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Engineering Methylotrophic Yeasts for Biotechnology Applications

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Because some yeast has evolved a methylotrophic lifestyle, they can use the single-carbon molecule methanol as a source of carbon and energy. Pichia pastoris (also known as Komagataella sp.) is one of them and is commonly employed for the generation of heterologous proteins as well as a model organism for organelle research. Our present understanding of the methylotrophic lifestyle is primarily based on extensive biochemical investigations that discovered numerous important methanol utilisation enzymes and their localization to the peroxisomes, including alcohol oxidase and dihydroxyacetone synthase. The pentose phosphate pathway is thought to be involved in C1

heme, caused by the strong induction of alcohol oxidase, dihydroxyacetone synthase, formaldehyde and formate

Introduction

Methylotrophic yeasts can take carbon from a variety of sources. Similar e ciency is achieved when using multicarbon sources like sugars and sugar alcohols like glucose, glycerol, or mannitol as opposed to decreased C1-compounds like methanol. In addition to the cells being properly equipped with the enzymes required for substrate metabolism, their coordinated expression is a requirement for the e cient use of various carbon and energy sources. Several recombinant proteins are created using the methylotrophic yeast Pichia pastoris (syn. Komagataella sp.), and a growing number of biopharmaceuticals and industrial enzymes are also made using this method. Lately, P. pastoris has been used more frequently as a model organism for [1-5] the growth of peroxisomes and secretory organelles. Its development has been primarily driven by the methylotrophic lifestyle, which includes peroxisomes—specialized organelles—as well as strong, tightly controlled promoters for the production of recombinant genes. Peroxisomes are described as intracellular organelles that house the enzyme catalase, which breaks down hydrogen peroxide (H2O2) and produces its own. Moreover, these organelles house the P. pastoris fatty acid beta-oxidation pathway. Alcohols, fatty acids, D-amino acids, and primary amines are just a few examples of the atypical carbon and nitrogen sources that yeast peroxisomal oxidases are primarily involved in the metabolism of. In methylotrophic yeasts, peroxisomes are relatively abundant in methanol-grown cells but drastically drop in both number and volume upon catabolite repression. Peroxisomes include the rst steps of the methanol utilisation pathway. Hansenula polymorpha, a di erent methylotrophic yeast, grows on glucose but only contains one tiny peroxisome, which can be used as a source for ssion-based cell division when induction is initiated by switching the cells to methanol. e expression of genes associated to methanol utilisation is highly stimulated by methanol, in addition to genes encoding structural peroxisomal proteins. Alcohol oxidase (AOX) converts methanol to formaldehyde in the rst phase of methanol assimilation, while dihydroxyacetone synthase, a unique transketolase, forms a C-C bond with the C1 molecule formaldehyde. ese two enzymes' reactions and their distribution in peroxisomes have been extensively studied. Pentose phosphate reactions are thought to be a part of the subsequent cycle of the absorption of methanol, but the speci cs are not yet fully understood. While numerous research have examined the cellular responses of P. pastoris to methanol induction in the context of the generation of recombinant proteins, it is mostly unknown how non-recombinant strains would react to the various carbon sources. As a result, we made the decision to look at how P. pastoris cells that aren't

making recombinant proteins react to the two most common cultivation substrates, methanol and glucose, respectively. e methanol cultures were co-fed with glycerol to enable the same chemostat-controlled constant speci c growth rates for direct comparison. A number of transcriptome regulatory studies of P. pastoris, examining the e ects of growth rate, unfolded protein response (UPR) activation, oxygen availability, osmotic stress, or heterologous protein production, were possible due to the availability of whole genome sequences. While numerous research have examined the cellular responses of P. pastoris to methanol induction in the context of the generation of recombinant proteins, it is mostly unknown how non-recombinant strains would react to the various carbon sources. As a result, we made the decision to look at how P. pastoris cells that aren't making recombinant proteins react to the two most common cultivation substrates, methanol and glucose, respectively. e methanol cultures were co-fed with glycerol to enable the same chemostat-controlled constant speci c growth rates for direct comparison. A number [5-10] of transcriptome regulatory studies of P. pastoris, examining the e ects of growth rate, unfolded protein response (UPR) activation, oxygen availability, osmotic stress, or heterologous protein production, were possible due to the availability of whole genome sequences. Further information about the traits of P. pastoris cultivated at various temperatures, osmolarity, UPR induction, and oxygen supply was revealed by analyses of the host proteome. More recently, using 2D-DIGE and subsequent mass spectrometry identi cation of di erentially abundant proteins, P. pastoris strains expressing an insulin precursor were examined for alterations in the cellular proteome as adaptive response to methanol

Protein totals are calculated

Cell pellets from a 2 mL chemostat culture were resuspended in 1 mL of PBS a er being washed with 0.9% NaCl (pH 7.0). According to Verduyn, the protein extraction was carried out by adding NaOH and incubating at 95°C. Centrifugation was used to remove cell debris a er