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Abstract

Glucoamylases are important industrial enzymes used in the conversion of starches to syrups and in other fermentation processes. Previously, the gene encoding the major glucoamylase activity of *N. crassa* was characterized (Stone et al. 1993 Curr. Genet 24:205-211). Here we report the identification of a possible second glucoamylase (*gla-2*) that is similar to a member of a class of glucoamylases represented by the *GAM1*gene of *S. occidentalis* (Dohmen et al. 1990 Gene 95:111-121).

Identification of a gene from *Neurospora crassa* with similarity to a glucoamylase gene from *Schwanniomyces occidentalis*

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Glucoamylases are important industrial enzymes used in the conversion of starches to syrups and in other fermentation processes. Previously, the gene encoding the major glucoamylase activity of *N. crassa* was characterized (Stone et al. 1993 Curr. Genet 24:205-211). Here we report the identification of a possible second glucoamylase (*gla-2*) that is similar to a member of a class of glucoamylases represented by the *GAM1* gene of *S. occidentalis* (Dohmen et al. 1990 Gene 95:111-121).

In our analysis of the *rco-3* gene of *N. crassa* we identified *gla-2* through partial sequencing of the DNA adjacent to *rco-3* from two sites in the clone. Homology to *GAM1* was found through a BLAST search using the translated single-stranded DNA sequences. An alignment by the Wisconsin GCG program 'Bestfit' is shown in Fig. 1. *gla-2* and *GAM1* are sufficiently similar in the sequenced regions to suggest that *gla-2* is likely a functional glucoamylase with properties similar to *GAM1*. The 3' end of the *N. crassa gla-2* coding region was sequenced and the predicted protein has a 39 amino acid extension relative to *GAM1*. There is no significant sequence similarity between *gla-2* and *gla-1* of *N. crassa*.

```
                      Sequence 1
  287
So  LYFFSGPTPKDAIQQYVKEIGLPAFQPYWSLGYHQCRWGYDTIEKLSEVVENFKKF
                 : :    :::: : :: :: :::::::        :: :: ::
Nc  SISCTLEDLLRYHQVYQQYVGLPAMQQYWTLGFHQCRWGYSNWTVVKDVVDNFRKF

                                                       392
So  NIPLETIWSDIDYMDSYKDFTYDPHRFPLDEYRKFLDELHKNNQHYVPIL
     :::::::::::::  ::::  ::  :   :   :: :::::
Nc  GIPLETIWSDIDYMKGYRDFENDPD.FSYEEGARFLEELHKNHHMTCRLS

                      Sequence 2
  908                                                  958
So  VTILGVGHKPKSVKFENANVDFTYKKST.....VFVTGLDKYTKDGAFSKDFTITW*
    ::::::   :  :                      :: :    : :      :  :
Nc  VTILGVAEAPLGVAINSHLLGSASWSYDSEGEVLSVTELQDNFKEGGWASNWTLSW

Nc  NSASNSGSSPVQGGGGRLEFSSPICSMQLLSASFLAACL*
```

Figure 1. The *S. occidentalis* (So) *GAM1* sequence is numbered with respect to the published initiator Met codon. Two regions of the *N. crassa* (Nc) gene were sequenced and the predicted polypeptide regions were aligned to *GAM1*. Identical residues are indicated by a colon (:). The C-termini of the proteins predicted from DNA sequence data are indicated by an asterisk (*).

The *gla-2* gene and *rco-3* are present in cosmid X10:E5 of the hygromycin cosmid library constructed by M. Orbach and M. Sachs (Orbach, M. J. (1994). Gene 150, 159-162; Orbach, M. J., and Sachs, M. S. (1991). Fungal Genet. Newsl. 38, 97.). A 10 Kb *Xba*I fragment from this cosmid contains both *rco-3* and *gla-2* with the gene organization shown (Fig 2). The cosmid was mapped by RFLP analysis and confirmed by genetic mapping of *rco-3* with respect to *his-3* (3/65) and the mating type locus (1/65) as being near the centromere of Linkage Group I (Fig. 3). The data suggest that X10:E5 could be placed on either side of *mei-3*.

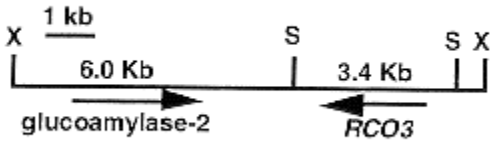

Figure 2. Organization of the *gla-2* and *rco-3* genes of *N. crassa*. The 10 Kb *Xba*I (X) fragment contains a 3.4 Kb *Sma*I fragment (S).

```
AP31a.1         MMMMOOMMMMOOMMMMMMM-MMOOOOOOOOOMOOO--M
AP8v.1          MMMMOOMMMMOOMMMMOMMMMMOOOOOOOOOMMOOOMMM
mei-3           MMMMMOMMMMOOMMMMMMMMMMMOOOOOOOOOMMOOOOMM
X10:E5          MMM-MOMMMMOOMM-MMMOM-M--OOOOOOOMOOOOMM
un-2, his-2     MMMMOOMMMMOOMMMMMMMMMMMOOOOOOOOOMMOOOOMM
lys-4           MMMMOOMMMMOOMMMMMMMMMMMOOMOOOOOMMOOOOMM
Fsr-33          MMMMOMMMMMOOMMMMMMMMMMMOOMOOOOOMMOOOOMM
```

Figure 3. RFLP mapping of cosmid X10:E5. The entire cosmid was labeled and used to probe *Bam*HI digested chromosomal DNA from progeny of the Oak Ridge x Mauriceville RFLP mapping cross.

The complete sequence of *gla-2* will be of interest to those interested in the structure and function of glucoamylases. A plasmid (pLMN1) containing the 10 Kb *Xba*I fragment has been deposited in the FGSC. Please contact DJE by e-mail at dje0282@zeus.tamu.edu for DNA sequence files.

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