

# Nucleotide sequence of the $\alpha$ -amylase gene (*ALP1*) in the yeast *Saccharomycopsis fibuligera*

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The complete nucleotide sequence of the secretable  $\alpha$ -amylase gene *ALP1* from the yeast *Saccharomycopsis fibuligera* has been determined. The *ALP1* DNA hybridized to a polyadenylated RNA of 2.0 kilobases. A single open reading frame encodes a 494-amino acid protein which is highly homologous with  $\alpha$ -amylase (Taka-amylase) of a fungus *Aspergillus oryzae*.

Yeast;  $\alpha$ -Amylase; DNA sequence

## 1. INTRODUCTION

$\alpha$ -Amylase (EC 3.2.1.1) plays an important role in the liquefaction of starchy molecules and is widely distributed among various organisms from microorganisms to vertebrates and plants. Several yeasts including *Saccharomycopsis fibuligera* secrete  $\alpha$ -amylase extracellularly [1–3], but the brewing yeast *Saccharomyces cerevisiae* does not. For the rationalization of the fermentation process, introduction of  $\alpha$ -amylase genes into *S. cerevisiae* would be of great value, since transformed yeast cells would produce ethanol directly from starchy materials. Recently, we have succeeded in cloning and isolating the expression in *S. cerevisiae* of a putative secretable  $\alpha$ -amylase gene (*ALP1*) from *Sa. fibuligera* [3].

In this paper, we describe the complete

nucleotide sequence of *ALP1* gene. The deduced protein sequence was highly homologous with  $\alpha$ -amylase (Taka-amylase) from *Aspergillus oryzae*, suggesting that *ALP1* is a structural gene for  $\alpha$ -amylase of *Sa. fibuligera*.

## 2. MATERIALS AND METHODS

### 2.1. DNA sequence analysis

DNA was sequenced from M13 subclones by the dideoxy chain termination method of Sanger et al. [4].

### 2.2. Northern blot analysis

Cells of *Sa. fibuligera* strain HUT7212 [3] were cultured in 100 ml of YPS medium (1% yeast extract, 2% polypeptone and 3% soluble starch) to an absorbance at 660 nm of 1.0, harvested, and washed with sterile water. RNA was extracted from the cells as described by Jensen et al. [5]. Poly(A)<sup>+</sup> RNA was isolated with oligo(dT) cellulose as recommended by the supplier (Collaborative Research Inc., Waltham, MA). The RNA (10  $\mu$ g per lane) was fractionated in agarose (1%) gels as described by McMaster and Carmichael [6], transferred to nitrocellulose paper, and hybridized as described by Thomas [7] with a

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The nucleotide sequence presented here has been submitted to the EMBL/GenBank database under the accession number Y00683

BamHI/SauIA -1520 -1500 -1480 -1460 -1440  
 GATCACA ATACTAAAG ACGGCTAGT ACCTGATAGC GTCACCCCT AGAGTTAGC CTACTCTTG TGGTACTT TATCCGAG ATGTACTTT TTGAGATCC  
 -1420 -1400 -1380 -1360 -1340 -1320  
 GTGAGCTAC TAGTGGAC TTGTTGATAT ATGGGAGAT TCACAGTGT TTTTATTCAT CAGTATGTT GTCTCTGCA TAAATTTGA TTGAGCAAT ATGGCATG GCTTTTAC  
 -1280 -1260 -1240 -1220 -1200 PstII  
 CTGATGTTT AGGCGGGG GCAATGAG AGGGGTTCT TGTGTGCAAT GGTGGAGCT AACCTAATT CGAGATTTT CGAGTCTAA AGACCAATG CGAGCTGGC CACTACTTA TGTGAGAC  
 -1160 -1140 -1120 -1100 -1080 -1060  
 CAATTTCTC CTTCTGTAG ATACTACAA TTGTGTGAA ATGGCACTT TAGGAGGAA AGGGAAGGG CTTCTGAT TTACAAAAT GTTAATGAC TAGGGGAC CGGGGAAC AGTAATTAG  
 -1020 -1000 -980 -960 -940 -920  
 TTGAGAGC AATCTTGA ATTCGTCTG TTGTTGAC ACTTGATTC TGATCTTCT GGCATTTT AGTTGTGCA TATTCAGG CCGTAGTA CTCTTTGA AGGAGTAT GCAAAATCA  
 -900 -880 -860 -840 -820 -800 PstI  
 GAAATGAC TCATAGCT TGCAATTA TTGTGAAA AGACAAATG AAGTGTCTT ACCGAGGCG TGAGAGCTG AATTAATAT CACAAATTG CCGAGGAA CACTACTCA GTTAATGCT  
 -760 -740 -720 -700 -680 -660  
 TTGAGGTA GUTAGAGC ATGACTCTA CTTGTGTC GATTAATAT AATATATAT TTGATATTT GTGACTGCA GTTCTCTG CAATTTGAG AGTTAGTC AGTCTGAG GAGCAATA  
 -640 -620 -600 -580 -560 -540  
 AAGACCTA AATGTGCA GTGGGAGA CAAAAACAC GGGGATCTA GAGGTTGTA ATATTAGAC TTAATAGTG ACTTTGCT ATAAATGCT CATTTTGA AATAGTCT TAAATAGG  
 -500 -480 -460 -440 -420 -400  
 ATTTGATC ATATATAG GCAAACTT CTTGTGTA AGTGATTTA TACGGGGT ATATTAGCT AATGTGAGC ATTTGCTAT CTGAACAT TTTTGTGT CCGTGTGAA CTAATAGG  
 -380 -360 -340 -320 -300 -280  
 TTAGGGTCT TAGTGTCTA TTTTGTGCT TGTGGAGAT GACTTGTAT ATGTTGTTT TGTGTGCT GAGGTTGCT CACTATGCA TATTAACAA TATTTTCTT ACTTGAGCT  
 -240 -220 -200 -180 -160 -140  
 GTTCTGCT CATCATGAT ATCAACTT TGTCTTTC ATAGGGGCT TGTAAATGG TAGGAGAGC GATTAACCT TGAGGTTGA AATGGGCTT TATGTGCT TTTTATGCT ATCAAGAGG  
 -120 -100 -80 -60 -40 -20  
 CAGCTATGT TAAATAGCT TTTGAGAT ATAAATAGG ACACATGCA CTTCTGACA AAGACATTC TCAATATGA AATGCTGAA TGGAGTAA CATCTTAA CTTACTGAT TTTAATAT  
 -10 -1  
 ATG CAA ATT TCA AAA GCT GCT TTG CTT GGC TCA TTG GCT GGC CTT GTT TAT GCT CAA CCA GTG ACT CTA TTC AAA AGA GAA ACT AAT GCT GAT AAA TGG AGA TCA  
 Met Gln Ile Ser Lys Ala Ala Leu Leu Ala Ser Leu Ala Ala Leu Val Tyr Ala Gln Pro Val Thr Leu Phe Lys Arg Glu Thr Asn Ala Asp Lys Trp Arg Ser  
 10 20 30 40 50 60 70  
 CAG TCT ATT TAT CAA ATT GTC ACT GAC AGA TTT GCT AGA ACC GAT GGT GAT ACA AGT GCT TCC TGT AAC ACA GAA GAT AGA CTT TAC TGT GGT GGT TCT TTC CAA  
 Gln Ser Ile Tyr Gln Ile Val Thr Asp Arg Phe Ala Arg Thr Asp Gly Asp Thr Ser Ala Ser Cys Asn Thr Glu Asp Arg Leu Tyr Cys Gly Gly Ser Phe Gln  
 40 50 60 70  
 GGC ATC ATA AAG AAG TTG GAT TAC ATC AAA GAT ATG GGC TTT ACT GCT ATT TGG ATT TCT CCA GTT GTT GAA AAC ATT CCG GAT AAC ACA GCA TAT GGT TAT GCT  
 Gly Val Ser Ala Ser Lys Leu Asp Tyr Ile Lys Asp Met Gly Phe Thr Ala Ile Trp Ile Ser Pro Val Val Glu Asn Ile Pro Asp Asn Thr Ala Tyr Gly Tyr Ala  
 80 90 100  
 TAT CAT GCT TAC TGG ATG AAG AAC ATA TAC AAA ATT AAT GAA AAC TTT GCT ACT GCT GAT GAT TTG AAG TCT TTG CCA CAA GAA TTG CAC GAT GAT GAT ATG TTG  
 Tyr His Gly Tyr Trp Met Lys Asn Ile Tyr Lys Ile Asn Glu Asn Phe Gly Thr Ala Asp Asp Leu Lys Ser Leu Ala Gln Glu Leu His Asp Arg Asp Met Leu  
 110 120 130 140  
 TTA ATG GTG GAT ATC GTT ACC AAC CAT TAC GGC AGT GAT GGC AGT GGA GAT AGT ATC GAT TAC TCA GAG TAC ACC CCG TTC AAC GAC CAA AAG TAC TTC CAT AAC  
 Leu Met Val Asp Ile Val Thr Asn His Tyr Gly Ser Asp Gly Ser Gly Asp Ser Ile Asp Tyr Ser Glu Tyr Thr Pro Phe Asn Asp Gln Lys Tyr Phe His Asn  
 150 160 170  
 TAC TGT CTT ATT TCA AAC TAT GAT GAC CAA GCT CAG GTT CAA AGT TCC TGG GAA GGT GAC TCT TCA GTT CCA TTA CCA GAT TTG AGA AGC GAA GAT AGC GAC GTG  
 Tyr Cys Leu Ile Ser Asn Tyr Asp Asp Gln Ala Gln Val Gln Ser Cys Trp Glu Gly Asp Ser Ser Val Ala Leu Pro Asp Leu Arg Thr Glu Asp Ser Asp Val  
 180 190 200 210  
 GGC TCA GTT TTC AAT TCT TGG GTT AAA GAT TTT GTT GGC AAT TAC TCA ATT GAT GGT TTA AGA ATT GAT AGT GCT AAA CAT GTG GAC CAA GGC TTT TTC CCG GAT  
 Ala Ser Val Phe Asn Ser Trp Val Lys Asp Phe Val Gly Asn Tyr Ser Ile Asp Gly Leu Arg Ile Asp Ser Ala Lys His Val Asp Gln Gly Phe Phe Pro Asp  
 220 230 240  
 TTT GTT AGT CCA TCT GGA GTT TAC TCA GTA GGC GAA GTT TTC CAA GGA GAC CCA GCT TAT ACA TCC CCA TAC CAA AAT TAC ATT CCA GGG GTT AGT AAT TAT CCA  
 Phe Val Ser Ala Ser Gly Val Tyr Ser Val Gly Glu Val Phe Gln Gly Asp Pro Ala Tyr Thr Cys Pro Tyr Gln Asn Tyr Ile Pro Gly Val Ser Asn Tyr Pro  
 250 260 270 280  
 TTG TAC TAC CCA ACC ACG AGA TTT TTT AAA ACT ACT GAT TCA AGT TCC AGT GAG TTG ACT CAA ATG ATT TCA AGC GTT GCT TCC AGT TGT TGG GAT CCA ACT TTG  
 Leu Tyr Tyr Pro Asn Thr Arg Phe Phe Lys Thr Thr Asp Ser Ser Ser Ser Ser Glu Leu Thr Gln Met Ile Ser Ser Val Ala Ser Cys Ser Asp Pro Thr Leu  
 290 300 310  
 HincII  
 TTG CAA AAC TTT GTA GAA AAT CAC GAT AAT GAA AGG TTC GCT TCA ATG ACC AGC GAC CAA AGT TTG ATT TCT AAT GCT ATT CCA TTT GTC CTT TTG GGT GAT GGT  
 Leu Thr Asn Phe Val Glu Asn His Asp Asn Glu Arg Phe Ala Ser Met Thr Ser Asp Gln Ser Leu Ile Ser Asn Ala Ile Ala Phe Val Leu Leu Gly Asp Gly  
 320 330 340 350  
 ATT CCT GTC ATT TAC TAT GGA CAA GAA CAA GGC TTG AGC GGA AAA AGT GAC CCA AAC AAC AGA GAG GGC TTG TGG TTA TCC GGC TAC AAC AAA GAG AGT GAC TAT  
 Ile Pro Val Ile Tyr Tyr Gly Gln Glu Gln Gly Leu Ser Gly Lys Ser Asp Pro Asn Asn Arg Glu Ala Leu Trp Leu Ser Gly Tyr Asn Lys Glu Ser Asp Tyr  
 360 370 380  
 TAC AAG CTC ATT GGC AAA GCT AAT GCT GGC AGA AAC GGC GGC GTT TAT CAA GAC TCA AGC TAT GGC ACC TGG CAG CTT TCT GTG ATC TTT TCA AAT GAC CAT GTT  
 Tyr Lys Leu Ile Ala Lys Ala Asn Ala Ala Arg Asn Ala Val Tyr Gln Asp Ser Ser Tyr Ala Thr Ser Gln Leu Ser Val Ile Phe Ser Asn Asp His Val  
 390 400 410 420  
 ATT GCA ACA AAA AGA GGC ACC GTT GTT TCT GTT TTC AAC AAC CTT GCT TCC AGC GGT TCT TCT GAT GTG ACT ATT TCC AAC ACA GAT TAC ACT TCC GGT GAG GAT  
 Ile Ala Thr Lys Arg Gly Ser Val Val Ser Val Phe Asn Asn Leu Gly Ser Ser Gly Ser Ser Asp Val Thr Ile Ser Asn Thr Gly Tyr Ser Ser Gly Glu Asp  
 430 440 450  
 HincII  
 TTG GTA GAA GTT TTG ACA TCC AGT ACT GTT AGC GGC AGC TCT GAC TTA CAA GTT TCT ATC CAA GGT GGT CAA CCA CAA ATC TTT GTT CTT GCT AAA TAT GCT TCT  
 Leu Val Glu Val Leu Thr Cys Ser Thr Val Ser Gly Ser Ser Asp Leu Gln Val Ser Ile Gln Gly Gly Gln Pro Gln Ile Phe Val Pro Ala Lys Tyr Ala Ser  
 460 470 480 490  
 GAC ATT TGT TCA TGA  
 Asp Ile Cys Ser Stop.  
 494  
 TGGCTGG GGTGGTTTT TATCTTTGAA CTGTGCTCA GGTTCATCA ACTTTTCTT TTTCTTTT AGACTGTTT TCTTACTT TGGGACTT AGTTATCTG TGTATGAT ATTTPACTT  
 XbaI +140 +160 +180 +200 +220 +240 +260  
 CTGATATTT ATCTCTTTT ACCTTCTAT GAATGCTCA TTGACAAAT TATATTAACA CACTGTCTG TTGTAATCTT TAATCTTTT TTGATATCC ATATGAAC TTAATTTGT GTGGGATGC  
 +280 +300 +320 +340 +360 +380  
 AATATATAT ATGTGTAGG GGTGAGTAT AAGGGTCT CATCAAGGA AACATTAATA ATAAATGAA AATATGAC TACTGAAAT GGAAGGCTT TGTATCTTT TTAGAGGAC ATAAAGACA  
 +400 +420 +440 +460 +480 +500 +520  
 CTTTGTGAT CTTTGTATTT ATGATTTCA AGGCATCTA TTAATGACT GAAAATAC TATACATCT CACAGAGCA TGTGATTC CAAACATTC GGTGATTTA AATCTGCTG CACTTGTGCT  
 +540 +560 +580 +600 +620 +640  
 CGAGGGTCT GGGGCTGAT ATATCAAGA AGATCAATC CATGGAGGG ATATCTGCA AATATGAT GAGTGAATC AKTGTCTA TTGTGGGGA TAGAAACT GAGAGTCTA GGTTTCTGC  
 +660 +680 +700 +720 +740 +760 +780  
 ACGAAGGG TTGATATCT TTTTATTTA AGGGAGGG ATGTGAGT TCATAGGAA GTGATTTCC GGGGAAGTA TTTTGACTT GTCTTCAGA GCACAAATG CATCAAGAC GTTGGTAGT  
 +800 +820 +840 +860 +880 +900  
 GAGATCTGC GGTCTACAC AGGATGACA AATTTGAG GACTAATAT CTGCTTTCA AGAATATCT GCGGAGCAA CAGAGGGGT GGTATGCTG GTGGAGAGT ACCTTGGAG CGAGATTTG  
 +920 +940 +960 +980 +1000 +1020 +1040  
 CAGATATCT TAACTGAC GAGGATAT CATAGATAG TTGAGATCT CAGAGAGCT TCGTATGAC TACGGAGGG TCGAGGAAA GATTGAAA GCGAATGTA TGGAGATCC CAGTGTCTA  
 +1060 +1080 +1100 +1120 +1140 +1160  
 TCGAATTTG GTTACGATTT CCGTAAAG AGAGATCTA TTGAGTGG ACTACTACA TGTAGCTA TCGAGACT ACGAGAGT TCGCAAGGT GGTCTTGA TGTGATGA GTTGAAGAG  
 +1180  
 TTTAGCACA AACATGAG TATAGAGCA TCC  
 BamHI

Fig.1. Sequence of the *ALPI* gene. Potential *N*-glycosylation site is boxed.

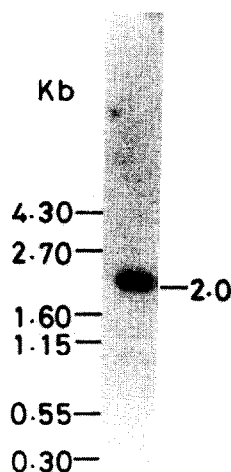


Fig.2. Northern blot analysis of the polyadenylated RNA from *Sa. fibuligera*.

nick-translated  $^{32}\text{P}$ -labelled M13mp11 containing a *StuI-PstI* fragment of *ALPI*. Restriction fragments derived from both M13mp11 and the probe DNAs were used as size markers.

### 3. RESULTS AND DISCUSSION

Fig.1 shows the complete nucleotide sequence of a 4223-base-pair-long DNA fragment which is essential for  $\alpha$ -amylase production in *S. cerevisiae*. A unique open reading frame of 1495 base pairs was found. A corresponding peptide sequence consisting of 494 amino acids is also shown in fig.1. The length of the coding region is in reasonable agreement with the observation that *ALPI*-specific mRNA was 2.0 kilobases in size (fig.2). The predicted protein carries one potentially glycosylated asparagine residue [8] (boxed in fig.1) and a hydrophobic amino-terminal segment of ~20

Sf ( 1)	MQISKAALLASLAALVYAQPVTLFKRETNADKWRSSQSIYQIVTDRFARTDGDTSAS	QNTTE	( 60)
Ao ( 1)	A-P--	-----FLL-----S-T-T--A	( 33)
Sf ( 61)	DRLYGGSFQGIKKLDYIKDMGFTAIWISPVVENIPDNTAYGYAYHGYWMKNIYKINEN		(120)
Ao ( 34)	-QK--TW---D---QG-----T--TAQL-QDC---D--T---QTD--SL---		( 93)
		* 1	
Sf (121)	FGTADDLKSLAQELHQRDMLLMVDIVTNIYVSGDGSIDYSEYTPFNDQKYFHNYS	CLIS	(180)
Ao ( 94)	Y-----A-SSA--E-G-Y--V-A-M-Y--A-S-V---VFK--SS-D--PF--F-Q		(153)
		S1	
Sf (181)	NYDDQAQVQS	WEGDSSVALPDLRTESDVASVFNSWVKDFVGNYSIDGLRIDS	AKHVDQ (240)
Ao (154)	--E--T--ED--L--NT-S---D-TKDV-KNEWYD--GSL-S---	TV--QK	(213)
		* 2	
Sf (241)	GFFPDFVSASGVYSVGEVFGDPAITCPYQNYIPGVSNYPLYPTTRFFKTTDSSSSELT		(300)
Ao (214)	D-W-GYNK-A---CI--LD-----VMD--L---I---LLNA--S-SG-MDD-Y		(273)
		S3	
Sf (301)	QMISSVASSCS	DPDLLTNEVENHDMERFASMTSDQSLISNAIAFVLLGDGIPVIYYGQEQ	(360)
Ao (274)	N--NT-K-D-P-S---GT-----P---Y-N-IA-AK-VA--II-N--L-I--A---		(333)
		S4	
Sf (361)	GLSGKSDTNNREALWLSGYNKESDYKLIAKANAARNAAVYQDSSYATSQLSVIFSNDHV		(420)
Ao (334)	HYA-GN--A--T-----PTD-EL-----S---I--Y-ISK-TGFV-YKNPY-KDDTT		(391)
		* *	
Sf (421)	IATKRGSVVSV	FNNLGSSG SSDVTISNTGYSSGEDLVEVLTCTVSGSSDLQVSI	(475)
Ao (392)	--MRK-TDG-QIVTILS-K-A--D-YTSLSL-GAS-TA-QQ-T--IG--T--TVG--GN-PV		(452)
		M ← C	
Sf (476)	QGGQPQIFVP AKYA SDICS		(494)
Ao (453)	PMA--L-RVLY-TE-L-G-K--DSS		(478)
		4	

Fig.3. Comparison of amino acid sequence between *Sa. fibuligera* (Sf) and *A. oryzae* (Ao)  $\alpha$ -amylases. Identical amino acid residues to those in the top sequence are indicated by dashes. Asterisks and arrowheads indicate substrate-binding and catalytic residues in Taka-amylase, respectively. Pairs of cysteine residues are indicated by boxes with identical numbers. The *N*-glycosylation site is indicated by -CHO. Four highly homologous segments (S1-S4) are also boxed.

The main (M) and C-terminal (C) domains of *A. oryzae*  $\alpha$ -amylase are indicated by hooked arrows.

amino acids which resembles signal sequences found in a wide variety of secretory protein precursors [9].

Comparative studies revealed amino acid sequence homology between *Sa. fibuligera* and *A. oryzae*  $\alpha$ -amylases as shown in fig.3. From X-ray analysis of Taka-amylase, it has been found that Glu-230 and Asp-297 residues (indicated by arrowheads in fig.3) play a central role for enzymatic activity, and that other amino acid residues indicated by asterisks in fig.3 serve for binding of substrate [10]. These residues are well conserved in the *ALPI* protein. Moreover, Taka-amylase carries 4 pairs of cysteine residues for disulfide bonds (boxed with numbers in fig.3) and one *N*-glycosylated asparagine residue (*N*-glycoside was indicated by -CHO in fig.3). Such amino acid residues were also conserved in the *ALPI* protein. Kusunoki et al. [11] have reported that Taka-amylase consists of two globular domains, main and C-terminal ones (indicated by hooked arrows in fig.3). The function of the C-terminal domain has not yet been understood. The amino acid sequence of the C-terminal region of *ALPI* protein showed very little homology with that of Taka-amylase.

A comparison of *ALPI* protein with other  $\alpha$ -amylases from *Bacillus* sp. [12–14], human salivary glands and pancreas [15] and barley [16] showed only 4 short homologous segments (S1–S4 in fig.3) which are proposed to be essential for the catalytic reaction of Taka-amylase.

These results suggest that *ALPI* is a structural gene for  $\alpha$ -amylase of *Sa. fibuligera*.

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