one mutant was noticed out of 72 colonies tested. Hopwood et al., (1985) suggested that a dose causing highest mortality in cultures is best suited for strain improvement as the few survivors would undergo more extensive mutations which may lead to enhancement in their productivity.

Survival rate of the yeast cells under different treatments has been presented in Fig.-1. It is noticeable that survival declined regularly with increasing concentration of DES. The survival decreased to 8.9% at the highest concentration of 3mM as compared to 98.2% in control. Similar effects of chemical mutagens have been reported by Zambare (2010) while working with Trichoderma reesei. DES is known as a highly potent mutagen, frequently used for induction of mutations in plant, insect and microbial cells. Besides being mutagenic, DES is toxic to most cell types and the toxic potential depends on the target genome and the treatment conditions (Bull and Wilke, 2008; Kava-Cardeiro et al., 1995).

Saccharomyces cerevisiae is yeast of extensive industrial application. It is used widely for production of alcohol, organic acids and bakery items. Besides, it is also used in genetic research because many cellular processes such as genetic recombination etc. are conserved between the yeasts and larger eukaryotes. Its relatively small genome can be manipulated and analyzed readily. Strains of S. cerevisiae are known to produce enzymes. In this study, we propose to generate industrially useful mutants of S. cerevisiae and characterize them by molecular procedures, especially PCR. The aim is to identify molecular techniques that may yield strain-specific genetic markers useful for selecting strains for various industrial applications.

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