CHAPTER VI

CONCLUSION
Like most of the dimorphic human and plant pathogenic fungi, *B. poitrasii* exhibit glucose and temperature dependent morphological transition. Factors such as temperature, pH, glucose and metal ion Zn\(^{2+}\) found to affect morphological transition in *B. poitrasii*. During the sequential exposure to temperature and glucose and *vice-versa* the temperature exposure reversed the glucose effect. This can be attributed to the common signaling pathway operated by both, temperature and glucose triggers. Morphological shift was accompanied with the change in glutamate dehydrogenase activities. ODC enzyme level was also found to alter with the morphological change induced by all factors studied. Furthermore, ODC activity was significantly correlated with glutamate dehydrogenase activity. Previously, Khale and Deshpande (1993) suggested the role of cAMP in the regulation of glutamate dehydrogenases in *B. poitrasii*. The GDH activities were found to be influenced by the addition of Ca-CAM inhibitor, TFP or H-7, a cAMP dependent protein kinase inhibitor. TFP reversed the effect of temperature exposure and H-7 reversed the effect of glucose exposure. The yeast cells pre-incubated with the different inhibitors affected the levels of NAD- and NADP-dependent glutamate dehydrogenases activities and in turn influenced the germ tube formation. These observations suggest the role of signal transduction pathway components such as cAMP and Ca-CAM in the regulation of GDH activities and Y-H transition in *B. poitrasii*. ODC regulates the levels of polyamines, putrescine, spermidine and spermine in the cell. These polyamines were reported to be involved in the signal transduction pathways (Bachrach *et al.*, 2001). Polyamines essential for the normal cell growth, were found to control GDH activity in mammalian tissue (Jarzyna, *et al.*, 1994; Kuo *et al.*, 1994). In *B. poitrasii*, it was observed that putrescine and spermidine affected glutamate dehydrogenases especially yeast form specific NADP-GDH activity in *B. poitrasii*. From the present findings the tentative scheme of biochemical events is suggested (Fig 6.1)
Chapter VI

External triggers

- Ca
- CaM
- cAMP
- Glucose
- Polyamines (?)

Morphological outcome

- Budding
- Germlube formation

Fig 6.1 The possible biochemical events in the yeast cells of *B. poitrasii* triggered by temperature and glucose conditions leading to the different morphological outcome.

The morphological shift in fungi was previously evaluated for its potential use in biotechnology (Doiphode *et al.*, 2008). *B. poitrasii* was previously studied for its ability to produce ethanol (Srinivasan *et al.*, 1986). Surface characters are important in determining the fungal interactions such as flocculation, an important character in ethanol biotechnology (Verstrepen *et al.*, 2003). In the present investigation, flocculation studies showed that the parent and mutant yeast form cells flocculated faster when they were grown in the presence of 2-ketoglutarate and isopthalic acid than glutamate. Effect of 2-ketoglutarate, glutamate and isopthalic acid were observed on the cell wall properties and nitrogen metabolizing enzymes NAD- and NADP-GDH that participate in chitin metabolism. Activities of these enzymes were found to be correlated with the change in the cell surface properties. AFM analysis revealed that yeast cells having more mannan contents were rougher as compared to other cells. Mannan and chitin are the two major fungal cell wall polymers which have fructose-6-phosphate (F6P) as a common precursor. In *S. cerevisiae*, the levels of F6P

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were reported to be regulated by NAD- and NADP-GDH (Boles et al., 1993). The cell wall composition showed that a decrease in the NAD-GDH activity was associated with the increase in mannan contents, surface roughness and flocculation. The decrease of NAD-GDH activity was associated with increased mannan content of the cell wall. The morphological change in response to the environmental conditions could be associated with the change in cell wall polymer contents of chitin and mannan that affect the cell surface. NAD-GDH activity modulating substances could have potential use as flocculation inducing agent, important for ethanol biotechnology. The study of the cell surface properties will help in understanding the role of cell wall at different interfaces of fungal interactions such as flocculation and fungus-host interactions.

NAD-GDH of B. poitrasii was purified to the homogeneity, and it was extensively characterized. Like most of the other fungal NAD-GDH, it has four identical subunits (97 KDa) and native structure is of 371 KDa. Kinetic study suggested that it played a role towards the synthesis of L-glutamate, whereas most of fungal NAD-GDH was found to act towards the breaking down of L-glutamate. Histidine residue was important for the activity of NAD-GDH. The histidine kinase has been suggested to play an important role in the phosphorylation-dephosphorylation of NAD-GDH. In the molecular study of NADP-GDH from the B. poitrasii, it was observed that the gene fragment isolated from the yeast mRNA down regulated in hyphal form cells which indicate the importance of NADP-GDH in Y-H transition of B. poitrasii. From the differential expression study of NADP-GDH gene, it can be suggested to have cause-effect relationship with Y-H transition in B. poitrasii observed.

As NAD-GDH was found to be biochemically correlated with Y-H transition in B. poitrasii, it was considered as a new target for the development of antifungal agents. The compounds were effective in halting Y-H transition as well as inhibiting activity of purified NAD-GDH. From this observation it can be suggested that NAD-GDH plays an important role in the morphological transition of B. poitrasii and it can be used as an effective target for the screening of antifungal compounds against dimorphic pathogenic fungi.
During the course of the work it has been found that nitrogen metabolizing enzymes such as NAD-, NADP-GDH and ODC were important in the Y-H transition of *B. poitrasii*. Purified NAD-GDH showed that unlike other fungal NAD-GDH it has novel properties of catalyzing conversion of 2-ketoglutarate to glutamate whereas, there is form specific differential expression of NADP-GDH gene. It has also been found that NAD-GDH is an important biochemical correlate which was effective in the screening of antifungal compounds against dimorphic *B. poitrasii*. The differential expression of NADP-GDH gene during Y-H transition suggests it’s important in morphological shift of *B. poitrasii*. It was observed during the course of the investigation that there is a cause-effect relationship of glutamate dehydrogenase enzymes with Y-H transition in *B. poitrasii*. 