ABSTRACT

Saccharomyces cerevisiae, the best known and studied yeast, has been used in food and alcoholic beverages for centuries as it is safe, efficient and easy to use during fermentation. This unicellular ascomycete also serves as an excellent experimental model to study eukaryotic genome and is most preferred organism in genetic engineering and heterologous gene expression for productive purposes, owing to its biological safety and easy manipulation.

Flocculation, an asexual aggregation of the cells, is a widespread phenomenon observed in many yeast genera. Flocculation in *S. cerevisiae* is proposed to be mediated by lectin like proteins situated on the cell surface, which upon activation with calcium ions interact with carbohydrate moieties on the cell surfaces. Although much work has been carried out to understand the complex phenomenon of flocculation, few attempts have been made to isolate the active lectin and controversies regarding the role of lectin still persist.

An important prerequisite for yeast strains used in industrial fermentations on completion of the process is flocculation. Further flocculent strains permit continuous ethanolic fermentation in tower type reactors without recourse to centrifugation for cell recovery and recycle. The killer character in yeasts is another beneficial property since it prevents contamination of wild yeasts during continuous fermentation over prolonged periods.

The present investigation deals with purification and characterisation of cell wall lectin and establishment of its role in flocculation. The second part deals with the improvement of highly flocculent brewer's yeast strain with respect to the killer character.

Chapter I : General Introduction

The main focus of the chapter is on flocculent yeasts presenting a literature survey with reference to the factors governing flocculation,

measurement of flocculation, genetics of flocculation, mechanism of flocculation and various proteins, including lectins associated with flocculation. Industrial importance of flocculation and strain improvement procedures carried out to improve flocculent strains are also reviewed.

Chapter II : Studies on flocculation characteristics of *Saccharomyces cerevisiae* NCIM 3528

S. cerevisiae NCIM 3528, a highly flocculent strain which constitutively flocculates in a calcium dependent manner was characterised with respect to its flocculation. Flocculation of the strain showed exclusive mannose specificity with methyl α- mannoside being a potent inhibitor of flocculation. The strain could flocculate well in broad pH range of 3-10. Flocculation was sensitive to the temperatures above 60°C as well as to the action of broad specificity proteases such as pronase E and proteinase K but relatively resistant to chymotrypsin. Tunicamycin did not inhibit flocculence of this strain. The strain showed most of the flocculation characteristics similar to the Flo1 phenotype.

Chapter III : Extraction, purification and characterisation of the cell wall lectin from the flocculent yeast *Saccharomyces cerevisiae* NCIM 3528

A cell surface lectin was isolated and purified to homogeneity from the cell walls of a highly flocculent strain of *Saccharomyces cerevisiae* NCIM 3528 by chromatography on DEAE-cellulose, phenyl Sepharose and Sephacryl S-300. It showed a molecular mass of 40 kDa on SDS PAGE. It is an acidic protein with a pI of 4.0 and contains 44% hydrophobic amino acids. N-terminal sequence up to 10 amino acid residues showed at least 70% homology with the predicted N-terminal sequence of the putative *FLO1* gene product. Mannose binding nature of the lectin was indicated by its high affinity and specificity towards branched trisaccharide of mannose, a ligand which also inhibits flocculation of the yeast cells. Immunofluorescence studies confirmed presence of the lectin on the cell

surface and lectin specific IgGs prevented flocculation of the cells. This cell surface mannose specific lectin probably plays an important role in flocculation, with the branched trimannoside on cell wall being the apparent carbohydrate receptor.

Chapter IV : Strain improvement of highly flocculent *Saccharomyces cerevisiae* NCIM 3528 : Construction of flocculent yeast with a killer character by protoplast fusion

Conditions were optimised for rapid release and improved regeneration of protoplasts of *Saccharomyces cerevisiae* NCIM 3458. Rapid protoplast release was also obtained with representatives of several other yeast genera under the modified conditions of treatment. Application of protoplast fusion technique in construction of a highly flocculent *Saccharomyces cerevisiae* with a killer character is described. Fusion was effected between UV-killed protoplasts of *S. cerevisiae* NCIM 3578 with a killer character and live protoplasts of the highly flocculent *S. cerevisiae* NCIM 3528 in the presence of polyethylene glycol 6000. Stable fusants were obtained using benomyl resistance as a marker, the killer toxin producer rather than the highly flocculent yeast being resistant to the fungicide at a concentration of 100 μg/ml. Fusants were also characterised by their DNA content, capacity for the ethanolic fermentation of the molasses sugar and the levels of invertase, alcohol dehydrogenase and pyruvate decarboxylase activities.

Chapter V : General Discussion

In this chapter the salient features of the thesis have been discussed with respect to the current state of the knowledge on yeast flocculation and industrial yeast strain improvement.