Pichia pastoris **a viable heterologous expression system for steroidogenic P450s.**

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The methylotrophic yeast, *Pichia pastoris*, has been used with success for the large scale expression of mammalian proteins. In this study the suitability of this expression system for membrane bound cytochrome P450-dependent enzymes was investigated. The *CYP19* gene encodes the cytochrome $P450_{arom}$ ($P450_{arom}$), the enzyme responsible for estrogen biosynthesis from androgens. Breast cancer is believed to develop and progress as result of abnormal expression of $P450_{arom}$. $P450_{arom}$ mRNA has been localized in peripheral tissues, however, the protein levels remain to be determined. Therefore, for quantification and localization of $P450_{arom}$, it is necessary to raise antibodies against the protein. An expression vector was constructed containing the *CYP19* gene. The methylotrophic yeast, *Pichia pastoris*, was transformed with the *CYP19* gene; whereafter $P450_{arom}$ was expressed from the cells. The results from these expression studies will be discussed.

The *CYP17* gene encodes for the cytochrome P450c17 enzyme (P450c17). In women this enzyme is primarily expressed in the ovary and adrenal cortex. P450c17 mediates both steroid 17-hydroxylase and 17,20-lyase activities. Progesterone and pregnenolone are converted to 17OH-progesterone and 17OH-pregnenolone, respectively, by the P450c17 17-hydroxylase activity. 17OH-progesterone is then converted to androstenedione and 17OH-pregnenolone to DHEA. These reactions are mediated by the P450c17 17,20-lyase activity. *Pichia pastoris* was previously transformed with the human *CYP17* gene. Kinetic studies with *CYP17* in *P. pastoris* were done and the V_{max} and K_m values determined and compared to human P450c17 previously expressed in COS 1 cells.