J Ind Microbiol Biotechnol (2003) 30: 175–182
DOI 10.1007/s10295-003-0028-2

ORIGINAL PAPER

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Degradation of DNA during the autolysis of *Saccharomyces cerevisiae*

Received: 6 May 2002 / Accepted: 5 January 2003 / Published online: 22 February 2003
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Abstract The autolysis of yeast cells has practical implications in the production of fermented foods and beverages and flavourants for food processing. Protein and RNA degradation during yeast autolysis are well described but the fate of DNA is unclear. Yeast cells (*Saccharomyces cerevisiae*) were autolysed by incubating suspensions at 30–60°C (pH 7.0), and at pH 4.0–7.0 (40°C) for 10–14 days. Up to 55% of total DNA was degraded, with consequent leakage into the extracellular environment of mainly 3′- and 5′-deoxyribonucleotides, and lesser amounts of polynucleotides. The rate and extent of DNA degradation, composition of the DNA degradation products and DNase activity were affected by temperature and pH. The highest amount of DNA degradation occurred at 40°C and pH 7.0, where the highest DNase activity was recorded. DNase activity was lowest at 60°C and pH 4.0, where the proportion of polynucleotides in the degradation products was higher.

Keywords Autolysis · Yeasts · *Saccharomyces cerevisiae* · DNA degradation

Introduction

Autolysis of yeast cells occurs after they have completed their life cycle and entered the death phase. It is characterized by a loss of cell membrane permeability, alteration of cell wall porosity, hydrolysis of cellular macromolecules by endogenous enzymes, and subsequent leakage of the breakdown products into the extracellular environment. Although a naturally occurring event, autolysis can be initiated by exposing yeasts to elevated temperatures (40–60°C), organic solvents or detergents [2]. The initiation of yeast autolysis and the chemical and cytological changes that occur during this reaction have been reviewed [2,3,5,6,9,12]. Most studies, to date, have focussed on autolysis of *Saccharomyces cerevisiae*.

Yeast autolysis occurs in many foods and beverages, where it may affect their sensory quality and commercial acceptability. Autolysis of yeasts during the maturation and ageing of beer [21] and sparkling wines [5,19,20,31] causes the release of amino acids, proteins, nucleic acid degradation products, free fatty acids and polysaccharides that affect the sensory properties of these products, and serve as nutrients for the growth of bacterial contaminants. Autolysis of baker's yeast during storage reduces its fermentative ability [29,30]. Yeast autolysates are used in the food industry as flavour enhancers [7,27], where key components include nucleic acid degradation products such as 5′-guanosine monophosphate (5′-GMP) [23, 24].

Despite the commercial significance of yeast autolysis, the biochemical changes that occur during this process are not completely understood. Degradation of proteins has received the most study, but changes to other cell components such as nucleic acids have been examined to a lesser extent. Yeast cells contain 5–15% RNA and 0.1–1.5% DNA on a cell dry weight basis [17]. Early studies on yeast autolysis reported the solubilisation of phosphorus and concluded that this originated from the degradation of nucleic acids [15,16]. Subsequent studies showed that RNA was extensively degraded by RNase action during yeast autolysis [12,17,32]. However, the fate of DNA during yeast autolysis is not clear. Hough and Maddox [14] and Suomalainen [29] reported almost complete degradation of DNA during the autolysis of brewing and baking

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strains of *S. cerevisiae*, respectively. In contrast, Trevelyan [33] found that the DNA content of baker's yeast did not decrease during autolysis. Recently, we showed that about 40% of the cellular DNA was lost during the autolysis of *S. cerevisiae* and *Candida stellata*, and only about 25% was lost during autolysis of *Kloeckera apiculata* [13]. The association of DNase activity with yeast autolysis and the products of autolytic degradation of DNA have not been reported.

Here, we report the degradation of DNA, changes in DNase activity and the DNA degradation products that are produced during the autolysis of *S. cerevisiae*. The effects of pH and temperature on the autolytic degradation of DNA are described.

Materials and methods

Yeast strain, cultivation and autolysis

S. cerevisiae (haploid strain ×2180a) was obtained from the H.J. Phaff culture collection, Department of Food Science and Technology, University of California (Davis, Calif.). The yeast was maintained on malt extract agar (Oxoid, CM59, Melbourne, Australia). Cells for autolysis experiments were grown in 5% glucose-0.5% yeast extract broth. Inoculum (100 ml) was grown at 25°C for 24 h, then inoculated into the experimental culture (1 l in a 2 l conical flask), which was incubated at 25°C for 48 h (end of exponential growth) with orbital shaking (200 rpm). Cells were harvested by centrifugation at 5,000 *g* for 10 min at 4°C, washed three times with sterile 0.2 M phosphate-citric acid buffer, pH 7.0, and resuspended in the same buffer at either pH 4.0, 5.0, 6.0 or 7.0 to give suspensions containing 10^8–10^9 cells/ml, as determined microscopically. Biomass concentration in each suspension was determined by dry weight measurement. To initiate autolysis, cell suspensions were incubated at either 30°C, 40°C, 50°C or 60°C for up to 14 days with orbital shaking (200 rpm). Samples (50 ml) of the autolysing suspensions were withdrawn at intervals and separated by centrifugation (5,000 *g* for 5 min at 4°C) into cell pellet and autolysate (supernatant) fractions. The autolysate fraction was filtered through a 0.45 μ pore size membrane (Millipore, Bedford, Mass.). Both fractions were analysed for DNA and DNase activities. Autolysate fractions were also analysed for deoxyribonucleotides, deoxyribonucleosides and nucleic acid bases. Three independent experiments were run for each autolytic condition. The results of typical experiments are presented. Aseptic conditions were maintained throughout the experiments and autolysing suspensions were checked by agar culture to ensure freedom from bacterial contamination.

Cell dry weight and viability

Cell suspensions (1.0 ml) were centrifuged at 5,000 *g* for 5 min to sediment the cells, which were then washed twice with 5 ml sterile distilled water and finally resuspended in 1.0 ml distilled water. The suspension was poured onto a pre-weighed membrane of 0.45 μ pore size and dried at 60°C in a vacuum oven to constant weight [29]. The viability of cells in autolysing suspensions was determined by spread inoculating 0.1 ml samples over the surface of plates of malt extract agar. The plates were incubated at 25°C for 3 days, after which colonies were counted.

DNA and DNase assays

DNA and DNase activity were measured by the fluorometric method described by Labarca and Paigen [18] using the fluorescence dye, bisbenzimidazole (Hoechst 33258). DNA standard (0–5 μg/ml) was prepared from salmon sperm DNA (Boehringer-Mannheim, Germany). To measure DNA in the yeast cells, 5 ml cell suspension was centrifuged and the sediment was washed (as described previously), resuspended in 5 ml phosphate-buffered saline (PBS), pH 7.3 (Oxoid, BR14a) and mixed with 5 g glass beads (0.45 mm diameter). The mixture was homogenized with cooling for 2 min in a Braun homogenizer (Braun, Melsungen, Germany) to break the cells (> 95% disruption) and centrifuged at 5,000 *g* for 5 min at 4°C to sediment the glass beads and cell debris. The cell homogenate (1 ml) was pipetted out and mixed with 4 ml PBS (pH 7.3). The diluted homogenate (0.5 ml) was mixed with 4 ml of the same buffer and 0.5 ml of Hoechst 33258 dye solution (10 μg/ml in the same buffer). The fluorescence intensity of the mixture was measured in a Toshiba fluorescence spectrophotometer with an excitation wavelength of 334 nm and an emission wavelength of 475 nm. DNA concentration in autolysates was measured in the same way except that the cell homogenate was substituted by the autolysate. In separate experiments, it was determined that no significant degradation of DNA occurred during the period between cell disruption and DNA assay. In these experiments, 50 mM EDTA was included in the homogenizing buffer to inhibit DNases.

Intracellular DNase activity was measured by mixing the supernatant of the cell homogenate (0.5 ml) with 0.5 ml 10 mM $MnCl_2$ and 0.5 ml salmon sperm DNA solution (50 μg/ml in PBS, pH 7.0), incubating the mixture at 37°C for 60 min, then terminating the reaction with the addition of 0.5 ml 50 mM EDTA. The fluorescence intensity of the mixture was measured and compared with controls, which were similarly treated except that the substrate and enzyme solutions were incubated separately. The difference in fluorescence readings between the control and the sample was due to degradation of DNA and was taken as the measurement of DNase activity. One unit of DNase activity was defined as the activity in the enzyme solution that gave a reduction in fluorescence intensity of 0.01 under the conditions described. DNase activity in the autolysates was measured by the same procedure.

All DNA and DNase assays were performed in triplicate and mean values were used. Standard deviation and coefficient of variation values for the DNA assays were of the order 0.02 and 4%, respectively. To enable comparisons between experiments, data for DNA content and DNase activity were calculated for a constant dry weight of cells.

Analysis of DNA degradation products

DNA degradation products in autolysates were separated and quantified by two HPLC procedures [35,36]. Briefly, nucleotides were separated by reversed-phase ion-pairing HPLC using a Waters μBONDAPAK C18 10 μm stainless steel column (30 cm×3.9 mm I.D.). Elution was performed with a gradient system consisting of a mixture of eluent A (50 mM $K_2H\ PO_4$, 5 mM Waters PIC A, pH 5.45 and eluent B (100% methanol). Elution was commenced with eluent A, with eluent B being increased from 0% at the start to 10% over 10 min. The injection volume was 20 μl and the elution rate was 1.5 ml/min. Detection was with a Waters (Model 440) absorbance detector at 254 nm and 280 nm.

Nucleosides and bases were separated by reversed-phase HPLC using a Waters Resolve C18 5 μm Radial-Pak cartridge (100 mm×8 mm I.D.) equipped with a Waters RCM-100 cartridge holder. Elution was performed with a gradient system consisting of eluent A (0.02 M K_2HPO_4, pH 6.30) and eluent B [60% methanol in water (v/v)]. Elution was commenced with eluent A and the amount of eluent B was increased from 0% at the start to 40% over 18 min. The injection volume was 20 μl and the elution rate was 3.0 ml/min. Detection was as described above.

Quantification of separated compounds was achieved by comparing their peak area with standard curves obtained by injection of individual standard nucleotides, nucleosides and bases (US Biochemical Corporation, Cleveland, Ohio) into the HPLC systems. Detection sensitivity was 0.1 μg/ml.

Protein assay

The concentration of protein in autolysates was determined by the Coomassie Brilliant Blue method using the Bio-Rad protein assay kit (Cat. no. 500-0002; Bio-Rad, Hercules, Calif.).

Results

Cell death and protein release

Autolysing cell suspensions were characterized by cell death and release of protein into the autolysates. At pH 7.0, these reactions occurred rapidly at 50°C and 60°C, but were more gradual at 30°C and 40°C (Fig. 1). For autolysis at 40°C, there was little difference in death kinetics at pH 4.0, 5.0, 6.0 or 7.0 (data not shown). Protein release was greatest at pH 5.0 and 7.0 (130–140 μg/ml at 10 days) and least at pH 4.0 (87 μg/ml at 10 days).

Changes in DNA content

The initial DNA content of yeast cells was about 0.4% of cell dry weight, and decreased by 35–55% after 10 days of autolysis (Fig. 2). Most DNA degradation occurred in the first 4–5 days. Cells autolysed at 40°C gave the greatest degradation while those autolysed at 50°C and 60°C gave least degradation (Fig. 2a). The degradation of DNA was only slightly affected by pH.

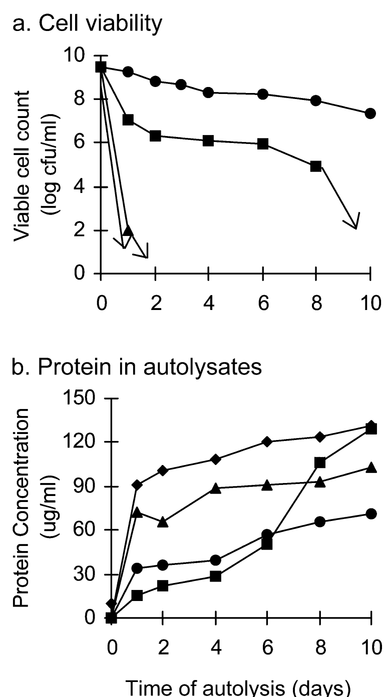
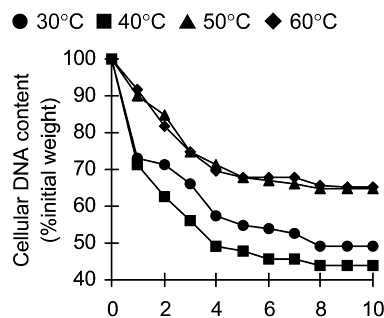


Fig. 1 Changes in cell viability (**a**) and protein content in autolysates (**b**) during autolysis of *Saccharomyces cerevisiae* at different temperatures and pH 7.0. • 30°C, ■ 40°C, ▲ 50°C, ◆ 60°C. *Arrows* denote counts less than 10 cfu/ml. Data were adjusted to the same initial cell concentration of 3.05×10^9/ml



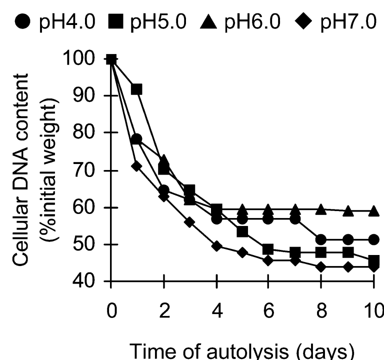


Fig. 2 Changes in cellular DNA content during autolysis of *S. cerevisiae* at different temperatures (**a**) and pH values (**b**)

After autolysis at 40°C for 10 days, the cellular content of DNA was about 60% of its initial weight at pH 6.0 and 45% at pH 5.0, with intermediate values at pH 4.0 and 7.0 (Fig. 2b).

Despite the loss of DNA in the cell, very little was detected in the autolysates (data not shown). Greatest recovery of DNA in the autolysates occurred when autolysis was conducted at 60°C, but this represented only 3–4% of the DNA initially present in the cell. Lesser values (about 2%) were found for autolysis at 30°C and 40°C. The release of DNA into the autolysates occurred mainly in the first 1–2 days.

Intracellular DNase activity

DNase activity increased during the initial stages of autolysis, then decreased to non-detectable levels. DNase activity was affected by autolysis temperature (Fig. 3). The highest activity was found in cells autolysed at 30°C and 40°C, where it increased by 4–5-fold during the first 3 days, then decreased to almost no activity by day 10. The lowest activity was observed during autolysis at 50°C and 60°C where, after a small 2-fold increase during the first 2 days, it decreased to non-detectable levels.

Intracellular DNase activity was also influenced by pH (Fig. 4). The initial increase in DNase activity was least at pH 4.0, and decreased to non-detectable levels

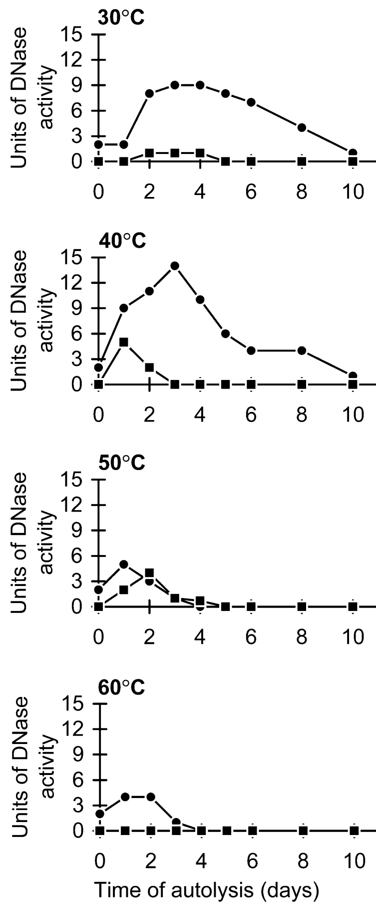


Fig. 3 Effect of temperature on DNase activity during autolysis of *S. cerevisiae*. • DNase activity within cells, ■ DNase activity in autolysates. Autolysis was conducted at pH 7.0

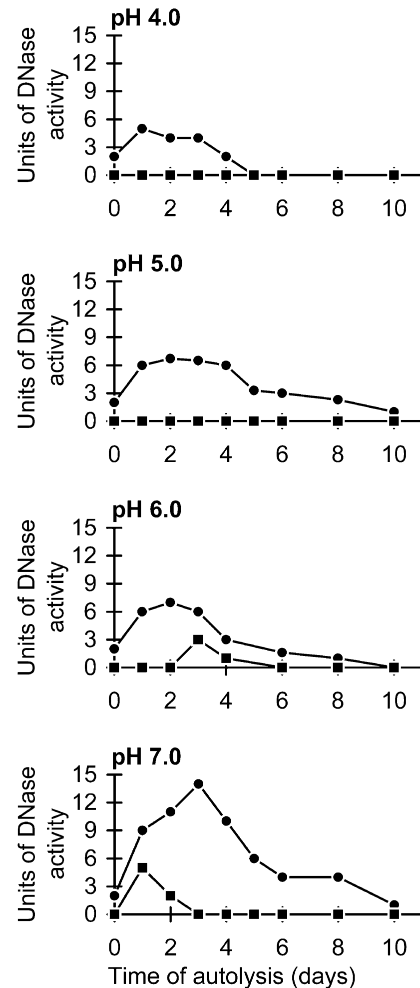


Fig. 4 Effect of pH on DNase activity during autolysis of *S. cerevisiae*. • DNase activity within cells, ■ DNase activity in autolysates. Autolysis was conducted at 40°C

by day 5 (Fig. 4). The highest activity was found at pH 7.0, but this also decreased to low levels by day 10. DNase activities for autolysis at pH 5.0 and 6.0 were only slightly higher than that at pH 4.0, but low levels were still detectable after 8–10 days.

DNase activity in autolysates

DNase activity was detected in autolysates depending on temperature and pH (Figs. 3, 4). No DNase activity was found in autolysates when autolysis was conducted at 60°C, or at pH 4.0 or 5.0. For other conditions, the activity increased during the first 1–3 days, then rapidly decreased to non-detectable values. For any conditions, no activity was observed in autolysates after 5 days. Highest activity was found in autolysates produced at 40°C or 50°C, and pH 7.0 or 6.0.

DNA degradation products in autolysates

The HPLC methods [35,36] gave separation and quantitation of the eight deoxyribonucleotides, four deoxyribonucleosides and four nucleobases produced from DNA degradation. Figure 5 shows the profiles of these products in autolysates produced at 50°C and pH 7.0. Data for the bases adenine, cytosine and guanine are not presented since the greater proportion of these compounds arise from RNA degradation. The concentrations of most products increased rapidly during the first 1–5 days, generally reaching a maximum level by day 10. Occasionally (where noted), the concentration of some products decreased during the later stages of autolysis.

Tables 1 and 2 show the general composition of autolysates after 1 and 10 days. Deoxyribonucleotides were the most predominant components for all autolytic conditions, and accounted for 53–92% of the products. Lower temperatures (30–40°C) and higher pH values favoured the formation of deoxyribonucleotides, while higher temperatures (50–60°C) and lower pH (pH 4.7) gave increased proportions of polynucleotides.

Only small amounts of deoxyribonucleosides were observed in the autolysates, with the highest proportions (15–30%) being produced at 50–60°C. Thymine was not

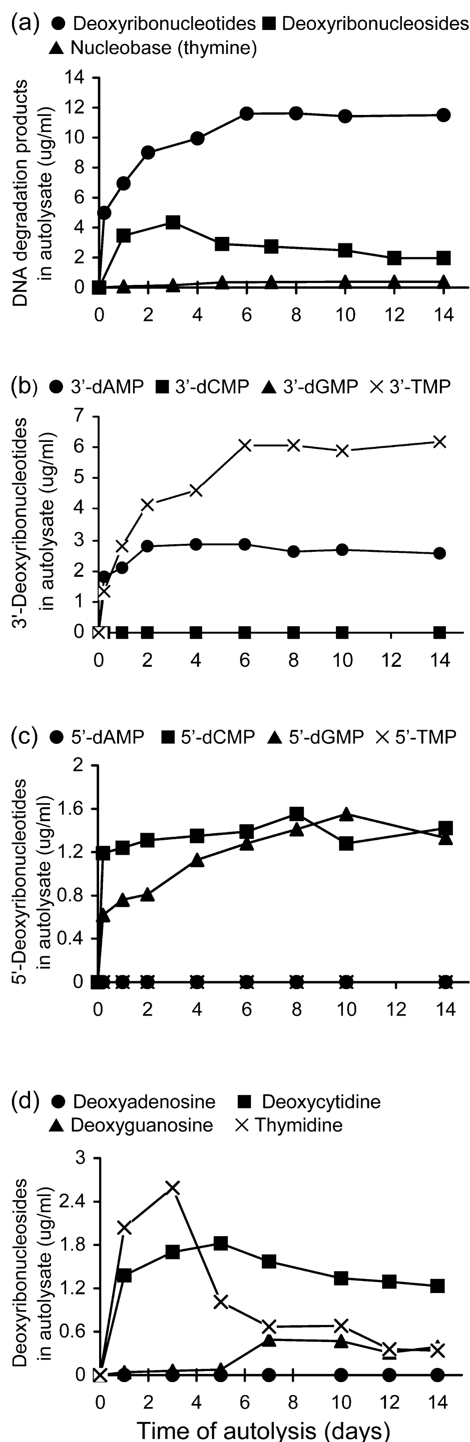


Fig. 5 Changes in the concentration of DNA degradation products in the autolysates of *S. cerevisiae* during autolysis at 50°C, pH 7.0; 4.2 mg/ml cells (dry weight) was used for autolysis

detected, except in autolysates produced at 50°C and pH 7.0, where trace amounts were found (Table 1, Fig. 5). Its increase in concentration corresponded to a decrease in the concentration of thymidine.

Tables 3 and 4 show the composition of deoxyribonucleotides in the autolysates after 1 and 10 days. The proportions of individual 3′- and 5′-deoxyribonucleotides varied with the conditions of autolysis. At 30°C (pH 7.0), 3′-dAMP and 3′-dCMP were predominant, while at 40°C, no 3′-deoxyribonucleotides were detected and 5′-TMP was the most obvious product (Table 3). At 50°C, 3′-TMP and 3′-dAMP accounted for 68–75% of the total deoxyribonucleotides. At 60°C, 3′-dAMP and 5′-TMP were most evident.

At pH 4.0 (40°C), the main products were 3′-dAMP, 3′-dCMP, 5′-dCMP, 5′-dGMP and 5′-TMP, with the 5′ isomers being present in greater amounts at the earlier stages of autolysis (Tables 4). At pH 5.0, there were approximately equal amounts of 3′-dCMP, 3′-dGMP, 5′-dAMP, 5′-dCMP, 5′-dGMP and 5′-TMP. At pH 6.0, 3′-dAMP, 3′-dCMP and 5′-TMP were mainly present. No 3′-deoxyribonucleotides were detected for autolysis at pH 7.0 (40°C) where 5′-TMP was predominant.

Of the deoxyribonucleosides (data not shown), deoxycitidine was the most prevalent and represented approximately 80% of the total deoxyribonucleosides found, except for autolysis conducted at pH 4.0, 40°C and pH 7.0, 50°C where the proportion was about 55%. At pH 4.0 and 40°C, deoxyguanosine was also present at approximately 50% and, at pH 7.0 and 50°C, thymidine represented 50% of these products.

Discussion

As reported elsewhere [2,3,12,19,20] and confirmed by us, cell death and protein release are key reactions of yeast autolysis that are enhanced as the temperature is increased to 60°C. However, unlike protein and RNA degradation, DNA was only partly degraded during autolysis of *S. cerevisiae*. Its degradation correlated with the occurrence of DNase activity. Most degradation (55%) occurred during autolysis at 40°C, pH 7.0, where the highest activity for intracellular DNase was observed. Less DNA degradation occurred for autolysis at the higher temperature (60°C) and lower pH values, where less DNase activity was found. These findings are consistent with our previous study [13] where we used the diphenylamine method to determine the concentration of DNA. They also agree with reports that autolysed yeasts always contain detectable amounts of DNA [32,33]. The complexing of DNA with proteins [34] could affect their susceptibility to hydrolysis by DNases, and might explain why partial degradation is observed. Also, DNase activity was not stable (Figs. 3, 4), and this may account for the incomplete DNA degradation. We also observed incomplete degradation of cell DNA in other experiments in which pulsed-field gel electrophoresis was used to monitor changes in DNA during autolysis (data not shown). The intensity of the chromosomal DNA bands in the gels decreased during autolysis. While many large-sized bands (greater than 680 kb) had disappeared by day 10, smaller-sized bands were still present.

Table 1 Composition of DNA degradation products in autolysates of *Saccharomyces cerevisiae* after autolysis for 1 and 10 days at different temperatures at pH 7.0

Degradation product	Composition (% of total degradation products)								Max. SEM[d]
	30°C		40°C		50°C		60°C		
	Day 1	Day 10	Day 1	Day 10	Day 1	Day 10	Day 1	Day 10	
Deoxyribonucleotides[a]	92	88	90	84	63	69	53	54	4.2
Deoxyribonucleosides[a]	N.D.[c]	7	4	7	31	15	16	13	1.2
Base (thymine)[a]	N.D.	N.D.	N.D.	N.D.	1	2	N.D.	N.D.	0.1
Polynucleotides[b]	8	5	6	9	5	14	31	33	1.0

[a]Nucleotides, nucleosides and bases are measured by HPLC [35,36]. The data were mean values of duplicate measurements
[b]Polynucleotides were measured by the fluorometric method of Labarca and Paigen [18]. The data were mean results of triplicate measurements
[c]Not detected
[d]Maximum standard error of means for data within the same row

Table 2 Composition of DNA degradation products in autolysates of *S. cerevisiae* after autolysis for 1 and 10 days at different pH values, 40°C

Degradation product	Composition (% of total degradation products)								Max. SEM[d]
	pH 4.0		pH 5.0		pH 6.0		pH 7.0		
	Day 1	Day 10	Day 1	Day 10	Day 1	Day 10	Day 1	Day 10	
Deoxyribonucleotides[a]	70	75	88	85	91	89	90	84	3.7
Deoxyribonucleosides[a]	7	3	6	4	1	3	4	7	0.4
Base (thymine)[a]	N.D.[c]	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	–[e]
Polynucleotides[b]	23	22	6	11	8	8	6	9	1.3

[a]Nucleotides, nucleosides and bases were measured by HPLC [35,36]. The data were mean values of duplicate measurements
[b]Polynucleotides were measured by the fluorometric method of Labarca and Paigen [18]. The data were mean results of triplicate measurements
[c]Not detected
[d]Maximum standard error of means for data within the same row
[e]Standard error of means could not be calculated

Table 3 Composition of deoxyribonucleotides in autolysates of *S. cerevisiae* after autolysis for 1 and 10 days at different temperatures, pH 7.0

Compound[a]	Composition (% of total deoxyribonucleotides)								Max. SEM[b]
	30°C		40°C		50°C		60°C		
	Day 1	Day 10	Day 1	Day 10	Day 1	Day 10	Day 1	Day 10	
Total 3′-deoxyribonucleotides	80	70	N.D.[c]	N.D.	68	75	32	52	4.0
3′-dAMP	60	32	N.D.	N.D.	23	23	32	52	3.7
3′-dCMP	20	38	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	2.6
3′-dGMP	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	–
3′-TMP	N.D.	N.D.	N.D.	N.D.	45	52	N.D.	N.D.	3.2
Total 5′-deoxyribonucleotides	20	30	100	100	32	25	68	48	4.5
5′-dAMP	N.D.	N.D.	17	13	N.D.	N.D.	10	N.D.	0.6
5′-dCMP	N.D.	N.D.	21	10	20	11	21	15	1.0
5′-dGMP	5	7	14	6	12	14	11	10	0.5
5′-TMP	15	23	48	71	N.D.	N.D.	26	23	3.1

[a]Nucleotides were measured by HPLC [35,36]. The data were mean values of duplicate measurements
[b]Maximum standard error of means for data within the same row
[c]Not detected

The fluorimetric-dye method for DNA measurement does not detect oligonucleotides, deoxyribonucleotides or deoxyribonucleosides since the dye binds only to relatively long segments of DNA [18]. With this method, only trace amounts of DNA were detected in the extracellular autolysates, suggesting that most of

Table 4 Composition of deoxyribonucleotides in autolysates of *S. cerevisiae* after autolysis for 1 and 10 days at different pH values, 40°C

Compound[a]	Composition (% of total deoxyribonucleotides)								Max. SEM[b]
	pH 4.0		pH 5.0		pH 6.0		pH 7.0		
	Day 1	Day 10	Day 1	Day 10	Day 1	Day 10	Day 1	Day 10	
Total 3′-deoxyribonucleotides	15	63	18	43	47	48	N.D.[c]	N.D.	3.2
3′-dAMP	15	37	N.D.	1	15	21	N.D.	N.D.	1.0
3′-dCMP	N.D.	26	18	21	32	26	N.D.	N.D.	1.1
3′-dGMP	N.D.	N.D.	N.D.	21	N.D.	N.D.	N.D.	N.D.	1.2
3′-TMP	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	–
Total 5′-deoxyribonucleotides	85	37	82	57	53	52	100	100	5.4
5′-dAMP	N.D.	N.D.	23	11	7	5	16	13	1.3
5′-dCMP	24	11	27	N.D.	10	10	21	10	1.0
5′-dGMP	15	6	19	27	9	N.D.	15	6	1.5
5′-TMP	46	20	13	19	27	37	48	71	2.1

[a]Nucleotides were measured by HPLC [35,36]. The data were mean values of duplicate measurements
[b]Maximum standard error of means for data within the same row
[c]Not detected

the degradation products were oligonucleotides, nucleotides and nucleosides—as confirmed by HPLC analysis. Autolysis at 60°C, and at pH 4.0 were the only conditions where large segments of DNA constituted a substantial proportion of the degradation products, and this corresponded to the conditions where DNase activity was lowest. Furthermore, these were the only autolytic conditions where we detected DNA fragments in autolysates by agarose gel electrophoresis (data not shown). These fragments had a molecular mass of about 10^6–10^7 Da and demonstrated that the functionality of the plasma membrane and the porosity of the cell wall had been altered to allow exit of such molecules.

Degradation of DNA involves the activity of several enzymes [1]. The main linkage hydrolysed is the phosphodiester bond between adjacent deoxyribose units. Endonucleases hydrolyse this bond to produce poly- and oligo-deoxyribonucleotides. Exonucleases hydrolyse this bond in a step-wise manner from one end of the polymer to produce mono-deoxyribonucleotides. For both types of enzymes, there are sub-classes that hydrolyse at either the 5′ or 3′ side of the phosphate, generating nucleotides with either a 3′- or a 5′-phosphate group, respectively. For the endonucleases, the base type may determine which phosphodiester linkage is cleaved. Phosphomonoesterases (nucleotidases with 3′ and 5′ specificity) act on the oligonucleotides and nucleotides to liberate phosphate and the nucleoside. Finally, nucleosidases hydrolyse the nucleosides to liberate nucleobases and deoxyribose [1,24]. As a consequence of the array of enzymes involved, there is likely to be substantial diversity in the products of DNA degradation, and this is reflected in the products shown in Tables 1, 2, 3 and 4.

The predominance of deoxyribonucleotides in the autolysates suggests that endonucleases and exonucleases were principally responsible for the degradation of DNA. However, at higher temperature (50–60°C) or lower pH (pH 4.0), increased concentrations of polynucleotides were found in autolysates, indicating a lesser contribution from exonucleases. As noted already, less total DNase activity was also observed at these conditions (Figs. 3, 4). Higher proportions of deoxyribonucleosides were found for autolysis at 50–60°C, suggesting that the nucleotidases may be more active at these temperatures. Nucleosidases appear not to be very active in DNA degradation since we could not detect thymine in the autolysates, except for autolysis at 50°C and pH 7.0, where only trace amounts were recovered.

The concentrations of individual deoxyribonucleotides in autolysates varied with the conditions and, presumably, reflect the relative activities of specific endonucleases and exonucleases. Conditions (e.g. 40°C, pH 7.0) that give a predominance of 5′-deoxyribonucleotides in the products indicate the action of endonucleases that generate 3′-hydroxyl end-groups, which, in turn, yield the 5′-deoxyribonucleotide end-product on cleavage by exonuclease. In contrast, conditions (e.g. 30°C, 50°C, pH 7.0) that give a predominance of 3′-deoxyribonucleotides suggest stronger activities of endonucleases that generate 5′-hydroxyl end groups. The fact that some deoxyribonucleotides were often found at higher concentrations than others (e.g. cf. 5′-TMP and 5′-dGMP) suggests enzyme specificities for particular bases. This conclusion might also apply to the activity of the nucleosidases, because deoxycytidine was the main component of the deoxyribonucleosides. There are numerous reports on the occurrence of nucleases in *S. cerevisiae*. Most of these enzymes are endonucleases that are located in the nucleus or mitochondria [see e.g. 8,10,11,26,28]. Their pH optima vary between pH 5.0 and 7.8 and some are unstable at 50°C [4]. Some have been reported to produce oligonucleotides with 5′-phosphate termini [22,25,28]. They mainly function in the repair and replication of DNA, but probably contribute to autolytic degradation of DNA when subcellular organisation has been disrupted.

In summary, cellular DNA is partially degraded during yeast autolysis and further study is needed to determine why some portion of the DNA is resistant to autolytic degradation. DNases are involved in the autolytic degradation and, judging from the diversity of the degradation products, a wide array of DNase, nucleotidase and nucleosidase enzymes are probably active. Further research is needed to determine the specific enzymes involved and factors that affect their relative activities. Such information would help to control the composition of nucleic acid degradation products in yeast autolysates as commercially produced for the food industry, although it is recognised that DNA represents only a small proportion of the cell dry weight.

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