

## 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*.

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Sequence 1
287
So  LYFFSGPTPKDAIQQYVKEIGLPAFQPYWSLGYHQCRWGYDTIEKLSEVVENFKKF
      : :      : : : : : : : : : : : : : : : : : : : : : : : :
Nc  SISCTLEDLLRYHQVYQQYVGLPAMQQYWTLGFGHQCRWGYSNWTVVKDQVVDNFRKF

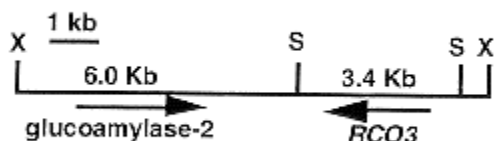
                                           392
So  NIPLETIWSDIDYMSYKDFTYDHRFPLDEYRKFLDELHKNNQHYPIL
      : : : : : : : : : : : : : : : : : : : : : : : :
Nc  GIPLETIWSDIDYMKGYRDFENDPD.FSYEEGARFLEELHKNNHMTCLRS

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

Nc  NSASNSGSSPVQGGGRLEFSSPICSMQLLSASFLAACL*
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**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 (S).

|             |                                                        |
|-------------|--------------------------------------------------------|
| AP31a.1     | MMMMOMMMMOMMMMMMMM-MMOOOOOOOOOO-MOOO--M                |
| AP8v.1      | MMMMOMMMMOMMMMMMMMMMMMOOOOOOO-MMOOOO-M                 |
| mei-3       | MMMMOMMMMOMMMMMMMMMMMMMMMMOOOOOOO-MMOOOO-M             |
| X10:E5      | MMM-MOMMMMMO-M-MMMOM-M--OOOOOOO-MMOOOO-M               |
| un-2, his-2 | MMMMOMMMMOMMMMMMMMMMMMMMMMMMMMOOOOOOO-MMOOOO-M         |
| lys-4       | MMMMOMMMMOMMMMMMMMMMMMMMMMMMMMMMMMOOOOOOO-MMOOOO-M     |
| Fsr-33      | MMMMOMMMMOMMMMMMMMMMMMMMMMMMMMMMMMMMMMOOOOOOO-MMOOOO-M |

**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](mailto:dje0282@zeus.tamu.edu) for DNA sequence files.

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