Volume 48(1-4):85, 2004
Acta Biologica Szegediensis
http://www.sci.u-szeged.hu/ABS

DISSERTATION SUMMARY

Development of a protein expression system

Gabriella Urbán

Bay Zoltán Foundation for Applied Research, Institute for Biotechnology, Szeged, Hungary

The aim of the work is to develop a protein expression system which can be used to overexpress proteins. The system would be based on a methylotrophic *Pichia* strain which is capable to utilise methanol as a sole carbon and energy source. The strain was isolated from nature by the co-workers of the Institute, so the system – if developed – could be used without any legal restriction.

Known alcohol oxidases (AO) were compared at the nucleic acid and amino acid levels, evolutionary conserved motifs were identified, degenerate oligonucleotides were synthesised accordingly and used in PCR reactions to amplify segments of the AO coding regions. The templates in these reactions were genomic DNAs isolated from the *Pichia* sp. and from two other methylotrophic *Candida boidinii* strains for comparison. The amplified products were cloned and sequenced. The partial sequences revealed the presence of two genes (*aoxA* and *aoxB*) in the *Pichia* sp. and one in each *Candida boidinii* strain. Gene specific PCR primers were synthesised on the bases of the sequence information gained, and the complete AO ORFs were subsequently isolated in several steps.

The expression of *aoxA* and *aoxB* was analysed by RT-PCR. RNAs for templates in these reactions were isolated from yeast grown on dextrose or methanol. In the presence of dextrose a constitutive, low-level expression of *aoxA* but not *aoxB* was detected. Both genes were induced in the presence of methanol, although to different extent.

The DNA segments that contain the promoter of the genes were isolated in several successive steps. The isolated genomic segment is 1920 bp long in the case of *aoxB* and 1661 bp long in the case of the *aoxA*. The 3' non-translated regions which probably contain the transcriptional terminators were also isolated as a 469 bp (*aoxA*) and a 430 bp (*aoxB*) long PCR fragments, respectively.

For the localisation of the functional elements of the promoter and for the identification of the minimal functional promoter, a number of transcriptional fusions were constructed. For a reporter in these fusions we used the fluorescent protein DsRed2.

For a functional expression system, some kind of a selection is essential. Plasmids, therefore, were constructed that indicate the successful transformation through the manifestation of a resistance gene (zeocin). Zeocin, as an antibiotics, though works at a laboratory level, might not be used for commercial productions. For future usage we also isolated the entire *his4* gene from the *Pichia* sp. which could be used for selection, provided a strain carrying a mutant version of this gene was isolated, too.

The effective transformation requires the stable maintenance of the transformed DNA, either through integration into the genome, or by replication. For integrative stabilisation the *his4* sequence can be used. The replicative stabilisation requires genomic regions the presence of which confers replication to the transformed DNA by the cellular functions. Such autonomously replicating sequences were isolated from the *Pichia* sp. and their ARS function were confirmed.

Supervisor: László Dorgai
E-mail: urga@bay.u-szeged.hu