

Evidence for *S. cerevisiae* Fermentation in Ancient Wine

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Abstract. *Saccharomyces cerevisiae* is the principal yeast used in modern fermentation processes, including winemaking, breadmaking, and brewing. From residue present inside one of the earliest known wine jars from Egypt, we have extracted, amplified, and sequenced ribosomal DNA from *S. cerevisiae*. These results indicate that this organism was probably responsible for wine fermentation by at least 3150 B.C. This inference has major implications for the evolution of bread and beer yeasts, since it suggests that *S. cerevisiae* yeast, which occurs naturally on the surface bloom of grapes, was also used as an inoculum to ferment cereal products.

Key words: Ancient DNA — Evolution — Fermentation — Ötztal Alps Iceman — *Saccharomyces cerevisiae* — Winemaking

Introduction

Hundreds, if not thousands, of years of experimentation in vine cultivation and winemaking technology are needed to explain the level of sophisticated and large-scale wine production that is displayed in

mountainous and upland Neolithic settlements of the ancient Near East. The earliest molecular archaeological evidence for large-scale wine production is from the site of Hajji Firuz Tepe in the northern Zagros Mountains, dated to ~5400 B.C. (McGovern et al. 1986). Even earlier chemical evidence, in association with what appear to be remains of domesticated grapes (*Vitis vinifera vinifera*), has been obtained from the early sixth millennium B.C. in the Neolithic village of Shulaveris-Gora in the Transcaucasus region of modern Georgia (Ramishvili 1983; McGovern, unpublished).

Winemaking enabled humans to produce a beverage with a high content of ethyl alcohol, whose mind-altering, analgesic, disinfectant, and preservative properties made it the most widespread drug and medicine of antiquity. Winemaking is consequently of great importance in the biocultural evolution of the human species and its societies. *Saccharomyces cerevisiae* is the principal agent in wine fermentation, converting the simple sugars of grapes to carbon dioxide and alcohol. Once the juices of the fruit have been exuded, an ideal breeding ground with the right mix of water and nutrients is created for *S. cerevisiae* to begin and sustain fermentation to produce an alcoholic beverage. By comparing the DNA sequences of modern *S. cerevisiae* with those of ancient wine yeasts, one stage in the human selection process of the microorganism can be reconstructed in a process likely extending back 8000 or more years.

Materials and Methods

The original experimental material consisted of residue inside one of the earliest wine jars from ancient Egypt, dated to 3000 B.C., which were recovered from tomb U-j of King Scorpion I (Dreyer 1993; McGovern et al. 1997; Hartung 2001). From residue inside jar 10/115 [catalog No. 156 in Hartung (2001)], three independent DNA extractions were carried out, each with a 500-mg sample. The DNA extractions employed a procedure designed for soil microorganisms (DNA Spin Kit; Bio101, LaJolla, CA). Strict laboratory protocols were followed throughout the DNA extractions and amplifications to prevent contamination from modern organisms.

All of the polymerase chain reaction (PCR) amplifications were carried out in a new, isolated laboratory at the University of Florence, using equipment not previously exposed to yeast or yeast DNA. The laboratory bench was cleaned of contamination by DNA or RNA using DNA-Zap (Ambion, Austin, TX). New, thoroughly cleaned Gilson pipettes (Gilson, Middleton, MI) were employed, and air-filtered tips were used for each PCR to prevent contamination from the aerosol. Samples were handled with clean latex gloves, and masks and laboratory gowns were worn at all times. Containers enclosing the samples were opened away from any possible source of yeast contamination, and the contents transferred to sterile Falcon tubes (Becton, Dickinson, Franklin Lakes, NJ). All the materials used for the extraction, including the mortars for crushing the samples, were thoroughly cleaned and sterilized.

The amplified DNA was subjected to gel electrophoresis using equipment not previously used for other yeast DNA samples. Freshly prepared gel buffers were used, and the gel cast apparatus was previously treated with DNA-Zap to eliminate contamination from modern DNA or RNA. DNA blank extractions, serving as negative controls, were carried out in parallel during the extraction and the PCR amplification. A positive control was also carried out by adding 50 ng of modern *S. cerevisiae* genomic DNA to an aliquot of the negative control prior to electrophoresis. The amplifications were performed using Hi-Fidelity DNA polymerase (Hoffmann-La Roche, Basel, Switzerland). The amplification reaction conditions were as described by Guillamón et al. (1998). The DNA amplifications were carried out twice at different times and in different laboratories and resulted in products that were indistinguishable from one sample to the next. After extraction of bands of the amplified DNA from the gel, DNA sequencing was carried out directly on the PCR products without prior cloning, employing the same primers as used for amplification and internal primers as needed. The putative amplification product from ancient *S. cerevisiae* was sequenced from samples from two different extractions.

The DNA was amplified with primers ITS1 (5'-GTTTCCGTAGGTGAACCTGC-3'), which corresponds to nucleotide coordinates 455966–455947 in the *S. cerevisiae* genomic sequence of chromosome 12 (genome-www.stanford.edu/cgi-bin/SGD/chromosomes), and ITS4 (3'-CGTATAGTTATTCGCCTCCT-5'), which corresponds to coordinates 455142–455123. These primers are located in the ribosomal DNA (rDNA) sequences and can amplify 394 bp of the ITS1 (internal transcribed spacer 1), the 158-bp sequence encoding 5.8S RNA (*RDN58-1*), and 288 bp of the ITS2 (Guillamón et al. 1998). The yeast genome contains 100–200 copies of this sequence, embedded in a tandemly repeated unit of 9.1 kb that encodes the 18S, 5.8S, and 25S rRNAs.

To confirm the putative *Saccharomyces* origin of the 840-bp product amplified by primers ITS1-ITS4, amplifications were also carried out with primers ITS5 (5'-GGTGAGAGATTTCTGTGC-3'), corresponding to coordinates 455776–455759, and ITS6 (3'-GGGTCTCCATTGTTTGTGTT-5'), corresponding to coordinates 455663–455644. These primers are nested inside the region amplified by ITS1 and ITS4. The IS5 and IS6 oligonucleotides were also used as primers for sequencing.

Results

The yellowish residue that we used for the DNA amplifications is present inside the earliest wine jars from ancient Egypt, as established by molecular archaeological analyses (McGovern et al. 1997, 2001; McGovern 2001). The date of the jars—3150 B.C.—is based on radiocarbon determinations (Dreyer 1993). The jar we studied, together with hundreds more, had been imported from the southern hill country of Palestine and the Jordan Valley, according to Instrumental Neutron Activation Analysis of the pottery fabrics (McGovern 2001), and deposited in tomb U-j of Scorpion I, one of the first kings of Egypt, at the site of Abydos along the middle Nile River. If all the jars were full when imported, as seems likely, they originally contained approximately 4500 liters of wine, equivalent in volume to 20 modern French Bordeaux barrels. The alcohol and other volatile substances had long ago disappeared, but the large quantity of solid material that had collected on the bottoms and sides of the jars was presumed to include dead cells and cellular debris from yeast.

Lees from the Scorpion I jar was subjected to DNA extractions and amplifications under conditions designed to minimize the risk of contamination with DNA from modern organisms, in a laboratory and using equipment previously unexposed to modern *S. cerevisiae*. The extracted DNA molecules exhibited a wide distribution of sizes ≥ 150 bp (Fig. 1A). The blank negative control, extracted using the same procedure, yielded no detectable DNA. The quantity of DNA recovered from the wine lees is an order of magnitude greater than that obtained from other ancient soft tissue samples (Pääbo 1989). Part of this high yield we attribute to the large number of dead yeast cells present in the lees. Yeast cells and debris typically make up about half of wine lees. Six liters of wine, approximately the volume of a Scorpion I jar, would contain as much as 12 mg of yeast DNA. A second reason for the high yield we attribute to the conditions of preservation. Because the tomb of Scorpion I was 3 m deep in desert sand, and was covered over with a roof and more sand in an extremely dry climate, the conditions for long-term organic preservation were excellent.

The extracted DNA was used as a template for PCR amplification using primers ITS1 and ITS4, which amplify a region of ~ 840 bp across the genomic region between the 18S and the 28S ribosomal RNA genes that includes ITS1 (internal transcribed spacer 1), 5.8S ribosomal DNA, and ITS2 (internal transcribed spacer 2). The ITS1 and ITS2 spacer regions differ among species of yeast and have recently been used to assess the phylogenetic relationship of strains belonging to the genus *Saccharomyces* and to assign strains to three closely related species of the

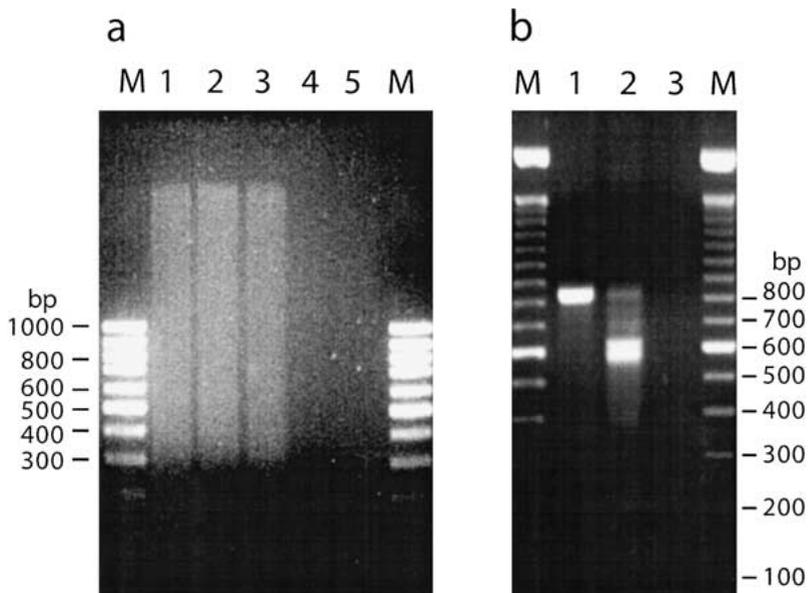


Fig. 1. Analysis of ancient DNA from Scorpion I wine jar residues. **A** Three aliquots of finely ground 500-mg samples were extracted and run on a gel. The DNA recovered is shown in lanes 1–3. A blank negative control, extracted using the same procedure, was run in lane 4. Lane 5 is empty. The molecular weight marker (Gene Ruler 100-bp DNA ladder, M-Medical Genenco, Florence, Italy) is shown in the M lanes. **B** The extracted DNA was replicated by PCR with primers ITS1 and ITS4 (lane 2); lane 1, 5 μ l of negative control + 50 ng of *S. cerevisiae* genomic DNA; lane 2, 5 μ l of amplified ancient DNA sample; lane 3, 5 μ l of negative control. A molecular weight marker with bands at 100-bp intervals from 100 to 800 bp (Invitrogen, Carlsbad, CA) is shown in the M lanes.

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T2709 AAATGAAGCCGGGAAACCGGTCCTTCTAACCCCTTGATTATCTTAATTTGTTGCTTTGGTG
Ab540 .....C.....A..C.C.....C.

T2709 GGCCGCGCAAGCACCGGCTTCGGCTGGATTGTGCCCGCCAGAGGACCACAACCTCTGTATT
Ab540 .....A...G.G....CAC...C..GA.....A.

T2709 AAATGTCGTCTGAGTACTATAAAATAGTTAAAACCTTTCACAACGGATCTCTTGGTTCTG
Ab540 .....T.....

T2709 GCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAAT
Ab540 .....T.....

T2709 CATCGAATCTTTGAACGCACATTGCGCCCTCTGGTATTCCGGAGGGCATGCCTGTTTCGAG
Ab540 .....

T2709 CGTCATAATGACCAAATCACCCCGTGGTGGACTTGGAGTTGG-CTGTACTAGCCTCTCT
Ab540 .....T..A.....C...-T.G.AA.....G.N...C.....C..

T2709 TAAAATCAGTGGCGGTGCTCTTAAAGCTCTACGCGTAGTA-ATTTTCTCGCTACAGGGTC
Ab540 ..N.....-N.C.....AT.....TC.....C..C.

T2709 TTGATTGTAAGTCCCAACACCCCAACTTATCATAAGGTTGACCTCGGATCAGGTAGGGA
Ab540 .A.G.G..G.....G.....TTC.C.....N..A....A..N...

T2709 TACCCGCTGAACCTTAA
Ab540 .....

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Fig. 2. BLAST alignment of the 540-bp fungal sequence from the Scorpion I wine jar from ancient Abydos (Ab540) compared with fungal clone T2709 (GenBank accession number X88771) from the Iceman's clothing. The sequence comprises 151 bp of ITS1, 157 bp of 5.8S rDNA, and 187 bp of ITS2.

genus: *S. cerevisiae*, *S. bayanus* (= *S. uvarum*), and *S. paradoxus* (= *S. douglasii*) (Montrocher et al. 1998).

Using the primer pair ITS1–ITS4, we obtained products of 540, 580, and 840 bp (Fig. 1B). These bands were purified from the gels and sequenced. A BLAST search (Altschul et al. 1990) of GenBank using the 540-bp sequence revealed a close match with that of an ITS1–5.8S rRNA–ITS2 sequence from fungal clone T2709 (Rollo et al. 1995) recovered from the clothing of The Iceman “Ötzi” discovered near the Hauslabjoch formation in the Ötztal Alps in 1991, and dated to 3350–3300 B.C. The alignment

between T2709 (GenBank accession number X88771) and our Abydos 540-bp product (Ab540) is shown in Fig. 2. Across 496 bp there is 90% identity between the sequences. The sequence of the 580-bp band was less interesting but was similar to those of ITS1–5.8S rRNA–ITS2 sequences from the fungi *Ampelomyces humuli* and *Phoma glomerata* (data not shown).

The observation that the 840-bp PCR product was faint (Fig. 1B) could be due to competition of the mold DNA sequences for the primers or to the rarity of long molecules of DNA in the ancient samples that are sufficiently undamaged by interstrand cross-links,

depurination, or other lesions to still support PCR amplification. The 840-bp fragment corresponds in size to the region encompassing *S. cerevisiae* chromosome 12 coordinates 455966–455123 comprising 394 bp of ITS1, 158 bp of 5.8S rDNA, and 288 bp of ITS2. To confirm the *Saccharomyces* origin of the 840-bp product, we designed new primers ITS5 and ITS6, internal to the 840-bp *S. cerevisiae* ITS1–5.8S rDNA–ITS2 sequence, which would specifically amplify sequences belonging to the genus *Saccharomyces* (Montrocher et al. 1998). Amplifications of the extracted DNA with the primer pair ITS1–ITS6 produced a band of ~320 bp, amplification with ITS5–ITS4 produced a band of ~650 bp, and amplification with ITS5–ITS6 produced a band of ~130 bp. These products correspond to overlapping regions within the 840-bp product and are of the predicted size. The bands were also more intense than the 840-bp band, suggesting that most of the molecules in the extracted DNA able to support amplification are smaller than 800 bp.

The bands of 840, 320, 130, and 650 bp were extracted from the gels and used to obtain a consensus sequence across the 840-bp region. A BLAST search of GenBank revealed that the 840-bp sequence most closely matches the sequence from modern *S. cerevisiae* (Fig. 3). This sequence has no homology with sequences from molds, and it shows no significant alignment longer than 100 bp with sequences of yeasts and other fungi outside of the genus *Saccharomyces*. The 748-bp region shown in Fig. 3 corresponds to *S. cerevisiae* chromosome 12 coordinates 455928–455181. The sequences denoted Sc, Sp, and Sb are from modern *S. cerevisiae*, *S. paradoxus*, and *S. bayanus*, respectively, and the nucleotides in boldface correspond to the 5.8S rDNA. The sequence denoted AW (ancient wine) is that from the 5000-year-old wine jar. Compared with the *S. bayanus* sequence, AW shows 24 nucleotide mismatches including 5 insertions/deletions of 1–2 bp. Compared with the *S. paradoxus* sequence, AW shows 10 nucleotide mismatches including 5 insertions/deletions of 1 bp. On the other hand, there are only four nucleotide mismatches with the sequence from *S. cerevisiae*, and the alignment requires no insertions/deletions. The sequences in Fig. 3 also include five phylogenetically informative sites in which the sequences of *S. paradoxus* and *S. bayanus* are identical but differ from that of *S. cerevisiae*, and these include two single-nucleotide insertions/deletions. At all five sites the ancient wine sequence AW matches that of *S. cerevisiae*.

The similarity between the AW sequence and modern *S. cerevisiae* sequences is illustrated in the unrooted maximum parsimony dendrogram in Fig. 4. In addition to AW, the sequences analyzed were *S. cerevisiae* strains CBS1171, HA6, CBS459,

CBS400, and CBS382; *S. paradoxus* strains CBS406 and CBS432; and *S. bayanus* strains CBS1513, CBS424, CBS380, IFO1127, and CBS1538. (See the legend to Fig. 4 for GenBank accession numbers.) The sequence comparisons were generated by ClustalX (Thompson et al. 1997), alignments of the ribosomal DNA region encompassing the region of ITS1–5.8S–ITS2 shown in Fig. 3. The number of bootstrap samples among 1000 replicates that support each node in the dendrogram is indicated. The clustering of the yeast strains of the three species is in agreement with that of Montrocher et al. (1998) and Oda et al. (1997). The results in Fig. 4 are also consistent with a dendrogram generated by neighbor joining (Saitou and Nei 1987).

The strong bootstrap support for the clusters in Fig. 4 implies that, based on the ITS1–5.8S–ITS2 sequence, the sequence AW should be regarded as coming from an ancient strain affiliated with modern *S. cerevisiae*. Hence we implicate *S. cerevisiae* in the production of the wine buried with Scorpion I.

Ancient DNA: Preservation and Possible Contamination

One of the major problems in acquiring information from archeological samples is to prevent contamination from modern samples. As stressed under Materials and Methods, all of the experiments were performed with the necessary precautions to prevent any modern contamination, in accordance with the suggestions recently summarized by Hofreiter et al. (2001). We also carried out three independent extractions and two independent sets of PCR amplifications with the appropriate negative and positive controls. Based on the reproducibility and consistency of the results, we believe that contamination with modern yeast can be ruled out.

But if modern laboratory contamination can be ruled out, how likely is other contamination, either during the postburial period of 5000 years or when the archaeologists collected the samples, admittedly without sterile gloves and masks? Postburial contamination seems unlikely in view of the large quantity of DNA extracted from the ancient Abydos samples. One would not expect contamination by stray microorganisms to yield an amount of DNA which, when extracted, could readily be visualized in a gel (Fig. 1A). A single Scorpion I jar would contain as much as 12 mg of yeast DNA, of which more than 7% consists of the ribosomal DNA repeats. We also attribute the large amount and relatively good quality of the extracted DNA to the exceptional environment in which the samples were preserved. The residues were inside closed pottery containers in an essentially zero-humidity, desert environment. The recovery of

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Sc ATTTAATAATTTTGAAAATGGATTTTTTTTGTTTTGGCAAGAGCATGAGAGCTTTTACTGG
AW .....T.....
Sp .....G.....
Sb .....T.T.....G.....

Sc GCAAGAAGACAAGAGATGGAGAGTCCAGCCGGGCTGCGCTTAAGTGC GCGGTCTTGCTA
AW .....
Sp .....T.....T
Sb .....

Sc GGCTTGTAAGTTTCTTTCTTGCTATTCCAAACGGTGAGAGATTTCTGTGCTTTTGTATA
AW .....A.C.....
Sp .....A.....
Sb .....C.....A.....C--.....C.T.....

Sc GGACAATTAAAACCGTTTCAATACAACACACTGTGGAGTTTTTCATATCTTTGCAACTTTT
AW .....
Sp .....
Sb .....T..CT.....

Sc TCTTTGGGCATTCGAGCAATCGGGGCCAGAGGTAACAAACACAAACAATTTTATTTATT
AW .....C.....
Sp .....A.....
Sb .....T-.....A.....

Sc CATTAAATTTTGTCAAAAACAAGAATTTTCGTAAGTGGAAATTTTAAAA-TA-TTAAAA
AW .....
Sp .....A.....
Sb .....A.A.....

Sc ACTTTCAACAACGGATCTCTTGGTTCTCGCATCGATGAAGAACGCAGCGAAATGCGATAC
AW .....
Sp .....
Sb .....

Sc GTAATGTGAATTGCAGAATTCGGTGAATCATCGAATCTTTGAACGCACATTGCGCCCCTT
AW .....
Sp .....
Sb .....

Sc GGTATTCAGGGGGCATGCCTGTTTGAGCGTCATTTCTTCTCAAACATTCTGTTTGTA
AW .....
Sp .....
Sb .....

Sc GTGAGTGATACTCTTTGGAGTTAACTTGAAATGCTGGCCTTTTCATTGGATGTTTTTTT
AW .....
Sp .....
Sb .....C.....

Sc TCCAAAGAGAGGTTTCTCTGCGTGCTTGAGGTATAATGCAAGTACGGTCGTTTTAGGTTT
AW .....
Sp .....
Sb .....YY.....

Sc TACCAACTGCGGCTAATCTTTTTTTATACTGAGCGTATTGGAACGTTATCGATAAGAAGA
AW .....
Sp .....G.....
Sb .....G.....A.....

Sc GAGCGTCTAGGCGAACAATGTT-CTTAAAGT
AW .....
Sp .....T.....
Sb .....

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Fig. 3. ClustalX alignment of the ribosomal DNA region encompassing ITS1–5.8S–ITS2 of the ancient wine sequence (AW) and the corresponding sequences of *S. cerevisiae* strain S288c (Sc), *S. paradoxus* strain CBS432 (Sp), and *S. bayanus* strain CBS380 (Sb). The region shown corresponds to *S. cerevisiae* chromosome 12 coordinates 455928–455181.

whole dried figs and raisins from some of the jars indicates how extraordinary the preservation conditions were inside the tomb. The presence of these

fruits also indicates that there was relatively little activity by microorganisms once the air and water present at the time of burial had been consumed.

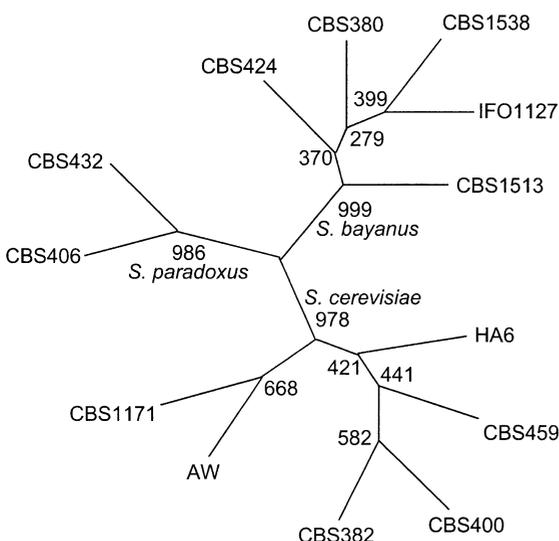


Fig. 4. Unrooted dendrogram based on maximum parsimony, with bootstrap scores based on 1000 replications indicated. In addition to the ancient wine sequence (AW), the other sequences are *S. cerevisiae* strains CBS1171 (GenBank accession number D89886), HA6 (U90327), CBS459 (Z95938), CBS400 (Z95939), and CBS382 (Z95936); *S. paradoxus* strains CBS406 (D89890) and CBS432 (Z95933); and *S. bayanus* strains CBS1513 (Z95949), CBS424 (Z95947), CBS380 (Z95945), IFO1127 (D89887), and CBS1538 (D89888).

Contamination by the archaeologists is also very unlikely, because *S. cerevisiae* is not airborne and is rare in nature, particularly in desert environments. Even grape skins rarely harbor *S. cerevisiae* cells unless broken to release the juice (Mortimer and Polsinelli 1999). Moreover, since wine yeasts do not live on human skin, the probability that the archaeologists transferred modern *S. cerevisiae* to the ancient samples is very low.

The ancient ITS1–5.8S–ITS2 sequence shown in Fig. 3 (AW) is very close to that of the modern *S. cerevisiae* strain S288c used for sequencing, and it clusters along with the sequences from other modern *S. cerevisiae* (Fig. 4). The close similarity to modern strains seems reasonable because, over 5000 years, one would not expect a large accumulation of changes in the 100–200 tandemly arrayed copies of the 9.1-kb repeating unit that encodes the 18S, 5.8S, and 25S rRNAs. Militating against rapid sequence evolution is the fact that in nature *S. cerevisiae* is typically a diploid organism, and it has a highly efficient DNA repair system in which a high rate of gene conversion and homologous recombination is likely to retard sequence evolution. In this context it should also be noted that there is a very close match between the mitochondrial DNA (mtDNA) sequences of 191 contemporary horses and those of 8 horses 12,000–18,000 years old as well as those of 8 horses 1000–2000 years old (Vila et al. 2001).

As an additional indication that the DNA extracted from the Abydos jars derived from ancient

organisms present at or introduced shortly after the time of burial, we note the striking similarity between the 540-bp fungal sequence in the Abydos samples and that recovered from the remains of the clothing of the 5000-year-old Iceman Ötzi discovered in the Ötztal Alps in 1991. This sequence has no significant similarities longer than 300 bp with sequences from modern molds. It seems likely that the fungal organism from which the 540-bp sequence derives, as well as the organism yielding the 580-bp sequence affiliated with the fungi *Ampeyomyces humuli* and *Phoma glomerata*, was present in the wine at the time of burial. At the beginning of natural fermentation, *S. cerevisiae* is usually in the minority. Later in the process, the fermentation of sugars by *S. cerevisiae* produces alcohol that, when the amount exceeds about 5% by volume, kills most of the other yeasts and molds. Under prolonged storage, however, the surviving molds can proliferate and produce repulsive odors and distasteful flavors.

Discussion and Conclusions

The finding that the lees inside a ~5000-year-old wine jar contain DNA from an organism that can confidently be assigned to *Saccharomyces cerevisiae* has important implications for the evolution of wine, bread, and beer yeasts, as well as for the emergence of long-term human settlement and civilization based on fermented grain and fruit staples as early as 8500 B.C. (McGovern et al. 1995; McGovern 2003). These results provide the oldest evidence of an association of a microorganism of the *S. cerevisiae* species with human activities. Although the chemical and archaeological evidence was compatible with the interpretation that the Scorpion I jar contained a fermented beverage—namely, grape wine—until the present study was carried out, it could not be established definitively what organism was responsible for the fermentation.

The Neolithic period, from about 8500 to 4000 B.C., is the first time in human prehistory when the necessary preconditions came together for the momentous innovation of viticulture, encompassing a knowledge of viticulture, winemaking, and fermentation along with methods for storage. Numerous year-round villages had been established by this time in the Near East, especially in upland regions bordering the “Fertile Crescent”—the foothills of the Zagros Mountains bordering the Tigris and Euphrates Rivers on the east, Transcaucasia to the north, and the upland plateaus descending from the Taurus Mountains in eastern Turkey. Using a variety of food processing techniques—in particular, fermentation, but also soaking, heating, and spic-

ing—Neolithic peoples produced the first wine, bread, and beer as well as an array of meat and grain entrées that we continue to enjoy today.

In trial-and-error fashion, it must have dawned upon the ancient beverage-maker that by adding grapes to any mixture of other less-sweet ingredients—berries, barley malt, wheat—the prospects of producing a fermented beverage were improved. It is even possible that the frothy yeast that bubbled on the surface of a mixed beverage of grapes and other materials was skimmed off and used in later fermentations. Gradually, in this seemingly nonhygienic, experimental setting, a single yeast species came to predominate and was shared by the whole range of fermented beverages: *S. cerevisiae*.

S. cerevisiae is also the bread and beer yeast. Because neither the organism nor its spores are airborne, a practical understanding of the use of *S. cerevisiae* in fermenting grapes, as well as dates, figs, or honey, probably preceded its use for grain-based foods and beverages. Ancient literary evidence implies that grapes generally provided the yeast for making barley beer (Civil 1964). It is thus likely that wine yeasts, which occur naturally in damaged grapes (Mortimer and Polsinelli 1999), were used to ferment other cereal products and, over centuries of human selection, this yeast eventually evolved into specific strains that became the fermenting agents of choice for human food and drink.

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