

Mutant *Hansenula polymorpha* Strain with Constitutive Alcohol Oxidase and Peroxisome Biosynthesis

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A mutant of the methylotrophic yeast *Hansenula polymorpha* with constitutive alcohol oxidase (AOX) and peroxisome biosynthesis was obtained after UV treatment followed by cell plating on a medium containing methanol and 2-deoxy-D-glucose (DOG). DOG-resistant colonies of mutants were insensitive to catabolic repression by glucose and methanol. A selection procedure is described that allows the isolation of a mutant exhibiting a constitutive phenotype of AOX involved in methanol utilization. Furthermore, additional features of the constitutive presence of peroxisomes are demonstrated. 562 DOG-resistant colonies were tested, 24 of them demonstrating constitutive AOX formation. Based on quantitative analysis, one of the strains – DOG-13 was selected and its growth, biochemical and ultrastructural characteristics were examined. Its specific enzyme activity when cultivated on a yeast nitrogen base + 1% glucose (YNB + 1% Glucose) was found to reach 145 nmol.min⁻¹.mg⁻¹protein (compared to zero of the parent strain) after the 20th hour of cultivation. This was confirmed by fine-structure analysis, showing typical peroxisomes, which number and size increased with the enzyme activity. This study demonstrates a constitutive AOX and peroxisome biosynthesis by the mutant strain *H. polymorpha* DOG-13 obtained.