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TRANSFORMATION OF BAKERS- AND WINE-YEAST USING THE DOMINANT FORMALDEHYDE RESISTANCE GENE *SFA1*. Martin Schmidt, Alp Cömer, Martin Grey and Martin Brendel. Institut für Mikrobiologie J.W.Goethe Universität Frankfurt - Germany.

The *SFA1* gene, and to a lesser degree the *ADH1* gene, were shown to cause hyper-resistance against formaldehyde when present on multi-copy vectors in yeast cells (WEHNER et al., Mol. Gen. Genet. 237, 351-358; 1993 GREY et al., Curr. Genet. 29, 437-440, 1996) and so represent dominant selection markers for genetic engineering of industrial strains of Saccaromyces cerevisiae. This selection method proved to be advantageous because the selective agent is cheap and metabolized completely at the end of batch cultures, avoiding problems of detoxification of fermentation products. We present protocols for and data of the transformation of genetically ill-defined bakers- and wine yeasts using formaldehyde-selectable vector systems. In this study, we managed to transform various industrially applicated yeast strains, selected them on their formaldehyde resistance and characterized the physiology of resulting transformants. Heterologous expression of the E. coli β-galactosidase resulted in blue colour of transformants growing on media containing X-Gal, and functionality of a yeast *ADH1* promoter fragment was proven by the expression of a *ADH1URA3* fusion in uracil auxotrophic laboratory strains. We show as well the additional formaldehyde detoxifying effect of an overexpressed *ADH1* gene, resulting in increased hyper-resistance.

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CONSTRUCTION OF BROAD HOST-RANGE VECTORS TO SCREEN THE AMAZONIAN BACTERIAL FLORA. Kipnis, A.[1]; Pedraça, E.B.[2]; Santos, M.M.[2]; Vicente, E.J.[3]; Santos, J.G.[2] & Astolfi-Filho, S.[1/2] 1-Lab. de Biol. Molecular-IB, UnB, Brasília-DF. 2-Lab. Microbiologia-ICB, FUA, Manaus-AM. 3-Depto. de Microbiologia-ICB, USP, São Paulo - SP.

The amazonian biodiversity is a rich source of microorganisms with biotechnological potential. In order to screen new microorganisms capable of express heterologous proteins it was developed a broad host-range vector (pMFL) introducing the easily expression detectable *Bacillus subtillis* α-amylase gene into the pMFY40 plasmid. In this construction the α-amylase gene is under control of a modified *lac* promoter. It were selected 20 Gram-negative bacteria, isolated from amazonian fishes, that were sensitive to most of the common antibiotics and were not amylase producers. After electroporation of these bacteria with the recombinant plasmid, it was obtained transformants from two distinct bacteria. The growth and α-amylase production of the transformants were analyzed, comparing with *Escherichia coli* (DH5α) cells transformed with the same plasmid. The levels of amylolythic activity in the culture's supernatant are similar between *E. coli* and the amazonian bacteria, however, the growth of the amazonian cells cultures is not affected by α-amylase production while it is severely affected in *E. coli* cells culture. The system here described to find new efficient bacteria hosts for heterologous gene expression has shown very effective and, in a preliminary analysis, it is possible to say that there is a big potential of isolating new microorganism with better biotechnological properties than those organism currently used today.

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C.350

ENGENHARIA GENÉTICA DE *PICHIA PASTORIS* VISANDO A OBTENÇÃO DE PROTEÍNA UNICELULAR A PARTIR DE SACAROSE

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A levedura metilotrófica *Pichia pastoris* apresenta um excelente potencial para o emprego como fonte protéica na alimentação animal. Este microrganismo pode ser cultivado em altas densidades celulares (até 150 g/L) e os teores de aminoácidos são superiores àqueles verificados em *Saccharomyces cerevisiae*.

Para tornar esta levedura capaz de crescer em sacarose de cana-de-açúcar, o gene codificador da enzima invertase de *S. cerevisiae* foi introduzido no seu genoma por integração cromossômica.

Nossos resultados mostram que a informação clonada é mantida com 100% de estabilidade na ausência de pressão seletiva, a quantidade de invertase produzida pelos clones recombinantes não é limitante para o crescimento em meio mínimo contendo 1% de sacarose como única fonte de carbono e, a quantidade de massa celular seca observada no final dos cultivos em agitador rotativo é sempre superior ao dobro daquela obtida com a levedura industrial *S. cerevisiae* FTPT.

Desse modo, o emprego do nosso sistema apresenta-se como uma alternativa bastante promissora para a produção de proteína unicelular a partir de sacarose. Em adição, abre-nos a perspectiva muito próxima da produção de proteínas heterólogas em cepas industriais de *P. pastoris*.

Apoio: CAPES, FINEP

C.351

EXPRESSION OF ANTISENSE VIRAL GENES IN TRANSGENIC BEANS RESULTS IN DELAYED OR ATTENUATED SYMPTOMS OF BEAN GOLDEN MOSAIC GEMINIVIRUS. F.J.L. Aragão[1], S.G.Ribeiro[1], J.C.Faria[2], E. Nogueira[2], G.R.Vianna[1], M. Lemos[1] and E.L.Rech[1]. [1]Centro Nacional de Pesquisa de Recursos Genéticos e Biotecnologia, EMBRAPA, P.O. Box 02372, Brasília, DF, 70770, Brazil. [2]Centro Nacional de Pesquisa de Arroz e Feijão, EMBRAPA, P.O. Box 179, Goiânia, GO, 74001, Brazil.

Bean golden mosaic virus (BGMV) is the causal agent of the major disease of beans (*Phaseolus vulgaris* L.) in South and Central America, and Caribbean Basin countries, causing losses up to 100%. The BGMV genome is divided in two components of circular single-stranded DNA (ssDNA), named DNA-A and DNA-B, both of which are required for infectivity. The A component contains the genes required for encapsulation of virions (coat-protein) and DNA replication (AC1, AC2 and AC3), and the B component contains two genes required for viral systemic movement (BC1, BR1) in the infected plant. In this work we have used the antisense strategy to produce genetic resistant plants against the Brazilian isolate of BGMV. Transgenic bean (*Phaseolus vulgaris* L.) plants were obtained using the biolistic process. These plant were transformed with the sequences of the AC123 and BC1 genes from BGMV in antisense orientation, controlled by the 35S CaMV promoter. Transgenic plants from the R3 and R4 generations were challenged against the virus. The results have shown delayed or attenuated symptoms of the virus upon BGMV whitefly-mediated inoculation. Virus titration on transgenic plants was performed by ELISA, Western and Southern blot. The results revealed a significant decrease in the virus content in plants with attenuated symptoms.

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