Chem-Bio Informatics Journal, Vol. 6, No. 2, pp.29-46(2006)

The evaluation of environmental waters using yeast DNA microarray

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(Received September 1, 2005; accepted May 16, 2006; published online August 25, 2006)

Abstract

We propose a new method for the evaluation of environmental water by using DNA microarray technology. Twenty-one types of environmental waters were sampled from incineration processes, some factories, and various soils. We performed a comprehensive analysis by DNA microarray, and attempted to classify the environmental water samples by hierarchical cluster analysis. These water samples were classified into clusters from A to F together with chemicals and physical stress conditions. The water sample that grouped into cluster A caused protein denaturation and oxidative stress, but not mutagenesis. The water samples that grouped into cluster B caused protein denaturation. The water samples that grouped into cluster C caused mutagenesis, protein denaturation, and cell wall damage. The water samples that grouped into cluster D caused mutagenesis. The water samples that grouped into cluster E caused protein denaturation and oxidative stress. The water samples that grouped into cluster F caused oxidative stress. Consequently, the potential influences of environmental water could be estimated by hierarchical cluster analysis.

Key Words: DNA microarray, hierarchical cluster analysis, environmental water, yeast

Area of Interest: Genome Wide Expressional Data Analysis

1. Introduction

It is said that this century is the age for creating a safe environment. In our country, we are required to secure our surroundings, especially in the case of our immensely important water resources. Wastewaters from households, storm sewage, and local area businesses are processed in sewage-treatment plants, and the processed (cleaned) wastewater is returned to the environment as safe (cleaned) water. Recently, a new technology, termed bioechoengineering, efficiently utilizing the degradative abilities of microorganisms, has begun to be introduced. This bioechoengineering technology has maximized the characteristics of microorganisms for use in water purification and septic tanks that have advanced purification processing performance, have been developed for removing nitrogen and phosphate contained in the wastewater drainage. Echoengineering technology has been developed that uses the original purification ability of ecology, including purification techniques that utilize water-cultured plants, and marshlands. Lately, large amounts of pollutants arising from overpopulation, and improvements in the standard of living, are being released by the urban society. The amounts of these pollutants are threatening to exceed the processing ability of the sewage-treatment plants. Thus, it is necessary to check and diagnose whether the wastewater can be safely processed in these treatment plants. In "environmental sciences", a bioassay is defined as, "a method used to estimate chemical toxicities and monitor pollutants in the environment by using cultured cells or microorganisms" [1][2]. There are some bioassay parameters, such as the growth inhibition constant (IC50), the frequency of back mutation (*Ames* test), the inhibitory effect of enzyme activities, and the induction of stress protein after treatment [3]. We may predict the effects of new chemicals or environmental water from these parameters. Although these data provide information on the safety of newly synthesized chemicals or environmental water, they tell us little about the potential influences other than the toxicity of the chemicals and the pollutants [4]. We attempted to diagnose the influences of environmental waters that were sampled from incinerators, factories, and soil samples by DNA microarray.

A DNA microarray comprises several thousand to tens of thousands of DNA probes arranged on a glass slide, making it possible to simultaneously analyze gene expression on a very large-scale [5][6]. We can investigate the cellular responses of an organism at the transcriptional level by DNA microarray analysis. Hence, we can gain large amounts of transcriptional data that reflect the effects of a stress, such as environmental water, unlike those available by a bioassay [7][8][9]. We used *Saccharomyces cerevisiae*, budding yeast, for evaluation of environmental water samples, because yeast is more suitable than other organisms, such as *Dophnia pulex*, *Chlosterium*, and *Oryzias latipes* for the following reasons. 1. The evaluation of environmental water by yeast can be carried out more rapidly than that by other organisms, because the cell doubling time is about 2h when yeast is cultivated at 30˚C. 2. Only a small volume of environmental water is needed for evaluation. 3. The reproducibility of the evaluation by yeast is better than that by other organisms. 4. The procedure for using yeast to evaluate environmental water is easy. We sampled 21 types of environmental water obtained from an incinerator, factories, and reclaimed grounds, and exposed yeast cells to these environmental water samples. In this paper, we compared the transcriptional patterns of the genes involved in cell rescue, defense, and virulence in MIPS for yeast exposed to environmental water samples and other chemical or physical conditions, and attempted to estimate the influence of environmental waters.

2. Material and Methods

2.1 Strain and growth conditions

A total of 21 types of environmental waters were sampled: "A" were eluted from reclaimed grounds or soil samples, "B" were collected during the cleaning process of an incinerator plant, and "C" were collected as industrial waste water from some factory plants. Each number indicates a different sample, and the same numbers with small letters indicate the samples taken before (a) or after (b) cleaning treatment. Normal YPD medium (1% Bacto Yeast Extract, 2% Polypeptone, 2% glucose in distilled water) was used as the control, and the YPD media for wastewater treatment were made with the various environmental water samples. *Saccharomyces cerevisiae* S288c (*MATα SUC2 mal mel gal2 CUP1*) was grown in YPD medium at 25ºC, shaken at 120-130 rpm to the late log phase (A_{660} = 1.0) and then transferred to YPD medium without (control) or with the environmental water samples. These cultures were incubated aerobically for 2h at 25ºC, and the yeast cells were then harvested by centrifugation at 2,300*g* for 3 min at 4ºC. After harvesting, the cells were flash-frozen and stored at -80ºC until they were processed for RNA extraction. The survival of the environmental water-treated cells was measured by counting the number of colony forming units (CFU) for non-treated (control) and treated yeast.

2.2 RNA extraction and mRNA purification

Total RNA was extracted from cells by the hot-phenol method [10]. The mRNA was purified from total RNA with an Oligotex-dT30 mRNA purification kit (Takara, Shiga, Japan).

2.3 Probe preparation and labeling

The probe was prepared by using the CyScribe first strand cDNA labeling kit (Amersham Biosciences). Briefly, 1 to 2 ug of mRNA was mixed with oligo(dT) primer, random primer, and dNTP nucleotide mix, and the reverse transcription was performed in the presence of 100 U of CyScribe reverse transcriptase (Amersham Bioscience), and 50 uM Cy3 or Cy5. In all experiments, Cy3-UTP (Amersham Bioscience) and Cy5-UTP (Amersham Bioscience) were used to label the control (A_{660} = 1.0) and experimental samples (environmental water), respectively. Each probe was purified by using AutoSeq G-50 (Amersham Bioscience).

2.4 Microarray hybridization and scanning

We used *S. cerevisiae* cDNA microarrays (DNA Chip Research, Kanagawa, Japan) containing targets for about 5,952 genes. We mixed 20 uL of the labeled cDNA, 40 uL of DEPC treated water, and 60 uL of x 2 hybridization solution (Cyscribe first strand cDNA labeling kit; Amersham Bioscience). After 3 min at 90ºC, the mixture was poured onto the array, and allowed to hybridize at 65ºC overnight. After hybridization, the microarray was washed and dried. Microarrays were scanned with a Scanarray 4000 scanner and GenePix 4000 software was used to locate spots in the microarray. To correct for variations (poor quality of some spots, background subtraction, and the different labeling efficiency between Cy3 and Cy5 on the same DNA microarray), we set the cut off at an average background value of + 2SD [11][12]. A Lowess curve was fit to the log-intensity versus log-ratio plot, whereby 20.0% of the data was used to calculate the Lowess fit at each point. This curve was used to adjust the control value for each measurement. If the control channel was lower than 10, then 10 was used instead. The gene expression level of each gene following

wastewater treatment was quantified as the ratio of fluorescence intensity Cy5/Cy3.

2.5 Hierarchical cluster analysis

To compare the transcriptional profiles of environmental waters with those of other conditions, we used the hierarchical cluster algorithm (GeneSpring; Silicon Genetics, U.S.A.; [13]). The transcriptional data for stress conditions such as herbicides, food additives, heavy metals, and physical stresses (high pressure and temperature changes) for cluster analysis are seen at the web site (http://kasumi.nibh.jp/~iwahashi/). We performed cluster analysis based on the 449 genes involved in cell rescue, defense, and virulence in the MIPS (Munich Information Center for Protein Sequences), because the transcriptional profiles of these genes reflected well the cellular responses when yeast was exposed to stress conditions. The similarity between transcriptional data of environmental waters and other stress conditions was measured by the standard correlation with adequate parameters (separation ratio: 1.0; minimum distance: 0.001). We estimated the influence of environmental waters from the expression profiles of stress conditions (i.e. chemicals or environmental stresses) that grouped into the same cluster. The median value of the ratio for each gene was calculated from the gene expression profiles of the several samples classified into the same cluster. The genes were aligned according to the magnitude of the median value. The genes associated closely among several samples classified into same cluster were arranged on top of the table. Therefore, we assessed the influences of the environmental waters from the characteristics of the genes arranged on the top of the table. In cluster analysis, the yeast cells were exposed to chemical and physical stress conditions as follows: Acrolein; 2 h with 0.2mM, Maneb; 2 h with 2ppm, Zineb; 2 h with 2ppm, o-Nitrophenol; 2 h with 3mM, Tetrachloroethylene; 2 h with 3.3mM, Lindane; 2 h with 1.25mM, m-Nitrophenol; 2 h with 5mM, p-Nitrophenol; 2 h with 1.5mM, Round Up; 2 h with 1500 times dilution, Sodium dodecyl sulfate (SDS); 2 h with 0.01%, Capsaicin; 2 h with 250ppm, Gingerol; 2 h with 400ppm, TPN; 2 h with 10uM, Patulin; 2 h with 0.05ppm, Cycloheximide; 2 h with 15uM, Mercury Chloride ($HgCl_2$); 2 h with 0.7mM, Pentachlorophenol; 2 h with 50uM, Temperature shift to 4˚C (12 h); 12 h after shift from 25˚C to 4˚C, Temperature shift to 4˚C (24 h); 24 h after shift from 25˚C to 4˚C, Temperature shift to 4˚C (6 h); 12 h after shift from 25˚C to 4˚C, Temperature shift to 4˚C (48 h); 48 h after shift from 25˚C to 4˚C, Supiculisporic Acid; 2 h with 0.16%, dimethyl sulfoxide (DMSO); 2 h with 10%, Gamma ray; The yeast cells (OD660=1) were irradiated by Co60 as the gamma irradiation source. The dose was 16Gy., α-Terpinene; 2 h with 0.02%, Cadmium Chloride ($CdCl_2$); 2 h with 0.3mM, Lead Chloride ($PbCl_2$); 2 h with 2mM, Manganese (II) Chloride ($MnCl_2$); 2 h with 2.5mM, Pentane; treatment 2 h with 1.5%, Paraquat; 2 h with 35ppm, Recovery from 30MPa; growth for 2hr under 0.1MPa after the incubation for 16 h at 25˚C under 30MPa, Thiram; 2 h with 75uM.

3. Results and Discussion

3.1 The effects of environmental water samples

The environmental waters were sampled from an incinerator, factories, and reclaimed grounds with the promise that the source would not be revealed. YPD medium was prepared with these environmental waters to obtain a maximum response in yeast cells. The incubation time equal to one doubling time was 2h, which was one of the optimum conditions for the stress response [14]. Thus, the CFU changes after 2h were measured. When the CFU of the control (non-treatment) after 2h was 200%, the CFU in environmental waters was calculated (Table 1). The viability of

yeast in the environmental waters was decreased in response to their toxicities (Table 1). But, some environmental waters had no influence on the yeast viability (Table 1). Therefore, we tried to analyze the yeast response at the transcriptional level by DNA microarray to estimate the influences other than the viability in the environmental waters.

3.2 The evaluation of 21 kinds of the environmental waters based on the transcription levels of genes involved in cell rescue, defense, and virulence.

We performed a comprehensive analysis by DNA microarray to evaluate the environmental waters. These transcriptional data are shown in the following web site (http://kasumi.nibh.jp/~iwahashi/). The expression profiles of the 449 genes that are involved in cell rescue and defense in MIPS well reflected the cellular responses to stress conditions. We performed the hierarchical cluster analysis based on these 449 genes. We attempted to compare the environmental waters with known chemical and physical stress conditions by the hierarchical cluster analysis.

Consequently, 21 kinds of environmental water were classified into several clusters together with chemical or physical stress conditions (Figure 1). We grouped the clusters as follows: Cluster A included B1 water; Cluster B included A4, A5, and B3 water; Cluster C included C1a, C1b, A2b, and A6 water; Cluster D included C3, C4, C6, and A2a water; Cluster E included B2, B4, and B5 water; and Cluster F included B6, A1a, A1b, F5, A3a, and A3b. We tried to evaluate the influences of the environmental waters from the gene expression profiling in each cluster.

Table1. Yeast Cells Viability by Environmental Waters

"A" water samples that were eluted from reclaimed ground or soils. "B" water samples that were collected from the incineration process. "C" water samples that were collected as industrial waste water from some factories. Each number indicates a different sample, and the same numbers with small letter indicates the sample taken before (a) or after (b) the cleaning process.

Environmental waters	CFU ratio (%, control as 200%)
B1	162
B2	195
B3	135
B4	206
B5	123
B6	154
A1a	not measured
A1b	not measured
C1a	not measured
C1b	not measured
C3	161
C4	155
C5	181
C6	200
A2a	200
A2b	205
A3a	140
A3b	175
A4	not measured
A5	not measured
A6	not measured

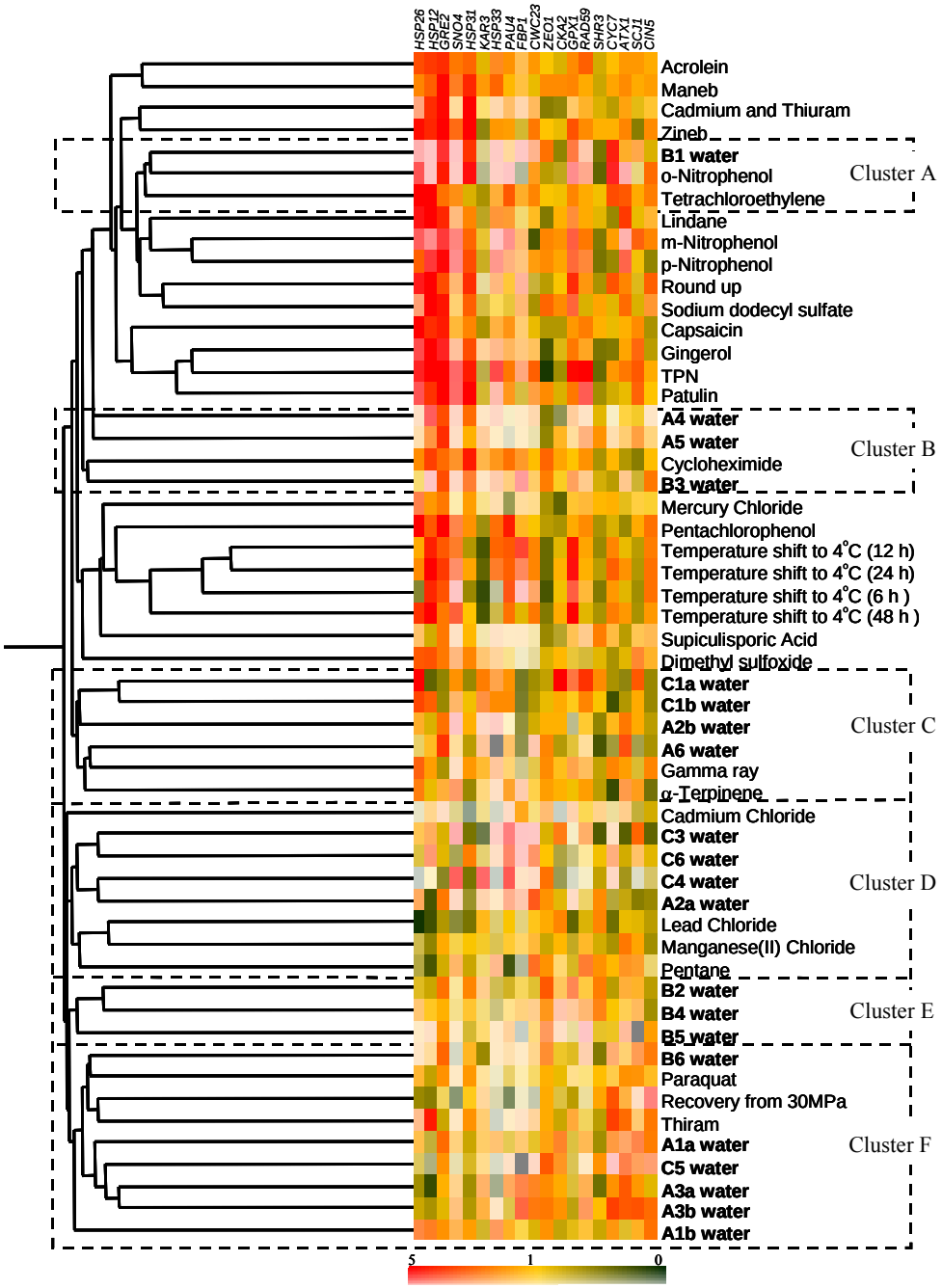

Figure 1. The hierarchical cluster analysis of 21 types of environmental water samples and 33 kinds of chemical and physical stress conditions based on the genes involved in cell rescue, cell defense, and virulence in MIPS. "A" water samples were eluted from the reclaimed ground or soils. "B" water samples were collected from the incineration process. "C" water samples were collected as industrial wastewater from some factories. Each number shows a different sample, and the same numbers with small letters indicate that the sample was taken before (a) or after (b) a cleaning process. Chemical or physical stress conditions were shown in Material and Methods.

3.3 Environmental waters that cause oxidative stress, and protein denaturation, but not mutagenesis.

The B1 water sample was collected from incinerator process [15]. In cluster A, B1 water was grouped into the cluster that contained o-Nitrophenol and Tetrachloroethlylene (Figure1).

Table 2. Cluster A; B1 water, tetra chloroethylene, and o-Nitrophenol

ORFs	B1 water Ratio	Tetrachloroethylen Ratio	o-Nitrophen Ratio	Median	Common	Description in MIPS
YBR072w	110.2	8.2	76.9	76.9	HSP26	heat shock protein
YMR175w		2.5	136.7	69.6	SIP18	osmotic stress protein
YFL014w	234.0	13.9	60.4	60.4	HSP12	heat shock protein
YOL052c-a		5.9	71.7	38.8	DDR2	heat shock protein
YMR169c		2.0	29.5	15.8	ALD3	stress inducible aldehyde dehydrogenase
YMR174c		3.1	26.2	14.6	PAI3	protease A inhibitor
YMR173w	23.9	1.5	10.1	10.1	DDR48	heat shock protein
YDR453c	10.1	1.3	11.8	10.1	TSA2	thiol peroxidase
YBR244w	7.7	0.6	13.4	7.7	GPX2	glutathione peroxidases
YCL035c	7.1	2.4	11.8	7.1	GRX1	glutaredoxin
YGR088w	6.9	1.1	16.4	6.9	CTT1	catalase T
YGR209c	6.5	1.5	9.6	6.5	TRX2	thioredoxin II
YDR256c	15.9	0.7	6.3	6.3	CTA1	catalase A
YMR251w-a		5.2	6.9	6.0	HOR7	hyperosmolarity-responsive protein
YBL064c	11.9	2.8	5.9	5.9	PRX1	mitochondrial isoform of thioredoxin peroxidase
YLR109w	7.4	1.3	5.3	5.3	AHP1	Alkyl hydroperoxide reducta
YEL039c	5.1	3.0	21.7	5.1	CYC7	cytochrome-c isoform 2
YGR008c	4.9	2.5	7.5	4.9	STF2	ATPase stabilizing factor
YIR038c	7.0	2.3	4.8	4.8	GTT1	glutathione S-transferase
YNL160w	4.7	2.4	7.8	4.7	YGP1	secreted glycoprotein
YOL151w	8.1	2.0	4.7	4.7	GRE2	methylglyoxal reductase
YHR104w	4.6	2.4	5.3	4.6	GRE3	aldose reductase
YPL280w	4.9	2.7	4.3	4.3	HSP32	heat shock protein
YMR250w	4.2	2.5	4.9	4.2	GAD1	similarity to glutamate decarboxylases
YDR043c	5.0	1.0	4.1	4.1	NRG1	transcriptional repressor for glucose repression of STA1 gene expression
YDR513w	4.9	1.4	4.1	4.1	TTR1	glutaredoxin
YJL165c	4.1	1.2	4.4	4.1	HAL5	ser/thr protein kinase
YJR104c	4.7	1.5	4.1	4.1	SOD1	copper-zinc superoxide
YOR391c	4.9	1.3	4.0	4.0	HSP33	heat shock protein
YDR258c	3.8	1.4	4.3	3.8	HSP78	heat shock protein
YDR533c	3.8	0.9	12.1	3.8	HSP31	heat shock protein
YNL241c	3.5	1.1	4.0	3.5	ZWF1	glucose-6-phosphate dehydrogenase
YLL060c	3.5	1.5	6.3	3.5	GTT2	glutathione S-transferase
YLL026w	3.3	1.5	14.3	3.3	HSP104	heat shock protein
YIR037w	3.2	1.8	9.0	3.2	HYR1	glutathione peroxidase

The genes were aligned according to the magnitude of the median value of the ratio, and the thirty-five genes within 449 genes are shown in Table 2. It was thought that these genes associated closely with the characteristics of cluster A. *o*-Nitrophenol is a strong oxidizing agent, which is used as a pesticide or insecticide. Tetrachloethylene is used in dry cleaning and metal degreasing. The genes coding for heat shock proteins (HSP), and the scavengers of reactive

oxygen species (ROS) are induced in yeast cells by these chemicals (Table 2). The major metals included in B1 water were calcium (Ca), natrium (Na), and potassium (K). Therefore, it was grouped into the cluster different from heavy metals such as Cd and Pb (Fig 1). The cells exposed to B1 water displayed a number of changed genes expressions, which were specially related to detoxification for reactive oxygen species, the intracellular signaling pathway, and DNA repair [15]. It was thought that denatured proteins, and oxidative stress were caused by the B1 water because the genes coding for same functions (*HSP26*, *HSP12*, *GPX1*, *GPX2*, and *CTA1* etc.) were also induced in B1 treated cells (Table 2).

3.4 Environmental waters that cause protein denaturation.

In cluster B, B3 water, which was sampled from the cleaning process of an incinerator plant, was grouped into the cluster that contained cycloheximide (Figure 1). Thirty-five of the genes within the 449 genes were aligned according to the magnitude of the median value of the ratio (Table 3). It was thought that these genes were associated closely with the characteristics of cluster B. cycloheximide is known as a protein synthesis inhibitor, because the genes encoding HSPs (*HSP31*, *HSP12*, *DDR48*, and *DDR2*) were induced by cycloheximide (Table 3). It was predicted that B3 water had caused the denaturation of protein, because the genes for HSPs (*HSP31*, *HSP12*, *DDR48*, and *DDR2*) were actually induced (Table 3). The A4 and A5 water samples were eluted from reclaimed lands and soil samples, respectively. These water samples were grouped within the cluster that contained Maneb, o-Nitrophenol, SDS, and Zineb (Figure 1). These compounds are known to cause oxidative stress and denaturation of protein [16] [17]. The A4 water and the A5 water were actually extracted from the soil that contained the surface-active agent and pesticides. Therefore it is thought that these waters have the same characterization such as SDS and Zineb. As shown in Table 3, it was predicted that A5 water would influence oxidative stress and protein denaturation, because the genes for ROSs scavengers and HSPs (*HSP12*, *HSP32*, *DDR48*, *GTT2*, *CTA1*, and *GPX2*) were induced by A5 water treatment (Table 3). In yeast cells treated with A4 water, the induction levels of the genes involved in oxidative stress (*GTT2*, and *GPX2*) were less than those in yeast cells treated with A5 water (Table 3). However, it seemed that A4 water potentially had the same toxicities as the chemicals contained in this cluster, because the A4 water was grouped into this cluster from the gene expression patterns, except for those related to oxidative stress.

3.5 Environmental waters that cause mutagenesis, protein denaturation, and cell wall damage.

The C1a, C1b, A2b, and A6 water samples were grouped together with gamma rays and α-Terpinene into cluster C (Figure 1). C1 water samples (C1a and C1b) were sampled as the industrial wastewater from a factory. A2b was an eluted water sample collected from the reclaimed ground, and A6 was eluted water sampled from the soil. The genes were aligned according to the magnitude of the median value of the ratio, and thirty-five of the genes within the 449 genes were shown in Table 4. It was thought that these genes are closely associated with the characteristics of cluster C. The genes involved in DNA repair (*RAD9*, *RAD54*, and *RAD17*), and HSPs (*HSP26*, *HSP33*, and *DDR48*) are shown in Table 4. The major influence of gamma rays is oxidative DNA damage [18]. *HUG1* was induced by 16Gy of gamma rays (data not shown), and Hug1p, the gene product, is the protein involved in the Mec1p-mediated checkpoint pathway that responds to DNA damage [19]. It is known that the levels of HSPs are increased by gamma rays or UV irradiation [20]. It was suggested that the environmental waters classified into cluster C caused DNA damage and protein denaturation, because the gene expression profiling of the gamma

Table 3. Cluster B; B3, A4, and A5, and cycloheximide

	B3 water	A4 water	A5 water	Cycloheximide			
ORFs	Ratio	Ratio	Ratio	Ratio	Median	Gene name	Description in MIPS
YFL014w	6.0	1.9	5.9	3.4	4.6	HSP12	heat shock protein
YOL151w	2.9	4.0	2.9	2.0	2.9	GRE2	methylglyoxal reductase
YPL280w	5.0	1.1	3.7	2.1	2.9	HSP32	heat shock protein
YMR322c	3.0	2.1	3.0	1.7	2.5	SNO4	strong similarity to YPL280w, YOR391c and YDR533c
YDR533c	2.4	1.1	2.1	4.8	2.2	HSP31	heat shock protein
YMR173w	2.0	1.9	3.0	2.3	2.2	DDR48	heat shock protein
YAR020c	5.7	1.3	2.5	1.6	2.1	PAU7	strong similarity to members of the Srp1p/Tip1p family
YFL059w	2.6	2.1	1.9	0.7	2.0	SNZ3	strong similarity to Para rubber tree ethylene-responsive
YLL060c	1.8	2.3	2.1	1.6	2.0	GTT2	glutathione S-transferase
YOR391c	2.4	1.1	2.8	1.4	1.9	HSP33	heat shock protein
YDR256c	7.2	0.8	2.2	1.5	1.8	CTA1	catalase A
YMR095c	2.0	1.5	2.6	0.8	1.8	SNO1	similarity to M.leprae hisH
YIR038c	1.8	2.1	1.6	0.7	1.7	GTT1	glutathione S-transferase
YLR350w	1.7	0.6	1.7	2.3	1.7	ORM2	strong similarity to YGR038w
YHL046c		1.7	1.0	2.8	1.7		strong similarity to members of the Srp1p/Tip1p family
YOR208w	0.8	1.8	2.5	1.5	1.7	PTP2	protein-tyrosine-phosphatase
YBL064c		1.7	0.8	1.9	1.7	PRX1	mitochondrial isoform of thioredoxin peroxidase
YGR008c	1.6	1.7	1.7	1.4	1.7	STF2	ATPase stabilizing factor
YDR263c	1.8	1.3	3.0	1.5	1.7	DIN7	DNA-damage inducible protein
YBR244w	0.9	2.6	2.3	1.0	1.6	GPX2	glutathione peroxidases
YPL223c	3.9	1.4	1.2	1.8	1.6	GRE1	induced by osmotic stress
YFR034c	1.7	2.4	1.5	0.9	1.6	PHO4	transcription factor
YJL165c	0.5	1.3	2.5	1.9	1.6	HAL5	ser/thr protein kinase
YDL022w	0.7	1.7	6.2	1.5	1.6	GPD1	glycerol-3-phosphate dehydrogenase
YMR096w	2.6	0.8	2.1	1.1	1.6	SNZ1	stationary phase protein
YAL028w	1.0	1.9	2.4	1.3	1.6	FRT2	Tail-anchored endoplasmic reticulum membrane protein
YOR279c	0.9	1.5	1.7	1.7	1.6	RFM1	hypothetical protein
YKL161c	1.9	0.5	1.6	1.5	1.6	(MLP1)	strong similarity to ser/thr-specific protein kinase Slt2p
YHR104w	1.7	1.4	3.3	1.4	1.6	GRE3	aldose reductase
YIL153w	0.6	4.1	2.1	1.0	1.6	RRD1	strong similarity to human phosphotyrosyl phosphatase activator
YDR059c	1.6	1.5	1.6	1.4	1.5	UBC5	E2 ubiquitin-conjugating
YDR032c	1.6	1.7	1.5	1.1	1.5	PST2	strong similarity to S.pombe obr1 brefeldin A resistance
YDR001c	1.0	0.7	2.3	2.0	1.5	NTH1	neutral trehalase
YDL013w	1.6	1.1	1.5	1.6	1.5	HEX3	hexose metabolism-related protein
YBR173c	1.4	1.6	1.7	0.8	1.5	UMP1	proteasome maturation factor

Table 4. Cluster C; C1a, C1b, A6, A2b, a-Terpinene, and gamma ray (16Gy)

	C1a water	C1b water	A2b water	A6 water	a-Terpinene	Gamma ray			
ORFs	Ratio	Ratio	Ratio	Ratio	Ratio	Ratio	Median	Gene name	Description in MIPS
YBR072w	6.8	3.1	1.2	0.8	1.8	2.6	2.2	HSP26	heat shock protein
YMR175w	2.9	0.6	16.4	2.1		1.7	2.1	SIP18	osmotic stress protein
YCR104w	2.2	1.9	7.3	1.5		1.3	1.9	PAU3	strong similarity to members of the Srp1/Tip1p family
YPR141c	1.7	2.1	2.7	2.1	1.1	1.1	1.9	KAR3	kinesin-related protein
YOR391c	1.6	1.5	4.6		1.8	2.0	1.8	HSP33	heat shock protein
YOR368w	5.2	2.2	1.1	2.1	0.9	1.6	1.8	RAD17	DNA damage checkpoint control protein
YMR173w	1.0	0.9	2.6	3.9	1.7	1.8	1.8	DDR48	heat shock protein
YFL020c	2.6	2.0	2.4	0.6	1.0	1.5	1.8	PAU5	strong similarity to members of the Srp1p/Tip1p family
YKL150w	3.5	3.4	1.3	1.9	1.0	1.5	1.7	MCR1	cytochrome-b5 reductase
YMR322c	1.3	1.5	13.4	1.1	2.5	1.8	1.6	SNO4	strong similarity to YPL280w, YOR391c and YDR533c
YNL333w	3.3	0.6	1.0	2.5	1.8	1.4	1.6	SNZ2	putative pyridoxine biosynthetic enzyme
YDR043c	2.5	1.1	1.0	1.8	1.4	2.6	1.6	NRG1	transcriptional repressor for glucose repression of STA1 gene expression
YBR082c	1.5	1.0	1.7	1.7	1.2	1.7	1.6	UBC4	E2 ubiquitin-conjugating enzyme
YBL009w	2.2	2.0	1.3	1.8	1.1	1.2	1.6		strong similarity to DNA damage responsive Alk1p
YMR273c	2.5	2.0	1.1			1.0	1.5	ZDS1	involved in negative regulation of cell polarity
YJL223c	2.1	7.6	1.1	0.7	1.7	1.3	1.5	PAU1	strong similarity to members of the Srp1p/Tip1p family
YAR020c	1.1	2.1		1.5	1.0	1.8	1.5	PAU7	strong similarity to members of the Srp1p/Tip1p family
YDL022w	5.0	2.0	1.1	3.3	0.8	1.1	1.5	GPD1	glycerol-3-phosphate dehydrogenase
YNL259c	0.5	0.7	1.8	3.1	1.9	1.2	1.5	ATX1	antioxidant protein and metal homeostasis factor
YKL086w	3.0	2.1	1.5		0.6	1.0	1.5	SRX1	strong similarity to hypothetical protein *S. pombe*
YIL153w	2.2	1.9	1.5	1.5	1.5	1.2	1.5	RRD1	strong similarity to human phosphotyrosyl phosphatase activator
YHR193c	2.5	1.2	1.2	1.7	0.8	1.8	1.5	EGD2	subunit of the nascent polypeptide-associated complex
YEL049w	2.3	3.0	0.7	0.9	1.4	1.5	1.5	PAU2	strong similarity to members of the Srp1p/Tip1p family
YER042w	1.3	2.1	1.0	1.5	2.1	1.3	1.4	MXR1	responsible for the reduction of methionine sulfoxide
YOR208w	1.4	1.8	1.1	1.5	1.5	1.1	1.4	PTP2	protein-tyrosine-phosphatase
YNL241c	1.5	2.6	3.0	0.7	1.4	1.0	1.4	ZWF1	glucose-6-phosphate dehydrogenase
YLR109w	0.5	0.7	1.6	2.3	1.3	1.7	1.4	AHP1	Alkyl hydroperoxide reductase
YDR217c	4.9	1.2	1.0		1.4	1.5	1.4	RAD9	DNA repair checkpoint protein
YDL220c	4.4	1.8	1.5	0.7	1.3	1.0	1.4	CDC13	cell division control protein
YJL160c	2.5	1.6	0.8		1.1	1.4	1.4		strong similarity to Pir1p/Hsp150p/Pir3p family
YGL163c	1.8	1.5	9.7	0.8	1.2	1.3	1.4	RAD54	DNA-dependent ATPase of the Snf2p family
YBR016w	1.7	2.2	1.1	1.4	1.3	1.0	1.4		strong similarity to hypothetical proteins YDL012c and YDR210w
YBL075c	1.4	1.3	0.8	2.7	1.5	1.4	1.4	SSA3	heat shock protein of HSP70 family
YDR263c	0.9	1.8	1.2	4.0	1.2	1.5	1.3	DIN7	DNA-damage inducible protein
YCR060w	2.2	1.0	1.3	1.4	0.9	1.4	1.3	TAH1	similarity to stress inducible protein Sti1p

ray-exposed yeast cells was grouped into cluster C. The induction of PAU genes (*PAU1*, *PAU2*, *PAU3*, *PAU5* and *PAU7*) was observed in cluster C (Table 4). The PAU genes encode seripauperin (Srp) proteins, which are generally considered to be stress proteins [21]. The function of seripauperin remains unknown. Another member of cluster C, α-Terpinene, influences the structure of the cell wall [22]. These environmental waters might damage the cell wall, because they cause denaturation of the cell wall proteins. C1a water was sampled prior to being cleaned by the sewage treatment process, and C1b water thaws sampled following cleaning by the sewage treatment process. The gene expression patterns did not change between the C1a and C1b water samples, although C1b water was treated by the sewage treatment process (Table 4). It has been indicated that the mutation sites were not removed by the sewage treatment process.

3.6 Environmental waters that cause mutagenesis.

The C3, C4, C6, and A2a water samples were grouped together with $PbCl_2$, $MnCl_2$, Pentane, and $CdCl_2$ into cluster D (Figure 1). The C3, C4, and C6 water samples were collected as industrial wastewater from three respective factories. The A2a water sample was eluted from the reclaimed ground. The genes associated with cluster D (35 genes) are given in Table 5. They include genes involved in DNA repair and DNA damage (*RAD16*, *XRS2*, *DDC1*, *LCD1*, *DIN7*, and *RAD24*). The chemicals (cadmium, lead, and pentane) that are also classified into this same cluster influence DNA damage (see supplement table). It is thought that these waters were classified into the cluster including the heavy metals, because C3 water, C4 water, and C6 water are industrial wastewater that contained the heavy metals. Oxidative stress causes the cell cycle to arrest in G1 or G2. DNA damage is repaired during the delay in cell cycle progression [23][24]. Yeast cells exposed to the environmental waters might incur cell cycle delay as a consequence of the induction of genes involved in DNA damage or repair. The A2a water was sampled prior to the purification processing, and the A2b water was sampled after the purification, although both A2a and A2b waters were sampled from the same reclaimed place (Table 1). These water samples were classified into the different clusters (Figure1, clusters C and D). Several of the genes, s among the induced genes differed between the A2a water and A2b water (Table 6). The genes involved in oxidative stress (*TRX1*, *TRX3*, *GPX1*, and *GTT2* etc.), protein degradation (*UBA1*, *RSP5*, and *QRI8* etc.), and stress responses (*TIR1*, *TIR2*, *TIR3*, and *PIR1* etc.) were induced in the A2a water (Table 6). It was predictable that A2a water is more harmful than A2b water from the functions of these genes; and it has been indicated that the mutation risks were removed by the cleaning (purification) process.

3.7 Environmental waters that cause protein denaturation and oxidative stress.

Cluster E included the B2, B4, and B5 waters (Figure 1). These waters were sampled from the incineration process. The genes associated with cluster E (35 genes) are listed in Table 7. *ZEO1* plays reciprocal roles with *MID2,* which functions as a stress sensor, in the modulation of the yeast PKC1-MPK1 cell integrity pathway [25]. The genes involved in DNA repair (*RAD59*), HSPs (*HSP82* and *HSP10*), and oxidative stress (*GPX1*, *TRX2*, and *GRX3*) are listed in Table 7. The genes associated with cluster E (DNA repair; *RAD59*, HSPs; *HSP82*, and oxidative stress; *TRX2*, and *GRX3*) were induced in the B5 water (Table 7). In contrast, induction of genes related to stress response was not observed in the B2 and B4 water samples (Table 7). It was thought that the B2 and B4 waters were safer than B5 water, because the induction levels of genes for the stress response in B2 and B4 waters were lower than that in B5 water. Actually, the CFU ratios in B2 water and B4 water were 195% and 206%, respectively, although the CFU ratio at 2h in B5 water was 123% (Table 1). This was a good example of the risk prediction for a sampled water

specimen by using both the gene expression profiles and the viability as CFU.

Table 5. Cluster D; C3 water, C4 water, C6 water, A2a water, $CdCl_2$, $PbCl_2$, $MnCl_2$, and Pentane

	C3 water	C4 water	C6 water	A2a water	$CdCl_2$	$PbCl_2$	$MnCl_2$	Pentane			
ORFs	Ratio	Ratio	Ratio	Ratio	Ratio	Ratio	Ratio	Ratio	Mediann	Gene name	Description in MIPS
YMR174c	7.8	11.5	4.5	1.1			1.3		4.5	PAI3	protease A inhibitor
YMR175w	10.5	2.9	2.6	2.4			1.4		2.6	SIP18	osmotic stress protein
YLR461w	16.5	5.6	3.7	2.8	2.4	1.0	1.0	0.2	2.6	PAU4	strong similarity to members of the Tir1p/Tip1p family
YLR337c	7.0	4.2	2.5	0.8			0.8		2.5	VRP1	verprolin
YGL128c	5.7	2.3	2.9	3.1	1.0	1.0	1.2	2.5	2.4	CWC23	weak similarity to dnaJ proteins
YJL223c	6.3	3.5	3.1	3.0	1.0	1.4	1.3	0.6	2.2	PAU1	strong similarity to members of the Srp1p/Tip1p family
YBR114w	2.5	9.8	1.8	2.3	1.0	1.3	1.1	2.1	2.0	RAD16	nucleotide excision repair
YDR369c	5.8	12.5	2.2	2.8	0.7	1.3	1.7	1.0	1.9	XRS2	DNA repair protein
YFR034c	4.0	2.4	3.5	1.7	1.4	2.1	1.2	1.1	1.9	PHO4	transcription factor
YBR275c	0.3	6.0	1.2	2.5	3.0	4.3	1.1	1.1	1.9	RIF1	RAP1-interacting factor 1
YPL194w	5.6	5.0	1.6	2.2	5.9	1.0	1.2	0.8	1.9	DDC1	DNA damage checkpoint
YJL095w	3.0		3.4	0.5	0.6	7.7	1.9	0.8	1.9	BCK1	ser/thr protein kinase of the MEKK family
YGL180w	3.7	7.7	2.1	2.0	1.0	1.1	1.3	0.8	1.7	ATG1	essential for autophagocytosis
YKL086w	1.6	8.5	1.7	3.7	0.7	3.5	0.8	1.1	1.7	SRX1	strong similarity to PIR:T39259 hypothetical protein *S. pombe*
YDR227w	2.1	4.5	2.0	0.9	1.9	1.3	1.1	1.3	1.6	SIR4	silencing regulatory and DNA-repair protein
YDR499w	1.4	2.6	1.3	1.8	2.1	1.1	0.9	3.0	1.6	LCD1	cell cycle checkpoint protein
YDL025c	1.8	5.7	2.4	1.9	1.1	0.6	1.0	1.3	1.5		similarity to probable protein kinase NPR1
YBL105c	1.6	1.6	1.7	0.8	2.6	1.0	1.2	1.5	1.5	PKC1	ser/thr protein kinase
YMR095c	8.5	0.9	3.1	0.7	1.8	4.0	1.2	1.1	1.5	SNO1	similarity to M.leprae hisH
YGL019w	1.7	4.2	0.9	2.5	1.6	1.3	1.0	0.2	1.5	CKB1	casein kinase II, beta subunit
YPL242c					1.3	1.6		1.5	1.5	IQG1	involved in cytokinesis
YER012w	4.8	1.6	1.0	1.9	12.5	1.3	1.0	1.1	1.4	PRE1	20S proteasome subunit C11
YER062c					1.4				1.4	HOR2	DL-glycerol phosphatase
YDR263c	4.9		2.5	0.3	1.0	1.2	1.4	1.5	1.4	DIN7	DNA-damage inducible protein
YJR055w	1.3	4.6	1.5	0.7	0.8	1.7	1.5	1.1	1.4	HIT1	required for growth at high temperature
YPR026w	1.5	22.7	1.7	2.7	1.4	0.5	1.0	1.3	1.4	ATH1	acid trehalase
YER173w	0.8	9.7	1.5	0.3	1.4	1.4	1.0	2.1	1.4	RAD24	cell cycle checkpoint protein
YCR104w	1.9	10.8	0.0	1.8	0.3		0.9		1.4	PAU3	strong similarity to members of the Srp1/Tip1p family
YBL022c	7.0	1.8	1.4	2.0	0.7	1.0	1.4	0.7	1.4	PIM1	ATP-dependent protease
YEL030w	3.6	4.3	1.9	1.5	0.9	0.7	1.2	0.5	1.4	ECM10	HSP70 family
YLL010c	0.7	2.4	1.3	2.1	1.4	1.0	1.1	1.4	1.4	PSR1	plasma membrane phosphatase involved in sodium stress
YML058w-a		1.3	2.1	0.6			1.5		1.4	HUG1	hydroxyurea and UV and gamma radiation induced
YLR043c	1.6	1.0	1.6	2.2		1.1	0.9	1.4	1.4	TRX1	thioredoxin I
YNL334c	0.4	2.2	1.3	2.9	0.8	0.7	1.4	5.9	1.4	SNO2	strong similarity to hypothetical proteins YFL060c and
YJL073w	0.8	1.8	1.4	1.6	1.5	1.3	0.9	1.3	1.3	JEM1	similarity to heat shock proteins

Table 6. The differences of the induced genes between A2a water and A2b water

	A2a water	A2b water		
ORFs	Ratio	Ratio	Gene name	Description in MIPS
Protein degradation				
YDL190c	2.5	1.8	UFD2	ubiquitin fusion degradation
YER125w	2.3	0.9	RSP5	hect domain E3 ubiquitin-protein ligase
YMR022w	2.2	1.6	QRI8	E2 ubiquitin-conjugation enzyme
YKL210w	2.5	1.0	UBA1	E1-like (ubiquitin-activating)
YLL039c	2.2	1.0	UBI4	ubiquitin
YKL073w	2.1	0.9	LHS1	chaperone of the ER lumen
Oxidative stress				
YCR083w	2.1	0.9	TRX3	mitochondrial thioredoxin
YML028w	2.0	0.9	TSA1	thiol-specific antioxidant
YKL026c	2.4	0.4	GPX1	glutathione peroxidase
YKL086w	3.7	1.5	SRX1	strong similarity to PIR:T39259 hypothetical protein S. pombe
YLL060c	2.3	1.3	GTT2	glutathione S-transferase
YLR043c	2.2	0.9	TRX1	thioredoxin I
YPR026w	2.7	0.6	ATH1	acid trehalase, vacuolar
Other stress				
YER011w	5.4	0.9	TIR1	cold-shock induced protein of the Tir1p,Tip1p family
YIL011w	2.5	0.7	TIR3	strong similarity to members of the Srp1p/Tip1p family
YKL164c	2.5	1.2	PIR1	required for tolerance to heat
YOR010c	4.3	0.8	TIR2	cold shock induced protein
YLR157c	2.8	0.9	ASP3-2	L-asparaginase II
YLR158c	2.5	0.9	ASP3-3	L-asparaginase II
YLR160c	2.4	0.9	ASP3-4	L-asparaginase II
DNA repair				
YBL009w	2.3	1.3		strong similarity to DNA damage responsive Alk1p
YBR114w	2.3	1.0	RAD16	nucleotide excision repair protein
YDR369c	2.8	0.8	XRS2	DNA repair protein
YER095w	2.3	0.5	RAD51	DNA repair protein
YPL194w	2.2	1.3	DDC1	DNA damage checkpoint protein
YML032c	2.5	0.7	RAD52	recombination and DNA repair protein
PAU genes				
YJL223c	3.0	1.1	PAU1	strong similarity to members of the Srp1p/Tip1p family
YGL128c	3.1	0.8	CWC23	weak similarity to dnaJ proteins
YLR461w	2.8	1.0	PAU4	strong similarity to members of the Tir1p/Tip1p family
YNR076w	2.3	0.9	PAU6	strong similarity to members of the Tir1p/Tip1p family
YOL105c	3.3	1.0	WSC3	cell wall integrity and stress response component 3
HSPs				
YGL073w	2.9	1.1	HSF1	heat shock transcription factor
YNL007c	2.8	0.7	SIS1	heat shock protein
YOL052c-a	2.4	0.9	DDR2	heat shock protein DDRA2
YOR232w	2.1	0.7	MGE1	heat shock protein - chaperone
YPL106c	2.2	1.1	SSE1	heat shock protein of HSP70 family

Table 7. Cluster E; B2 water, B4 water, and B5 water

ORFs	B2 water Ratio	B4 water Ratio	B5 water Ratio	Median	Gene name	Description in MIPS
YOL109w	2.6	1.5	2.5	2.5	ZEO1	overexpression causes resistance to Zeocin
YOR061w	1.1	2.8	1.9	1.9	CKA2	may have a role in regulation and/or execution of the eukaryotic cell
YKL026c	2.9	1.8	0.8	1.8	GPX1	glutathione peroxidase paralogue
YDL059c	1.0	1.7	7.6	1.7	RAD59	Involved in mitotic recombination
YDL212w	1.6	1.8	0.9	1.6	SHR3	protein required for appearance of amino acid permeases on the cell surface
YGR008c	1.6	1.0	1.7	1.6	STF2	ATPase stabilizing factor
YOR020c	1.6	1.1	1.7	1.6	HSP10	heat shock protein
YPL152w	1.6	1.3	1.8	1.6	RRD2	Resistant to Rapamycin Deletion 2
YAR020c	2.0	1.1		1.6	PAU7	member of Pau1 family
YPL240c	1.5	1.5	2.0	1.5	HSP82	heat shock protein
YGR209c	1.5	1.2	2.2	1.5	TRX2	thioredoxin
YOR324c	1.5	1.5	1.2	1.5	FRT1	hypothetical ORF
YDR079w	1.5	1.8	0.5	1.5	PET100	cytochrome c oxidase-specific assembly factor
YCR008w	1.7	1.5	1.0	1.5	SAT4	protein with similarity to Npr1p protein kinase
YMR186w	1.5	1.3	1.5	1.5	HSC82	constitutively expressed heat shock protein
YPL084w	1.2	1.5	1.7	1.5	BRO1	BCK1-like resistance to osmotic shock
YER142c	1.6	1.2	1.5	1.5	MAG1	3-methyladenine DNA glycosylase
YDR369c	1.4	1.7	0.9	1.4	XRS2	required for DNA repair
YDR098c	1.4	0.9	3.2	1.4	GRX3	glutaredoxin subfamily
YJR055w	1.4	1.1	1.4	1.4	HIT1	protein required for growth at high temperature
YBR044c	1.4	1.3	1.5	1.4	TCM62	putative chaperone
YOL151w	1.9	1.1	1.4	1.4	GRE2	induced by osmotic stress
YBR244w	1.6	1.2		1.4	GPX2	glutathione peroxidase paralogue
YAL015c	1.4	1.3	1.5	1.4	NTG1	endonuclase III like glycosylase involved in DNA repair
YJL140w	1.4	1.0	1.4	1.4	RPB4	fourth-largest subunit of RNA polymerase II
YGR100w	1.4	1.5	0.9	1.4	MDR1	Mac1-dependent regulator
YDR043c	1.4	1.5	1.3	1.4	NRG1	involved in regulation of glucose repression
YBR169c	1.5	1.3	1.1	1.3	SSE2	HSP70 family member
YDL235c	1.4	1.3	1.1	1.3	YPD1	Ypd1p is an intermediate protein between Sln1p and Ssk1p in the phosphorelay reaction.
YDL200c	1.6	0.8	1.3	1.3	MGT1	6-O-methylguanine-DNA methylase
YBL022c	1.3	1.6	1.2	1.3	PIM1	mitochondrial ATP-dependent protease
YNL064c	0.9	1.3	1.5	1.3	YDJ1	yeast dnaJ homolog
YKR053c	2.6	0.9	1.3	1.3	YSR3	yeast Sphingolipid Resistance Gene
YOL064c	1.3	1.0	1.3	1.3	MET22	putative phosphatase gene involved in salt tolerance
YMR251w-a	1.8	1.3	1.0	1.3	HOR7	hyperosmolarity-responsive gene

3.8 Environmental waters that cause oxidative stress.

The B6, A1a, A1b, C5, A3a, and A3b water samples were grouped into the cluster including paraquat, 30Mpa, and Thiram (Figure 1). B6 water was sampled from the incineration process, and C5 water was sampled as industrial wastewater from a factory. A1 (A1a and A1b) and A3 (A3a and A3b) waters were samples eluted from different reclaimed grounds, respectively. The genes associated with cluster F (35 genes) are listed in Table 8. It is known that Paraquat, the superoxide generator, and the pesticide Thiram cause oxidative stress [26][27]. Actually, the genes involved in oxidative stress responses (*ATX1*, *TRX1*, *TTR1*, *GPX3*, *TRX2*, *FAP7*, *TRR1*, and *PRX1*) were observed in Table 8. Therefore, it was thought that the environmental waters included in cluster F caused oxidative stress.

A3a was water before undergoing the cleaning treatment, and A3b was the water after the cleaning treatment, although A3 waters were of the same origin. These waters were grouped together into the same cluster (Figure1 cluster F). The stress response genes (*ATH1*, *SIS1*, *TRX1*, and *SDP1,* etc.) were induced in the gene expression profiles in A3b water, although A3b was the treated water (Table 8). The reason for this is unknown. It is possible that this standard cleaning treatment might not be sufficient. A1a is the water analyzed before the cleaning process, and A1b is the water after the cleaning in a sewage treatment plant. The stress genes (*ATX1*, *SCJ1*, *COX20*, *STRE50*, *TIR4*, and *TIM9*, etc.) listed in Table 8 were induced in A1a water, but not A1b water. The genes for stress responses such as DNA repair (*RAD23*, *RAD57*, and *MEC3* etc.), oxidative stress (*GPX2*, *GPX3*, and *SOD1* etc.), and HSPs (*CIS3*, and *HSP30* etc.) were induced in A1a water, but not A1b water (shown in the supplementary table). The harmful A1a water was classified into the cluster including the A3a and A3b waters which also showed low viability, and the A1b water after undergoing the cleaning treatment was classified into this cluster from a more distant position (Figure1 cluster F). This result indicates that the A1a water was cleaned via an appropriate process in the sewage treatment plant. Thus, the present classification of the environmental waters by means of the hierarchical cluster analysis demonstrated the ability to characterize water.

4. Conclusions

We analyzed 21 types of environmental waters comprehensively by DNA microarray, and performed hierarchical cluster analysis based on the 449 genes involved in cell rescue, defense, and virulence in MIPS. The environmental waters were classified into the clusters from A to F on the basis of the chemical and physical stress conditions. These chemical and physical stress conditions well reflected the characteristics of the environmental waters included in the same cluster. Consequently, we were able to estimate the influences of the environmental waters from the classified cluster. It was thought that the characteristics of the environmental water are dependent on the gene expression profile. Therefore, the influence of environmental water could be evaluated by the hierarchical cluster analysis.

Table 8. Cluster F; B6, A1a, A1b, C5, A3a, A3b, Paraquat, 30Mpa, and Thiram

	B6 water	A1a water	A1b water	C5 water	A3a water	A3b water	Paraquat	Recovery from 30MPa	Thiram			
ORFs	Ratio	Ratio	Ratio	Ratio	Ratio	Ratio	Ratio	Ratio	Ratio	Median	Gene name	Description in MIPS
YEL039c	2.1	1.8	1.4	11.4	1.4	3.4	1.0	2.8	3.3	2.1	CYC7	cytochrome-c isoform 2
YNL259c	1.1	2.1	0.9	2.9	2.6	2.6	1.3	1.3	2.1	2.1	ATX1	antioxidant protein and metal homeostasis factor
YMR214w	2.1	2.6	1.2	2.1	1.3	2.7	1.4	2.0	1.2	2.0	SCJ1	homolog of E. coli DnaJ
YLR337c	2.0	1.2	1.0	2.6	1.9	2.6	1.5			1.9	VRP1	verprolin
YOR028c	1.8	1.6	1.9	2.7	0.9	2.0	1.1	5.7	1.8	1.8	CIN5	transcriptional activator
YOL052c-a	2.1	2.2	2.3	1.7	1.7	1.0	1.2			1.7	DDR2	heat shock protein DDRA2
YDR231c	1.7	2.7	0.9	1.7	1.8	1.0	1.3	2.1	1.2	1.7	COX20	assembly of cytochrome oxidase involved protein
YNL007c	1.7	1.5	1.5	3.0	1.8	3.1	0.8	2.1	0.8	1.7	SIS1	heat shock protein
YLR043c	1.7	1.7	1.4	2.1	1.6	2.6	1.5	1.7	1.0	1.7	TRX1	thioredoxin I
YIL113w	1.0	1.5	1.6	5.2	1.9	2.3	1.2	2.6	0.9	1.6	SDP1	stress-inducible dual specificity phosphatase
YDR513w	1.6	2.1	2.0	2.3	1.0	1.3	1.7	0.9	1.3	1.6	TTR1	glutaredoxin
YHR005c-a	1.6	1.7	1.3	2.0	1.6	2.6	1.4			1.6	MRS11	subunit of the Tim22-complex
YCL032w	1.5	2.1	1.3	3.6	0.8	1.9	1.0	2.5	1.6	1.6	STE50	pheromone response pathway
YOR009w	1.6	2.4	0.9	1.8	2.4	1.5	1.0	0.9	2.4	1.6	TIR4	similarity to Tir1p and Tir2p
YEL020w-a	1.6	3.6	1.1	3.7	1.4	2.1	1.1			1.6	TIM9	essential subunit for mitochondrial protein import
YDR519w	1.0	1.2	1.5	2.1	2.2	1.7	1.6	0.7	0.8	1.5	FPR2	FK506/rapamycin-binding protein of the ER
YMR251w-a	1.5	1.3	1.3	4.0	1.6	4.0	1.0			1.5	HOR7	hyperosmolarity-responsive
YML016c	0.8	0.8	1.5	2.6	2.3	2.2	1.2	0.9	1.6	1.5	PPZ1	ser/thr phosphatase required for normal osmoregulation
YDR404c	1.5	1.1	1.5	1.8	1.5	1.6	1.3	1.6	1.0	1.5	RPB7	DNA-directed RNA
YOR145c	1.6	1.6	0.8	2.0	2.4	1.5	1.2	1.3	1.4	1.5	PNO1	strong similarity to hypothtical S. pombe protein
YPL140c	1.6	1.6	0.9	1.9	1.5	1.7	1.1	0.8	1.0	1.5	MKK2	protein kinase of the MEK
YFL059w	3.6	2.3	1.3	2.0	1.1	1.1	1.4	1.6	0.8	1.4	SNZ3	strong similarity to Para rubber tree ethylene-responsive
YDR098c	1.4	3.4	1.1	1.0	1.8	1.5	1.1	1.7	1.1	1.4	GRX3	glutaredoxin
YGR209c	1.4	1.9	2.0	2.7	0.9	1.4	1.5	1.1	0.9	1.4	TRX2	thioredoxin II
YDL079c	1.9	0.9	1.4	3.8	1.3	1.1	1.4	3.4	5.5	1.4	MRK1	ser/thr protein kinase
YDL213c	2.5	1.2	1.0	1.4	1.0	1.7	1.2	2.2	2.4	1.4	NOP6	weak similarity to potato small nuclear ribonucleoprotein
YDL166c	2.8	2.6	0.8	1.7	0.8	0.9	1.1	1.6	1.4	1.4	FAP7	involved in the oxidative stress response
YLR150w	2.1	2.6	1.4	1.5	1.3	0.7	0.9	1.6	1.3	1.4	STM1	specific affinity for guanine-rich quadruplex nucleic acids
YHR081w	1.4	1.3	0.7	5.0	2.0	3.9	1.1	1.6	1.3	1.4	LRP1	involved in regulation of DNA repair and recombination
YDR353w	1.4	1.8	1.3	2.6	1.1	1.1	1.6	1.5	1.0	1.4	TRR1	thioredoxin reductase
YIL009c-a	1.4	0.7	0.9	1.4	1.4	2.1	1.1			1.4	EST3	subunit of the telomerase
YNL334c	1.2	1.2	0.7	3.9	2.5	3.2	1.4	1.2	1.4	1.4	SNO2	strong similarity to hypothetical proteins YFL060c
YBL064c	2.3	1.0	1.4	1.5	0.9	0.6	1.4	1.7	1.2	1.4	PRX1	mitochondrial isoform of thioredoxin peroxidase
YOL151w	2.2	2.1	1.4	1.4	1.1	0.9	1.4	0.9	0.8	1.4	GRE2	methylglyoxal reductase
YDR079w	1.4	0.8	0.8	2.2	0.7	1.8	1.1	1.6	1.5	1.4	PET100	cytochrome-c oxidase assembly protein

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