Genetic studies on xylose sugar utilization by yeast

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The importance of studying the yeastfermentation of pentose sugars is due to the possibility of reaching to a clean source of energy. Also, removing the residues in order to minimize the environmental contamination as the xylose sugar is the sugar which is produced from the analysis of hemicellulose material which is found mainly in residues as saw dust, paddy straw and other plant residues.This study aimed to obtain strain (s) of yeast which had the ability to ferment the xylose sugar and produce ethanol. Furthermore a trial was conducted to transfer the genes which control the production of associate enzymes in this operation into the bakery yeast (Saccharomyces cerevisiae) this yeast is known by its ability to produce alcohol from hexose sugars aiming to obtain a modifying strain. This strain may have the ability to produce alcohol from xylose. In order to achieve this goal, many experiments were made to induce mutations followed by selection besides obtaining new genotypes through protoplast fusion (nuclear fusion). The results of this study can be summarized as the follows:-1. A strain of Pichia, which has originated from Saccharomyces family was found to produce ethanol from xylose sugar and it is known as Pichia stipitis.782.UV-light was used in different doses for the induction of mutants in Pichia stipitis strain. Four selected auxtrophic mutants were obtained. Tests were made to determine its abilities for xylose sugar consumption and selecting the best of them for protoplast fusion experiments with the selected strains of Saccharomyces genus. These selected strains were auxtrophic mutant (73) for leu, his and cys and auxtrophic mutant 171 (M2) for his.3.Four trials were made to Protoplast fusion between each of Saccharomyces cerevisiae strain (S.c. GT 160-34 B) with auxtrophic mutant 73, (M1). The second trial was between Saccharomyces cerevisiae strain (S.c. GT 160-34 B) and auxtrophic mutant 171, (M2). The third trial was between Saccharomyces cerevisiae strain (S.c. XJ 133-1 B) and auxtrophic mutant 73, (M1). The fourth trial was between Saccharomyces cerevisiae strain (S.c. XJ 133-1 B) and auxtrophic mutant 171, (M2).The strains and mutants were grown on two different media (minimal media); one contained Gylcerol with the pentose sugar (xylose) while the other consisted of sorbitol with the pentose sugar (xylose). This step aimed to obtain the best results.4.Results showed that the ability of isolating 91 protoplast fusants from Saccharomyces cerevisiae through using protoplast fusion. These protoplast fusants were large-sized cell colonies than the parental strains.795. Tests showed the success of these protoplast fusants of Saccharomyces cerevisiae to synthesis xylose sugar with highefficiency.6.Through using refractometer instrument, it was able to test the ability of the 91 genetic fusants for consuming the xylose sugar and selecting the best of them for xylose sugar consumption aiming to use it in ethanol production experiments.7.Seventeen genetic out of the 91 fusants, which proved to be superior in xylose sugar consumption, were selected and used them beside their parents for ethanol production experiments.Some of the protoplast fusants for ethanol production showed superiority as compared to the original parents of Saccharomyces cerevisiae which were not able to use xylose sugar, and consequently they were not able to produce any ethanol amount from xylose. When these fusants compared with the original parent; Pichia stipitis and the two resultant mutants; M1 and M2, data showed that some fusants were also superior in ethanol production. Results obtained in this investigation refered to the ability to use the protoplast fusion technique as it considered to be one of the important methods in production of new genotypes for pure line selection.9. Growth behaviour of the 17 fusant yeast

strains and its parents were tested on different carbon sources, hexoses (glucose), disaccharides (lactose and cellobiose), trisaccharides (raffinose)and polysaccharides (starch) to elucidate the sugar utilization control by genome.Results indicated that fusant yeast strains and its parental strains normally grew on glucose.Using polysaccharides as carbon sources revealed that all tested strains did not show any growth on starch. These results may be due to the lack of the genes (STA and / or DEX) that code for the enzyme amyloglucosidase which hydrolyzes starch and dextrin to yield glucose.With lactose, all tested yeast strains [except two fusant strains (19 and 11) besides Pichia stipitis 171 (M2) which gave normal growth] did not show any growth.The two fusants (19 and 11) are descendents of Pichia stipitis 171 (M2) which can grow on lactose.Two fusant strains (19 and 11) which gave normal growth on lactose may be constructed a new strains to utilize lactose by intergeneric protoplast fusion techniques. The fusants are new strains which may have all the characters of Pichia stipitis parental strain.The present results also showed that all tested fusants and its parents did not give any growth on raffinose.It is well known that , baker’s yeast are unable to utilize the disaccharide melibiose due to the lack of the enzyme ? - galactosidase (melibiase) to cleavage it to glucose and galactose.With cellobiose all tested fusant strains and Pichia stipitis parental strains showed good growth except both of Saccharomyces cerevisiae (G T 160- 34 B and XJ 133-1B) which did not give any growth on cellobiose. This result means that, all the fusant tested strains may have all the characters of Pichia stipitis parental strain via intergeneric protoplast fusiontechniques.