In the current study, we have attempted to isolate and characterize gene(s) and regulatory pathway(s) that are involved in the AmB resistance of *S. cerevisiae* and *Candida* species. A better understanding of molecular mechanism(s) of AmB resistance is likely to help in devising strategies to minimize development of AmB resistance during therapy. Preliminary studies on AmB susceptibility revealed that yeast strains were phenotypically resistant to a higher concentration of AmB at high cell density. Since farnesol, a quorum sensing molecule of *C. albicans* accumulates in a cell-density dependent manner (Hornby et al., 2001), we tested its effect on AmB resistance of *S. cerevisiae* and *Candida* strains. Presence of farnesol increased AmB resistance at least 2 folds indicating importance of farnesol in mediating AmB resistance.

By screening of yeast transformed with a multicopy genomic library screen in presence of AmB and farnesol, we have identified *PMP3* gene in *S.cerevisiae* which enhance AmB resistance several fold. Through dilution spotting assay, it was observed that *PMP3* overexpression provides tolerance compared to wild type which is further increased in presence of farnesol while *pmp3Δ* strain was hypersensitive to AmB.

Orthologs of *PMP3* gene in pathogenic yeasts *C. albicans* and *C.glabrata* were cloned and expressed and were found to enhance AmB resistance. Deletion of *PMP3* orthologs in these species rendered them sensitive to AmB further confirming the importance of this gene in modulation of AmB resistance. Membrane localization study suggests that Scpmp3p localized on plasma membrane. We have shown that AmB susceptibility is not related to ion homeostasis in yeast since *S.cerevisiae* deletion mutants of genes involved in ion transport and regulation are not sensitive to AmB.

Further we had shown that sensitivity of *pmp3Δ* strain against AmB and HygB involved two different mechanisms. In support of this we have shown that presence of KCl does not affect HygB resistance while it affects AmB resistance. Moreover presence of calcium increases resistance to HygB but not to AmB. Further while farnesol increase AmB resistance, it does not have any effect on HygB. Susceptibility of site directed alanine mutants of Pmp3p to AmB and HygB was also not correlated. While almost all mutants were defective in AmB tolerance, only a few were affected in HygB resistance. Thus sensitivity against these two drugs seems to involve different mechanisms.

Depletion of ergosterol is known to increase resistance to AmB. Thus initially we speculated that farnesol might increase AmB resistance by modulating ergosterol levels. Total ergosterol quantification data showed that *PMP3* deletant and overexpression strain have comparable to parent strain while farnesol slightly decrease the ergosterol level in all the strains. Since decrease in ergosterol level increases resistance to other polyenes as well, and farnesol does not affect resistance to these polyenes, it can be concluded that farnesol effect on AmB resistance is not through modulation of ergosterol content. However filipin staining showed that ergosterol of *PMP3* delete strain was mostly in the plasma membrane unlike in wild type and overexpression strains, where it was found in intracellular bodies also. Increased plasma membrane localization is unlikely to be reason for higher AmB sensitivity of *PMP3* delete strain, since this strain showed resistance to other polyenes comparable to the parent strain. Moreover deletion mutants impaired in lipid storage and transport show no difference in AmB resistance in comparison to wild type strain. Mutants of gene(s) involved in farnesol mediated signaling or hyphal morphogenesis were not affected in AmB resistance, suggesting that fanesol does not modulate AmB resistance through these pathways. Increased AmB resistance of ergosterol biosynthetic pathway mutants is dependent on functional *HSP90* chaperone (Vincent et al., 2013). However for *PMP3* overexpression mediated AmB resistance, *HSP90* chaperone is not important since *PMP3* overexpression strain is not sensitive to *HSP90* inhibitor radicicol. More over deletion of *HSP90* encoding gene (*hsp82Δ* and *hsc82Δ*) does not affect *PMP3* mediated AmB tolerance.

Gene ontology analysis of *PMP3* interacting genes for biological processes showed that many of these genes participate in actin cytoskeleton organization and endocytosis. We found that strain deleted in *PMP3* is impaired in actin polarization and endocytosis. Multicopy *SUR7*, encoding an eisosome component, suppressed salt sensitivity, actin polarity and endocytic defect of *pmp3Δ* strain, but not AmB sensitivity of this strain. These results show that AmB susceptibility is not mediated by defects in actin polarity or endocytic defect of *pmp3Δ* strain. AmB resistance can also be due to changes in cell wall integrity. However deletion of *PMP3* or presence of farnesol does not affect cell wall integrity implying that *PMP3* or farnesol effect on AmB resistance does not involve cell wall integrity.

AmB known to increase reactive oxygen species (ROS) production which mediates yeast cell death by oxidizing plasma membrane lipids, nucleic acids and other cellular constituents (Ferreira et al., 2013). *PMP3* deletant had more ROS as such in comparison to the parent strain and sensitivity of this strain against H_2O_2 also suggests that this strain is under oxidative stress. To correlate ROS levels to AmB sensitivity, various yeast genes deletion mutants reported to have increased or decreased levels of ROS were screened and their MICs to AmB was comparable to that of parent strain. Thus, it appears that intracellular ROS content of cells is not correlated to AmB susceptibility. *PMP3* deletant was reported to overexcrete glutathione (Perrone et al., 2005), thus we measured its level in *pmp3Δ* strain and found that there was no significant change in glutathione content compared to parent. Moreover *gsh1Δ* strain has very less intracellular glutathione content but it is not sensitivity to AmB, which suggests that glutathione content does not influence AmB susceptibility.

Sphingolipids play important roles in cell biology of eukaryotic cells. Recently we had shown that down regulating sphingolipid biosynthesis by treating cells with myriocin sensitizes cells to AmB (Sharma et al., 2014). We have found that overexpression of Pmp3p increases resistance to myriocin while *pmp3Δ* strain is sensitive towards myriocin. This is specific to sphingolipid biosynthesis inhibition because addition of exogenous phytosphingosine (PHS), an intermediate of sphingolipid biosynthesis pathway, suppresses the myriocin sensitivity of *pmp3Δ* strain. Addition of PHS suppresses AmB sensitivity of *pmp3Δ* strain, indicating *PMP3* effect on AmB resistance is mediated through sphingolipid pathway. While overexpression of *PMP3* in wild type background increases AmB resistance by 4 to 8-fold, it increases resistance by only about 2-fold or less in mutants of sphingolipid biosynthetic pathway genes *FEN1* and *SUR4* or regulatory gene *YPK1*. If *PMP3* overexpression effect is independent of sphingolipid pathway, then fold-increase in AmB resistance by *PMP3* in these mutants should have been comparable to that of wild type, i.e., 4 to 8-fold. Only 2-fold increase in resistance shows that *PMP3* is dependent on this pathway.

Finally we have also identified that *SAC1* another gene involved in regulation of sphingolipid biosynthesis, is essential for increased AmB resistance conferred by overexpression of *PMP3* since deletion of *SAC1* result in decrease in AmB tolerance conferred by *PMP3* by upto 4 fold. Sac1p is known to modulate sphingolipid metabolism

and physical interaction of Pmp3p and Sac1p has been reported in a large-scale study (Miller et al., 2005). Thus it appears likely that Pmp3p modulates sphingolipid biosynthesis and AmB resistance by interacting with Sac1p.

We also explored if farnesol modulates sphingolipid biosynthesis. Deletion mutants impaired in sphingolipid biosynthesis and regulation were tested for their myriocin tolerance in presence of farnesol. Strains deleted in *YPK1* and *SLM1* were sensitive to myriocin compared to wild type control, and presence of farnesol did not increase the resistance of these strains to myriocin, indicating that Ypk1p and Slm1p are critical for farnesol mediated increase in myriocin resistance. These results also suggest that farnesol mediated increase in AmB resistance might also involve sphingolipid biosynthesis pathway.

We also tested the effect of farnesol for tolerance to aureobasidin A an inhibitor for complex sphingolipid biosynthesis. Farnesol increased AbA resistance of wild type cells by 8-16 fold suggesting that the effect of farnesol was more pronounced at the level of complex sphingolipid biosynthesis. Functional *YPK1* gene was found to be necessary for farnesol mediated increase in AbA resistance. It might be possible that depletion of complex sphingolipids upon AbA treatment activates Ypk1p which leads to restoration of sphingolipid biosynthesis. To conclude, this study has led to the identification of a novel role for *PMP3* in modulation of AmB resistance through sphingolipid biosynthetic pathway. Increase in AmB resistance by farnesol is also possibly mediated through sphingolipid biosynthetic pathway. Role of *PMP3* and farnesol in modulation of myriocin tolerance is a novel finding of this study. Farnesol mediated increase in AbA tolerance is another novel finding of this study, though precise mechanistic details remain to be worked out.