Abstract

The methylotrophic yeast *Komagataella phaffii* is considered a highly successful expression host for the large-scale production of recombinant proteins. The presence of the strong, yet tightly regulated, promoter $P_{AOX1}$ is one of the main reasons for this success. Typically, methanol is used to induce $P_{AOX1}$ activity. It also serves, at least in the wild type strain, as a source of carbon and energy. However, there are problems with the use of methanol on an industrial scale, which have prompted researchers to explore ways to minimize the amount of methanol used. Two popular strategies are a) the use of Mut (“Methanol utilization”) strains, which are compromised in their production of alcohol oxidase (AOX) and thus their ability to consume methanol and b) addition of secondary carbon sources to support growth and to reduce methanol requirement. The need to systematically study the influence of the different Mut phenotypes and of different secondary carbon sources on recombinant protein expression has been acknowledged in the literature. In this work, we tried to determine the optimal Mut phenotype and suitable secondary carbon sources by studying the production of a model recombinant protein, β-galactosidase. We observed significantly higher specific expression rates in a Mut$^+$ (high AOX) strain compared to Mut$^-$ (little AOX) and Mut$^-$ (no AOX) strains, suggesting that the Mut$^+$ should be the strain of choice for production of (at least non-secreted) recombinant proteins. Moreover, this observation led us to hypothesize that a downstream metabolite of methanol is involved in induction of $P_{AOX1}$. It was found that the metabolites formate and formaldehyde do act as potent inducers of $P_{AOX1}$. Since these compounds have several advantages over methanol in industrial protein production processes, this observation has important practical implications for large-scale production of recombinant proteins. The second goal of this work was to identify the best secondary carbon source. Comparable recombinant protein production was observed when either glycerol (a repressing carbon source) or sorbitol (a non-repressing carbon source) was used as the secondary carbon source along with methanol in continuous culture. This demonstrates that it is irrelevant whether a non-repressing or a repressing carbon source is used to support growth. Again, this result has significant practical implications, as it allows the secondary carbon source to be chosen solely on the basis of industrially relevant parameters.