

Isolation and cloning of the *Sordaria macrospora* *ura3* gene and its heterologous expression in *Aspergillus niger*

Minou Nowrousian and Ulrich Kück - Lehrstuhl für Allgemeine Botanik, Ruhr-Universität Bochum, 44780 Bochum, Germany

We report the isolation and molecular characterization of the *ura3* gene encoding orotidine monophosphate decarboxylase (OMPD) from *Sordaria macrospora*. Pulsed field gel electrophoresis and Southern hybridization revealed that the *ura3* gene is located on chromosomes VI / VII of *S. macrospora*. A functional analysis of the *S. macrospora* *ura3* gene was performed by DNA transformation using an *Aspergillus niger* *pyrA* mutant as recipient strain.

Sordaria macrospora can be regarded as a model organism in developmental mycology, for example to study fruit body maturation. In an approach to isolate selectable marker genes for DNA mediated transformation of *Sordaria macrospora* we succeeded in isolating the homologous *ura3* gene. Using an indexed cosmid library from *S. macrospora* (Pöggeler *et al.* 1997 J. Microbiol. Meth. 29: 49-61), we identified the *ura3* gene on a single cosmid clone by heterologous hybridization with the *pyr4* gene from *Neurospora crassa* (Buxton and Radford 1983, Mol. Gen. Genet. 190: 403-405). Sequence analysis revealed a continuous open reading frame of 1191 bp coding for a polypeptide of 397 amino acids showing significant homologies to the OMPD polypeptides from other eukaryotes (Figure 1). Highest homology is found between *URA3* and *PYR4* from *N. crassa* with 94 % amino acid identity. As shown in Figure 1, the *URA3* encoded polypeptide from *S. macrospora* contains an insertion of 90 amino acids that is typically found in pyrenomycetes and related imperfect fungi (Radford 1993 J. Mol. Evol. 38: 389-395).

Southern hybridization of total DNA indicates that the *ura3* gene is a single copy gene in *S. macrospora* (data not shown). As shown in Figure 2, chromosomes of two wild type isolates of *S. macrospora* were separated by pulsed field gel electrophoresis (Walz and Kück 1995 Curr. Genet. 29: 88-95) and probed with a DNA fragment carrying the *ura3* gene. In both strains, the high molecular chromosomal band representing chromosomes VI and VII hybridizes with the *ura3* gene specific probe.

In order to demonstrate the functionality of the *ura3* gene, the uracil auxotrophic strain N593 of *A. niger* was transformed with plasmid p20.26. This plasmid has a size of 6.6 kb and contains the complete open reading frame of the *S. macrospora* *ura3* gene with additional 5' and 3' nontranslated regions of 2.2 kb and 0.2 kb, respectively. The recipient strain *A. niger* N593 carries a mutation in the OMPD gene (*pyrA*, Goosen *et al.* 1987 Curr. Genet. 11: 499-503). It was transformed according to Mohr and Esser (1990 Appl. Microbiol. Biotechnol. 34: 63-70) with slight modifications as the transformants were selected for uracil prototrophy instead of antibiotic resistance. With a transformation rate of 14 transformants per μg DNA, 55 uracil prototrophic transformants were isolated. The autoradiograph in Figure 3 shows the molecular analysis of six randomly selected transformants. In all of them one or more copies of the *ura3* gene of *S. macrospora* are detectable, while the internal *pyrA* gene of the recipient strain is not labeled since hybridizations were performed under high stringency conditions. Our data prove that the isolated *ura3* gene of *S. macrospora* is functional and can be used as a selection marker in transformation experiments.

We thank Dr. S. Pöggeler for providing the indexed cosmid library from *S. macrospora* and Prof. Dr. Tudzynski for the gift of the *A. niger* *pyrA* strain. This work was funded by the 'Deutsche Forschungsgemeinschaft', Bonn - Bad Godesberg, Germany.

S.m. MSTTQ--QPHWSLQKSFARVESSESHPLTSYLFRLMEVKQSNLCLSDQVEHARELLALAKVGP 62
 N.c. MSTSQETQPHWSLQKSFARVESSESHPLTSYLFRLMEVKQSNLCLSDQVEHARELLALAKVGP 64
 T.r. -----MAPHPTLKATFAARSETATHPLTAYLFKIMDLKASNLCLSDQVPTARELLYLAKVGP 58
 A.c. -----MAPHPTLKSTYSTRAEVTTHLSAYLYKIMDLKASNLCLSDQVANARELLHVALKVG 58
 A.n. -----MSSKSQLTYTARASKHPNALAKRLFEIAEAKKTNTVTSADVTTTKELLDLAARLGP 56
 S.c. -----MSKATYKERAATHPSVAAKLFNIMHEKQTNLCLSDQVTTKELLLEVEALGP 53
 A.t. SKNFTGKKLFDIMLKKEINLCLADQVSTAELLDLADKVG 41
 B.t. GTHVAAKILRLIMOKKETNLCLSDQVSESERELLQADALGS 41

S.m. SIIVLKTHTYDLITGWDYHPHTGTGAKLAALARKHGFLLIFEDRKFDIGSTVQKQYTAGTARTVE 126
 N.c. SIIVLKTHTYDLITGWDYHPHTGTGAKLAALARKHGFLLIFEDRKFDIGSTVQKQYTAGTARTVE 128
 T.r. SIIVLKTHTYDMVSGTSHPETGTGAQLASLARKHGFLLIFEDRKFDIGSTVQKQYTAGTARTVE 122
 A.c. SIIVLKTHTYDMVAGWDFTPETGTGARLAKLARKHGFLLIFEDRKFDIGSTVQKQYTAGTARTVE 122
 A.n. YIAVVKTHIDILSDFSDETIEG---LEKALAKHNFLLIFEDRKFDIGSTVQKQYHRRGLRTE 116
 S.c. KICLLKTHVDILDFSM---EGTVKPLKALSAKYNFLIFEDRKFDIGSTVQKQYHRRGLRTE 114
 A.t. EICLLKTHVDILDPFT---PDFGSKLRADKHKFLIFEDRKFDIGSTVQKQYHRRGLRTE 101
 B.t. RUCCLKTHVDILNDFT---LDVMKELTTLAKRHEFLIFEDRKFDIGSTVQKQYHRRGLRTE 101

S.m. WAHITINADIHAGEAMVSAMAQAAQKWRERIPYEVKTSVSVGTPVADQFADE-----EAEDQVD 184
 N.c. WAHITINADIHAGEAMVSAMAQAAQKWRERIPYEVKTSVSVGTPVADQFADE-----EAEDQVE 186
 T.r. WAHITINVMVPGKASVASLAQGAQRWLERYPCEVKTSTVTGTPMTDSFDDADSRDAEPAGAVN 186
 A.c. WAHITINVMVPGKASVSLANAAKWLERYPYEVKTSVTVGTPTNDEDDDD-DEDDGNAGEMER 185
 A.n. WAHITINCSILPGEIGIVEALQATASAPDFSYP----- 148
 S.c. WADITINAHGVVGPVIGVGLKQAA---EEVTKE----- 143
 A.t. WADITINAHVISGPGIVDGLKLGMPR----- 127
 B.t. WADLVNAHAVPGSGVMVLEEVGLPL----- 127

S.m. ELRKIVPRENSTSKEKDTGGRKGSIVSITTTVTQTYEPADSPRLAKTISEGDEAVFPFIEEAPLD 248
 N.c. ELRKIVTRETSTT-TKDTDGRKSSIVSITTTVTQTYEPADSPRLVKTISEDDDEMVPFIEEAPLD 249
 T.r. QMGSGVLDKFIYSNRSGDGRKGSIVSITTTVTQTYEPADSPRLTKAIAEGDESIFPFIEEAPLS 250
 A.c. SHTFGLNGNNGVPIKESDGRKGSIVSITTTVTQTYEPADSPRLTKAIAEGDMLLAGLEEPLN 249
 A.n. -----E 149
 S.c. -----P 144
 A.t. -----G 128
 B.t. -----H 128

S.m. RGLLILAQMSKGCCLMDGKYTNECVKARKNKDFVMGYVAQQLNGITKEDLAPGYEDGETSTE 312
 N.c. RGLLILAQMSKGCCLMDGKYTNECVKARKNKDFVMGYVAQQLNGITKEDLAPGYEDGETSTE 317
 T.r. RGLLILAQMSKGCCLMDGKYTNECVKARKNKDFVMGYVAQQLNGITKEDLAPGYEDGETSTE 295
 A.c. RGLLILAQMSKGCCLMDGKYTNECVKARKNKDFVMGYVAQQLNGITKEDLAPGYEDGETSTE 294
 A.n. RGLLILAQMSKGCCLMDGKYTNECVKARKNKDFVMGYVAQQLNGITKEDLAPGYEDGETSTE 202
 S.c. RGLLILAQMSKGCCLMDGKYTNECVKARKNKDFVMGYVAQQLNGITKEDLAPGYEDGETSTE 190
 A.t. RGLLILAQMSKGCCLMDGKYTNECVKARKNKDFVMGYVAQQLNGITKEDLAPGYEDGETSTE 176
 B.t. RGLLILAQMSKGCCLMDGKYTNECVKARKNKDFVMGYVAQQLNGITKEDLAPGYEDGETSTE 175

S.m. EEAQADNFIIHMPGCKLPPPGEEAPQ-----GDGLGQQYNTPDNLVNIKSTDIATVGRGIITAS 371
 N.c. EEAQADNFIIHMPGCKLPPPGEEAPQ-----GDGLGQQYNTPDNLVNIKSTDIATVGRGIITAS 372
 T.r. -TEPDDAFIHMTPGCKLPPPEDEDQQTNGSVGSGDSGQQYNTPDNLVNIKSTDIATVGRGIITAS 358
 A.c. -SQPDDDFIHMTPGCKLPPPEHEEDAE---LRGDSGQQYNTPDNLVNIKSTDIATVGRGIITAS 354
 A.n. -PSDEEDFVVFITGVNISSK-----GDKLGQQYNTPDNLVNIKSTDIATVGRGIITAS 253
 S.c. -RDGEYDWLIMTPGVGLDDK-----GDALGQQYNTPDNLVNIKSTDIATVGRGIITAS 241
 A.t. -GVVYPSMIHATPGVQMVKG-----GDALGQQYNTPDNLVNIKSTDIATVGRGIITAS 228
 B.t. -----PEFLHLTPGVQLEAG-----GDNLGQQYNTPDNLVNIKSTDIATVGRGIITAS 223

S.m. -DPPAERERARRKAKAYQDRRERLA 396 S.m. = *Sordaria macrospora* (Z70291)
 N.c. -DPPAERERARRKAKAYQDRRERLA 397 N.c. = *Neurospora crassa* (M13448)
 T.r. -DPVEERERARRKAKAY--TERLLR 381 T.r. = *Trichoderma reesei* (S14132)
 A.c. -DLQHEERERARRKAKAY--TER-VR 376 A.c. = *Acremonium chrysogenum* (P14017)
 A.n. -DPQARQOQKQEGWEAYLARVGGN 277 A.n. = *Aspergillus niger* (P07817)
 S.c. -RDAKVEGERARRKAGWEAYLRRCQQN 267 S.c. = *Saccharomyces cerevisiae* (P03962)
 A.t. -NPAETARERARRKAGWEAYLEKCSQ 251 A.t. = *Arabidopsis thaliana* (Q42586)
 B.t. -NQLEARRKARRKAGWEAYLSRLAV 246 B.t. = *Bos taurus* (P31754)

Figure 1. (preceding page) Comparison of amino acid sequences from OMPD genes from different eukaryotes. The sources and corresponding accession numbers (EMBL data library) are given at the end of all sequences. In the cases of *Arabidopsis thaliana* and *Bos taurus*, only the OMPD domains of the polypeptides were used. Asterisks below the sequence indicate amino acid residues, which are conserved in all sequences. Dashes represent gaps introduced to obtain the best alignment. Amino acid residues are framed when less than two residues differ from the consensus sequence.

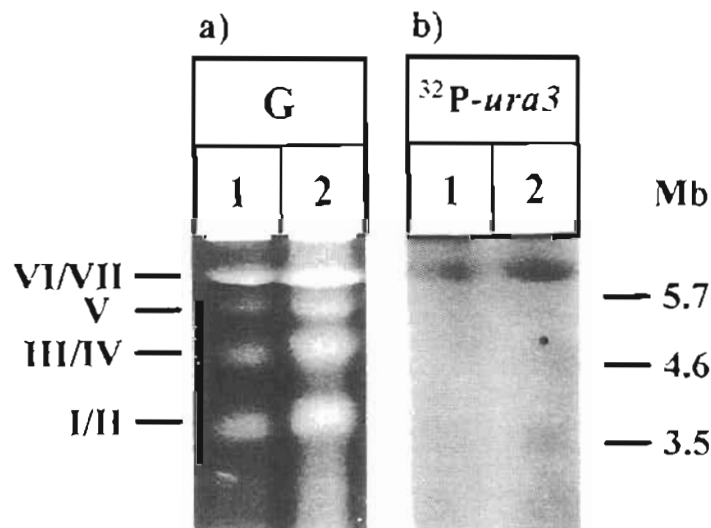


Figure 2. Chromosomal mapping of the *ura3* gene from *S. macrospora*. a) Chromosomes of two *S. macrospora* wild type strains (isolate S1957, lane 1; isolate L3346, lane 2) were separated using pulsed field gel electrophoresis. Roman numerals indicate positions of *S. macrospora* chromosomes. On the right, sizes of marker chromosomes are given in Mb. b) Autoradiograph showing the chromosomal localisation of the *ura3* gene. The filter bound chromosomal DNA was probed with a DNA fragment representing the open reading frame and adjacent regions of the *ura3* gene.

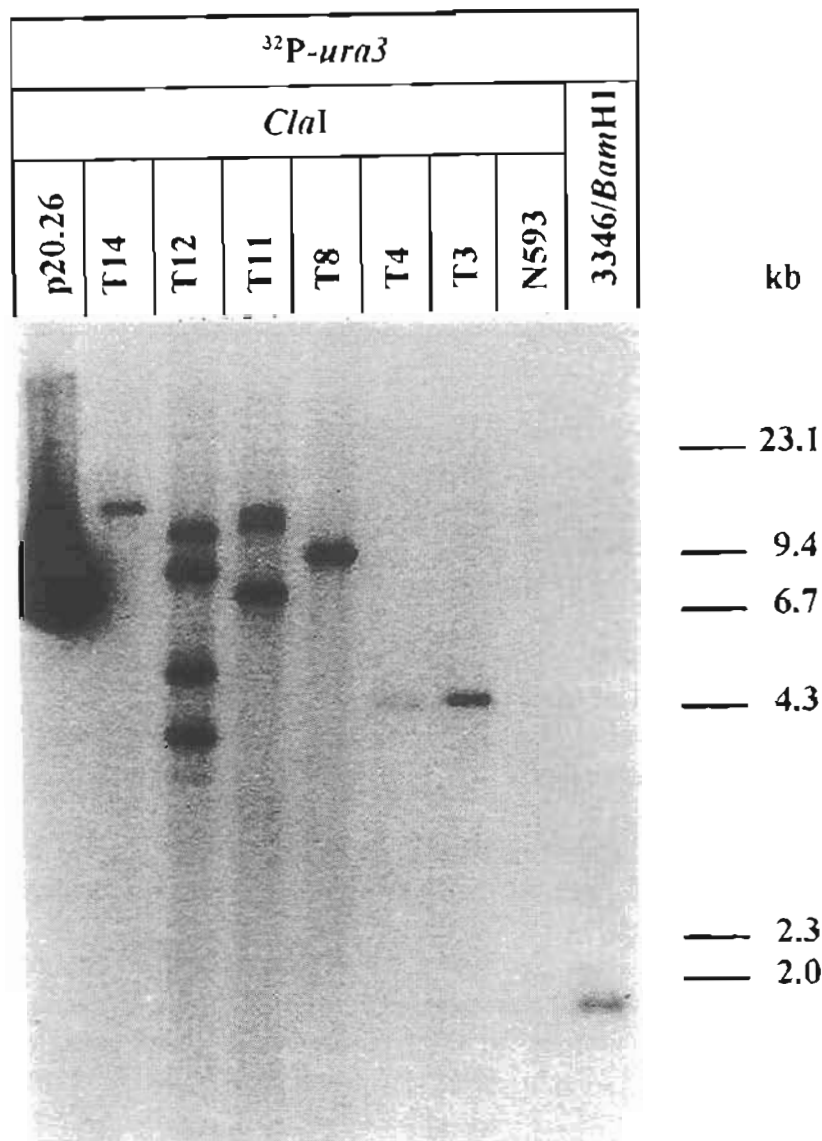


Figure 3. Autoradiograph from a Southern hybridization experiment with DNA from *A. niger* transformants. DNA from six transformants (T3, T4, T8, T11, T12 and T14) and from the recipient strain N593 was digested with *Cla*I and separated by gel electrophoresis. The Southern blot was probed with a DNA fragment, representing the open reading frame and adjacent regions of the *ura3* gene. As a control, the transforming plasmid p20.26 and DNA from a *S. macrospora* wild type strain (L3346) were used. Sizes of marker fragments are given on the right margin.