Application of Genome-Wide Expression Analysis To Identify Molecular Markers Useful in Monitoring Industrial Fermentations

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Genome-wide expression analysis of an industrial strain of *Saccharomyces cerevisiae* identified the *YOR387c* and *YGL258w* homologues as highly inducible in zinc-depleted conditions. Induction was specific for zinc deficiency and was dependent on Zap1p. The results indicate that these sequences may be valuable molecular markers for detecting zinc deficiency in industrial fermentations.

Deficiency of the essential nutrient zinc is a major contributor to retarded yeast fermentation in the brewing process (4). During the processing of wort, zinc ions can form complexes with polyphenols and can also form insoluble complexes of alpha acids (12). In this form the zinc is not accessible to yeast, and direct methods of measuring zinc in wort do not always accurately predict its availability to the yeast. Molecular markers have been used as an alternative method to indirectly monitor fermentation processes through yeast gene expression. Genes encoding heat shock (*HSP12*) and osmotic shock



FIG. 1. YOR387c and YGL258w differential gene expression is the most rapid and responsive to zinc depletion. Northern blot kinetic analysis of YOR387c and YGL258w, ZPS1, ZRT1, and ACT1 gene expression was performed with total RNA isolated from Lager 1 cells grown in LZMM with $(\Box; Zn^+)$ and without $(\Delta; Zn^-)$ 40 μ M ZnSO₄ added. Each Northern blot is a representative of a duplicate experiment, and the growth curves are the means of triplicate readings of duplicate experiments with lower than 12% standard errors. OD600nm, optical density at 600 nm.

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TABLE 1. Highly differentially expressed Lager 1 genes (more than five fold) after growth in zinc-depleted conditions compared to zinc-replete conditions

Induced by zine depletion Further Zap 1p target genes PURLN2 80 VOR.250w 280 VOR.250w 2401 VOR.250w 2401 VOR.250w 2401 VOR.250w 2401 VOR.250w 2401 VOR.250w 2411 VOR.255w 2417 VOR.256w 25 VOR.256w 25 VOR.256w 25 VOR.256w 25 VOR.256w 26 VMR.151c 23 VMR.152w 21	Open reading frame	Gene name	Fold induction or repression ^a	Gene or protein description ^b
YOR 255%80YGL 258wG3YGL 258wZPS1YGL 256wADH4YGL 256wADH4YGL 256wADH4YGL 256wZAP1Involved in zinc-responsive transcriptional regulationYGL 255wZRT1YGL 255wZRT1YGL 255wZRT2YGL 255wZRT1YGL 255wZRT2YGL 255wZRT1YGL 255wZRT1YDR24eDPP1GPG16.2GPG16.2GPG16.2YGL 255wCTT1YGR 268wCTT1YGR 268wCTT1YGR 268wCTT1YGR 268wCTT1YGR 268wCTT1YGR 268wCTT1YGR 268wGTT1YGR 268wGTT1YGR 268wGTT1YGR 278wHSP26YMR 275wSIP18YH 278wSIP18YGR 268cGTT1YH 278wSIP18YH 278wSIP18YH 278wSIP18YH 278wYADPH-dependent reductaseYH 278wYADPH-dependent reductaseYH 278wGN22YH 278wGN23YH 278wGN24YH 278wGN22YH 278wGN22YH 278wGN22YH 278wGN22YH 278wGN22YH 278wGN22YH 278wGN22YH 278wGN22YH 278wGN22YH 278wGN22 <td< td=""><td>Induced by zinc depletion Putative Zap 1p target genes</td><td></td><td></td><td></td></td<>	Induced by zinc depletion Putative Zap 1p target genes			
YOL 154w263YOL 154wZFSI28YOL 154wADFI413Alcohol dehydrogenase isoenzyme IVYIL 056YIL 056cZ/P110Inrobverk8.5YKL 175wZR718.1YKL 175wZR718.1YIL 136cZR718.1YIL 136cZR716.8Diacylglycerol pyrophosphate phosphataseYGL 121cGPGI6.2Or protein gammaPutative Msn2p-Msn4p target genesYPL 232cGRE127YPL 70000026YGR 700000025Heat shock protein 15 k DaYWR 700000026YWR 7000000026YWR 7000000000000000000000000000000000000	YOR387c		80	
YOL 154wZPS128YGL 256wADH413Alcohol dehydrogenase isoenzyme IVYUL056cZAP110Involved in zinc-responsive transcriptional regulationYUL057cZRT38.3Vacuolar Za refluxYUL75wZRT78.1High-affinity zinc transport proteinYUL75wZRT27.7Low-affinity zinc transport proteinYUR36cZRT27.7Low-affinity zinc transport proteinYUR284cDPP16.8Diacylglycerol prophosphate phosphataseYGL255wZRT26G protein gammaPutative Msn2p-Msn4p target genesYPL221cGRE127Hydrophilic polypeptide with no known homologyYGR058wCTT126Cytoplasmic catalase TYBR070c26Heat shock protein 26YMR175wSIP1817Salt-induced protein of 18 kDa-YWU128cGIN39.5YHR038cGTT110YHL128cGIN39.5YHR128c8YHR128c7.2YHR04w7.5YHR128c7.2YHR04w7.5YHR128c7.2YHR04wGRE3YHR128c7.3YHR128c7.4YHR128c7.4YHR128c7.4YHR128c7.4YHR128c7.4YHR128c7.4YHR128c7.4YHR128c7.4YHR128c7.4YHR128c7.4YHR128c	YGL258w		63	
$\dot{V}DL256w$ $\dot{AD144}$ 13Alcohol dehydrogenase isoenzyme IV $YILD56c$ $ZAPI$ 10Involved in zinc-responsive transcriptional regulation $YILL75w$ $ZR71$ 8.5Vacuolar Zn efflux $YGL255w$ $ZR71$ 8.1Higb-affinity zinc transport protein $YLR130c$ $ZR72$ 7.7Low-affinity zinc transport protein $TDR254c$ $DPP1$ 6.8Diacyleycerol pyrophosphate phosphatase $YDR254c$ $DPP1$ 6.8Diacyleycerol pyrophosphate phosphatase $YUL252c$ $GRE1$ 27Hydrophilic polypeptide with no known homology $YRL232c$ $GRE1$ 27Hydrophilic polypeptide with no known homology $YDR070c$ 26 $YWR181c$ 23 $YWR175w$ $SIP18$ 17 $YMR175w$ $SIP18$ 16 $YMR175w$ <	YOL154w	ZPS1	28	
TLD56c $ZAPI$ 10Involved in zinc-responsive transcriptional regulation $YILD56c$ $ZRT3$ 8.3Vacuolar Zn efflux $YILD57w$ $ZRT1$ 8.1High-affinity zinc transport protein $YILR130c$ $ZRT2$ 7.7Low-affinity zinc transport protein $YILR130c$ $ZRT2$ 7.7Low-affinity zinc transport protein $YILR130c$ $ZRT2$ 7.7Low-affinity zinc transport protein $YIL221c$ $GPCI$ 6.8Diacylgbycerol pyrophosphate phosphatase $YVL221c$ $GPCI$ 6.2G protein gammaPutative Msn2p-Msn4p target genes $YYL223c$ $CYtplasmic catalase TYVD223cCTTI26Cytplasmic catalase TYDR070c2625Heat shock protein 26YMR175wSIP1817Salt-induced protein of 18 kDaYOL053c-ADDR212Induced by heat shock, and oxidative sYIRJ23cGTT110Glutathione transferaseYMR175wSIP189.5Meiotic sister chromatid recombinationYIL128cGIN39.5Meiotic sister chromatid recombinationYIRJ23cRS75SSA37.7YIRJ38cR6T2YIRJ38cR6T2YIRJ38cR6YIRJ38cR6YIRJ38cR6YIRJ38cR6YIRJ38cR6YIRJ38cR6YIRJ38cR6YIRJ38cR6YIRJ38cYGL256wADH413Alcohol dehvdrogenase isoenzyme IV$	YGL256w	ADH4	13	Alcohol dehvdrogenase isoenzyme IV
WIL 10%L118.5WIL 10%ZRT18.5Vacuolar Zn effluxYGL255wYGL255wZRT18.1High-affinity zinc transport protein1PLR130cZRT27.7Low-affinity zinc transport protein1PUR254cDPP16.8Diardy ZerGRE127PUtative Msn2p-Msn4p target genes1PVL22cGRE127PVR070c26TVDR070c26WMR151c23YMR175wSIP1817Salt-induced protein of 18 kDaYMR175wSIP1817Salt-induced potein of 18 kDaYMR175wSIP18YML128cGIN3YMR175cSA3YMR175cSA43YMR175cSA43YMR175cSA43YMR107w2YHR107w6.1YHR107w6.1YHR107w72YHR107w6.4YHR107w72YHR107w74YHR106w74YHR106w74YHR106w74YHR106w74YHR106w74YHR106w74YHR107w74YHR106w <td>YIL 056c</td> <td>ZAP1</td> <td>10</td> <td>Involved in zinc-responsive transcriptional regulation</td>	YIL 056c	ZAP1	10	Involved in zinc-responsive transcriptional regulation
YEL 175:wZRT38.3Yacubar Zn effuxYGL 175:wZRT18.1High-affinity zinc transport proteinYLR136:wZRT27.7Low-affinity zinc transport proteinYDR284:wDPP16.8Diacylgycorel pyrophosphate phosphataseYCL121cGPG16.2G protein gammaPutative Msn2p-Msn4p target genesYPL223:wGREJ7YDR070:w26C tytoplasmic catalase TYDR070:w26SYMR175:wSP12Salt-induced protein of 18 kDaYMR175:wSP1817Salt-induced protein of 18 kDaGuatamic catalase TYDR070:w23YMR175:wSP18YMR175:wSP11YMR175:wSP11YMR175:wSalt-induced protein of 18 kDaYMR175:wGIN39.5Micotic sister chromatid recombinationYTL124:w9YMR175:w7.7YMR175:w7.7YMR175:wYMR175:w7.7YMR175:wYMR175:wYMR175:wYMR175:wYMR175:wYMR175:wYMR175:wYMR175:wYMR173:wYMR175:wYMR173:wYMR173:wYMR173:wYMR173:wYMR173:wYMR173:wYMR173:wYMR173:wYMR173:wYMR173:wYMR173:wYMR173:wYMR173:wYMR173:wYMR173:wYMR173:w <t< td=""><td>YBL 049w</td><td>221111</td><td>85</td><td>involved in zhie responsive transcriptional regulation</td></t<>	YBL 049w	221111	85	involved in zhie responsive transcriptional regulation
VGL255ZRT18.1High-affinity zine transport protein $YGL255$ ZRT27.7Low-affinity zine transport protein $YIR236c$ DPP16.8Diazylgtycerol pyrophosphate phosphatase $YGL2121c$ GPG16.2G protein gammaPutative Msn2p-Msn4p target genes $YYL232c$ GRE127 $YVL232c$ GRE127Hydrophilic polypeptide with no known homology $YGL0808w$ CTT126Cytoplasmic catalase T $YVL070rbc$ 2625Heat shock protein 26 $YRR072w$ HSP2623Salt-induced protein of 18 kDa $YOL073c-A$ DDR212Induced protein of 18 kDa $YOL073c-A$ DDR212Induced protein of 18 kDa $YR033c-A$ DDR212Induced protein of ransferase $YHL128c$ GH39.5Meiotic sister chromatid recombination $YFL014w$ HSP12912-kDa heat shock protein $YHL128c$ GN39.5Meiotic sister chromatid recombination $YHL128c$ GN26.172 $YHR060c$ HXT56.9 $YR017w$ 7.5Cytosolic member of the 70-kDa heat shock protein f $YHL104w$ GRE35.8 $YR116c$ 7.7 $YHR04w$ GRE35.2 $YR1106w$ GRE35.2 $YR1106w$ 8.7 $YR2150w$ SP115.6Component of cell wall $YLL150w$ MCR15.0 $YR1106w$ 8.2 $YR2150w$ PIR35.2 </td <td>YKL175w</td> <td>ZRT3</td> <td>83</td> <td>Vacuolar Zn efflux</td>	YKL175w	ZRT3	83	Vacuolar Zn efflux
ND220% VLR130cZRT15.1Importantly Junc Intrasport protein Protein DPP1VLR130cZRT27.7Low-affinity Junc Intrasport protein 	VCL 255w	ZR15 7RT1	8.1	High affinity zing transport protein
TDR234: TDR24: TDR24:DP1 GPC16.8 	VI R130c		77	Low affinity zinc transport protein
Dial ProcessDial Process<	VDP284c		6.8	Disculational purenhasehate phosphatase
Putative Msn2p-Msn4p target genes $VPL 23c$ $GRE1$ 27 Hydrophilic polypeptide with no known homology $VGR088w$ $CTTI$ 26 $Cytoplasmic catalase TVDR070c26YBR072wHSP2625YMR18Lc23YMR175wSIP1817Salt-induced protein of 18 kDaY0.053c-ADDR212Induced by heat shock, cosmotic shock, and oxidative sYR038cGTT110YR122cGIN39.5YML128cGIN39.5YML128cGIN39.5YBL075cSSA37.7YDL075cSSA37.7YRL078c8.6YBL075cSSA37.7YRL078cRE36.4YBL078cRE36.4YBL075cSSA37.7YRL078w9YL078cSA33YRL078w6.1YRL078w6.1YRL078w7.2YRL078w6.1YRL078w6.1YRL078w6.1YRL078wSR23YRL078wSR23YRL078wSR23YRL078wSR23YRL078wSR11SC256wGRE3YRL08wSP11SC30wSP11SC40wSC40wYRL172wAPC1SC30wSC20wYRL173w^{2}APC1$	YGL121c	GPG1	6.2	G protein gamma
YPL232 YGR088wCTT127 GRE1Hydrophilic polypeptide with no known homology Cytoplasmic catalase TYGR070c26YBR072wHSP2625YMR175wSIP18YMR18Lc23YMR175wSIP18YMR25cGTT1YGR038eGTT1Glutathione transferaseYMR175wSIP18YL128cGIN39.5Meiotic sister chromatid recombinationYFL014wHSP12912-kDa heat shock, somotic shock, and oxidative sYMR173kc86YBL075cSSA3YMR107w75YMR107w72YHR107w72YHR107w72YHR107w61YHR107w61YHR104wGRE3YHR105w57YHR104wGRE3YHR105w57YHR105wSP115.6Component of cell wallYKL150wMCR1YKL150wMCR1YKL150wMCR1YKL150wMCR1YKL173wAPC18.2Subunit of ubiquitin-protein ligaseYKL173wAPC18.2Subunit of ubiquitin-protein ligaseYKL173wAPC18.2Subunit of ubiquitin-proteinYMR173w-A64YMR173w-A64YMR173w-A64YMR173w-A64YMR173w-A64YMR173w-A64YMR173w-A64YMR173w-A64YMR173w-A<	Putative Msn2p-Msn4p target gene	es		
YGR088wCTT126Cytoplasmic catalase TYDR070c26YBR072wHSP2625YMR181c23YMR175wSIP1817Salt-induced protein of 18 kDaYOL053c-ADDR212Induced by heat shock, osmotic shock, and oxidative sYR038cGTT110Glutathione transferase10YML128cGIN39.5Mieotic sister chromatid recombinationYFL014wHSP129YDL124w9YDL124w9YHR138c8.6YBR07cSSA37.7YKL151c7.2YHR096cHXT56.9YDL204w6.1YHR104wGRE35.8YBR16c5.7YER150wSP11YKL150wMCR1YKL150wPIR3YKL150wSP11YKL150wACYFR150wSP11Stan-Keto-aldose reductaseYBR16c5.7YER150wMCR1YDL172wAPC1YOR374wALD4YL172wAPC1YDL32w7.8YUL173wDDR48YDL126c7.8YUL173wAPC1YDL1274w6.1YMR173w-A6.4YDL123b7.8YUL173wAPC1YDL204w7.8YUL239wLAP3YDL204w7.8YDL204w7.8YDL204w7.9YDL204w7.0 <tr< td=""><td>YPL223c</td><td>GRE1</td><td>27</td><td>Hydrophilic polypeptide with no known homology</td></tr<>	YPL223c	GRE1	27	Hydrophilic polypeptide with no known homology
YDR070c261.1.YBR072wHSP2625Hat shock protein 26YMR181c233YMR175wSIP1817Salt-induced protein of 18 kDa701.035c-AVDD21Induced by heat shock, somotic shock, and oxidative sYR038cGTT110Glutathione transferaseGIN3YML128cGIN39.5MillMSP129YL124w9YDL075cSSA37.7YR1078c7.5YR1078c7.2YHR178c8.6YR1078c7.2YHR096cHXT56.9HKR096cHXT56.9YDL204w6.1YHR104wGRE35.8~Creation of cell wall7.7YER150wSP115.6Component of cell wall7.8YKL153wMCR15.0YKL150wMCR15.0YKL150wMCR15.0YKL150wMCR15.0YKL150wMCR15.0YKL150wMCR15.0YKL150wMCR15.0YKL150wMCR15.0YKL150wMCR15.0YKL150w7.8YKL150w7.8YKL150w7.8YKL150wMCR1YKL150w7.8YKL150w7.8YKL150w7.8YKL150w7.8YKL150w7.8YKL150w7.8YKL150w7.8YKL150w7.8	YGR088w	CTT1	26	Cytoplasmic catalase T
YBR072wHSP2625Heat shock protein 26YMR181c23YMR175wSIP1817Salt-induced protein of 18 kDaYOL053c-ADDR212Induced by heat shock, somotic shock, and oxidative sYR038cGTT1YML128cGIN3YPL014wHSP12912-kDa heat shock proteinYPL014w9NADPH-dependent reductaseYHR138c8.6YHR075cSSA3YR107c7.5YKL151c7.2YHR096cHXT5YHR096cHXT5YHR104wGRE3YEL154w5.7YKL154w5.7YKL154w7.2YHR096cSP115.6Component of cell wallYKL154w5.7YKL154w9YER155wSP115.6Component of cell wallYKL150wMCR1S0NADH-cytochrome b_5 reductaseYKL150wAPC1S1.0NADH-cytochrome b_5 reductaseYKL155w7.8YUL230wAPC1YUL230w7.8YUL172wAPC1YUL173wDDR487.3Flocculent-specific proteinYMR173w-A6.46.9Induced by starvationYMR173w-A6.4YUL230w7.8YUL230wAPC1YUL230w7.8YUL230w7.8YUL230w11511G7Homologue of mammalian TIS11YUL73	YDR070c		26	
YMR181c23YMR175wSIP1817YML058c-ADDR212Induced by heat shock, osmotic shock, and oxidative sYMD38cGTT1YML128cGIN3YML128cGIN3YML128cGIN3YML128cYML128cYML128cGIN3YML128c9YML128c9YML128c9YML128c9YML128c9YML128c9YML128c9YML128c9YML128c72YMR107w75YKL151c72YMR107w75YKL151c72YHR096cHXT5GND26.1YHR104wGRE3S.8 α -Keto-aldose reductaseYDL204w5.1YER150wSP11S.6Component of cell wallYKL163wPIR3S.2Protein containing tandem internal repeatsYKL163wPIR3S.2Protein containing tandem internal repeatsYKL150wMCR1Other γ YOL374wALD5YL056w8.2YMR173wDDR487.3Flocculent-specific proteinYMR178c6.9Homologue of mammalian TIS11YMR178w-A6.4Overlaps DDR48YMR173w-X6.1YMR173w-X6.1YMR173w-X6.1YMR173be5.9YMR174c5.9YMR175c7.0<	YBR072w	HSP26	25	Heat shock protein 26
YMR/75wSIP1817Salt-induced protein of 18 kDaYOL053c-ADDR212Induced by heat shock, somotic shock, and oxidative sYR033cGTT110Glutathione transferaseYML128cGIN39.5Meiotic sister chromatid recombinationYFL014wHSP129I2-kDa heat shock proteinYPL124w9NADPH-dependent reductaseYHR138c8.6SK43YHR107w7.5YKL151c7.2YHR096cHX756.9YHR104w6.1YHR104wGRE35.8QC50wSP115.6YBR116c5.7YER150wSP11YKL163wPIR3YKL150wSP11YKL150wACYKL150wACYKL150wACYKL150wACYKL150wACYKL150wSP11S.6Component of cell wallYKL150wACYKL150wACYC0082w7.5YKL150wACYOL082w7.8YNL056w8.2YOL082w7.8YMR173wDDR48YMR173wDDR48YMR173wALD5YMR173wDDR48YMR173wDDR48YMR173wACYMR173wALD5YMR173wACYMR173wACYMR173wACYMR173wACYMR174w6.9YMR175c7.4YMR175c7.4 </td <td>YMR181c</td> <td></td> <td>23</td> <td></td>	YMR181c		23	
TODOSTODOTOYOL053c-ADDD212Induced by heat shock, smotic shock, and oxidative sYR038cGTT110Glutathione transferaseYML128cGIN39.5Meiotic sister chromatid recombinationYFL014wHSP12912-kDa heat shock proteinYDL124w9NADPH-dependent reductaseYBL075cSSA37.7YBL075cSSA37.7YKL151c7.2YHR096eHXT56.9YDL24w6.1YGR256wGND26.4G-Phosphogluconate dehydrogenaseYDL204w6.1YHR104wGRE35.8ac-Keto-aldose reductaseYHR1665.7YEX150wSP115.6YKL150wMCRIYKL163wP1R35.2YKL163wP1R35.2YKL163wALD424YOR374wALD59.5Mitochondrial aldehyde dehydrogenaseYNL056w7.8YNL056w7.8YMR173wDDR487.3YMR173wDDR487.3YMR173wDDR487.4YMR173wLDR487.4YMR173wLDR487.4YMR173wLDR487.4YMR173wLDR487.4YMR173wLDR487.4YMR173wLDR487.4YMR173wLDR487.5YMR173wLDR487.4YMR173wLDR487.4YMR173wLDR48	YMR175w	SIP18	17	Salt-induced protein of 18 kDa
TOLOGOVALDTRL12Inductor of the ansket, sindex, on outdative \cdot YIRU328cGIN39.5Meiotic sister chromatid recombinationYFL014wHSP12912-kDa heat shock proteinYDL124w9NADPH-dependent reductaseYHR138c8.6YBL075eSSA37.7YKL151c7.2YHR096cHXT56.9YDL204w6.1YHR104wGRE35.8acKeto-aldose reductase5.7YKL150wSP11S.6Component of cell wallYKL163wPIR3YKL163wPIR3YKL150wMCR1S.0NADH-eytochrome b_5 reductaseYKL150wMCR1YKL150wMCR1YKL150wALD4YKL150wALD4YKL150wALD4YKL150wALD4YKL150wALD4YKL150wS10YKL150wALD4YKL150wALD4YKL150wALD4YKL150wALD4YKL150wALD4YKL150wALD4YKL150wALD4YKL150wALD5YKL150wALD4YKL150wALD4YKL150wALD5YKL150wALD4YKL150wALD5YKL150wALD4YKL150wALD5YKL150wALD5YKL150wALD4YKL150wALD5YKL150wALD5YKL150wALD5YKL150wALD5 <td>$VOI 053c_{-}4$</td> <td></td> <td>12</td> <td>Induced by heat shock osmotic shock and oxidative stress</td>	$VOI 053c_{-}4$		12	Induced by heat shock osmotic shock and oxidative stress
ThroughOf ThToOf the differenceYML128cGTN39.5Meiotic sister chromatil recombinationYFL014wHSP129NADPH-dependent reductaseYDL124w9NADPH-dependent reductaseYBL075cSSA37.7YBL075cSSA37.7YHR138c7.2YHR096cHXT56.9YHR107w7.2YHR096cHXT56.9YHR104wGRE35.8QR256wGND26.46.1YHR104wYHR104wGRE35.8QR2510wSP115.6Component of cell wallYKL153wPIR35.2YKL153wPIR35.2YKL150wMCR15.0NADH-cytochrome b ₅ reductaseYKL150wALD59.5Mitochondrial aldehyde dehydrogenaseYNL056w8.2YNL056w8.2YMR173wDDR487.3Flocculent-specific proteinYMR136c11.51YMR136c6.1YMR136c7.3YMR136c7.3YML73w-A6.4YMR173w-A6.4YMR173w-A6.4YMR173w-A6.4YMR173w-A6.1YMR173w-A6.1YMR077c5.8YDR543c5.6YDR543c5.6YDR543c5.6YDR543c5.6YDR543c5.6YDR543c5.6YDR543c5.6YDR	VIR038c	GTT1	10	Glutathione transferase
TML120COT/S5.5Metode siter curonitation $YTL014w$ HSP12912-kDa heat shock protein $YDL124w$ 9NADPH-dependent reductase $YHR138c$ 8.6 $YBL075c$ SSA37.7 $YMR107w$ 7.5 $YKL151c$ 7.2 $YHR096c$ HXT56.9 $YDL204w$ 6.1 $YDL204w$ 6.1 $YHR104w$ GRE35.8 $YBR116c$ 5.7 $YER150w$ SP115.6 $YRL153w$ PIR35.2 $YKL153w$ PIR35.2 $YCR374w$ ALD424 $YL07374w$ ALD424 $YL07374w$ ALD59.5 $YL172w$ APC18.2 $YL172w$ APC18.2 $YMR173w$ DDR487.3 $YMR173w$ DDR487.3 $YMR173w$ LAP36.1 $YDR543c$ 5.6 $YDR543c$	VMI 1282	CIN2	10	Majotic sister chromatid recombination
TPLD14wHST29T2-AD heat shock proteinYDL124w9NADPH-dependent reductaseYHR138c8.6YBL075cSSA37.7YMR107w7.5YKL151c7.2YHR096cHXT56.9YOL204w6.1YHR104wGRE35.8YER150wSP115.6YER150wSP115.6YKL151w7.2YHR094w6.1YKL163wPIR35.2YER150wSP115.6YKL150wMCR15.0YKL163wPIR35.2YKL150wMCR15.0YKL150wAPC18.2SVR073wALD59.5YNL172wAPC18.2YNL056w7.8YNL173wDDR487.3YMR138c6.9Induced by starvationYMR138c115.16.7YMR138c6.9Induced by starvationYMR138c6.9Induced by starvationYMR138c6.7Homologue of mammalian TIS11YMR138c6.7Homologue of mammalian TIS11YMR138c5.85.6YDR543c5.65.6YDR543c5.6YertYDR543c5.6YertYDR543c5.6YertYDR543c5.4Yert	VEL 014		9.5	12 hDe heat sheek motion
The L124w9NADPH-dependent reductaseYHR138c8.6YBL075cSSA3YBL075cSSA3YKL151c7.5YKL151c7.2YHR096cHXT5YDL204w6.1YHR104wGRE3YBR116c5.7YER150wSPI1SOComponent of cell wallYKL151wMCR1SONADH-dependent reductaseYBR116c5.7YER150wSPI1SOSOYKL150wMCR1SONADH-cytochrome b_3 reductaseOther1000000000000000000000000000000000000	IFL014W VDL124	HSF12	9	12-KDa neat shock protein
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YNL056w8.2YOL082w7.8YMR173wDDR48YMR173wDDR48YMR118c6.9YLR136cTIS11YMR173w-A6.4YNL239wLAP3YNR077c5.8YDR543c5.6YET15.4YET15.4YACT5.4	YNL172w	APC1	8.2	Subunit of ubiquitin-protein ligase
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YNL239wLAP36.1Aminopeptidase of cysteine protease familyYNR077c5.8YDR543c5.6YET15.4	YMR173w-A		6.4	Overlaps DDR48
YNR077c 5.8 YDR543c 5.6 VET1 5.4	YNL239w	LAP3	6.1	Aminopeptidase of cysteine protease family
YDR543c 5.6 YETI 5.4 Vent endedemie estimitur 25 PD (consultant	YNR077c		5.8	
VVI 065a VET1 54 Vasat en deglacaria estimbra 251 D. (conserved)	VDR543c		5.6	
INLUUSC IEII 5.4 Yeast endoplasmic reticulum 25-kDa transmembrane	YKL065c	YET1	5.4	Yeast endoplasmic reticulum 25-kDa transmembrane
protein				protein
YBR214wSDS245.1Nuclear protein similar to Schizosaccharomyces pombe sYIR037wHYR15.1Putative glutathione peroxidase	YBR214w YIR037w	SDS24 HYR1	5.1 5.1	Nuclear protein similar to Schizosaccharomyces pombe sds23 Putative glutathione peroxidase
Repressed by zinc depletion	Repressed by zinc depletion			
YOL086c ADH1 15 Alcohol dehydrogenase	YOL086c	ADH1	15	Alcohol dehydrogenase
<i>YMR116c</i> ASC1 10 WD repeat protein (G-beta-like protein)	YMR116c	ASC1	10	WD repeat protein (G-beta-like protein)
YLR150w MPT4 9.2 Telomeric DNA binding activity	YLR150w	MPT4	9.2	Telomeric DNA binding activity
YBR299w MAL32 9.1 α-Glucosidase. hvdrolvses maltose	YBR299w	MAL.32	9.1	α -Glucosidase, hydrolyses maltose
YGR292w MALL2 85 α-Glucosidase hydrolyses maltose	YGR292w	MAL12	8.5	α -Glucosidase, hydrolyses maltose
YBR298c MAL31 7.2 High-affinity maltose permease	YBR298c	MAI.31	7.2	High-affinity maltose permease
YHR183w GND1 5.3 6.Phosphiconate debudragenase	YHR183w	GND1	53	6-Phosphogluconate dehvdrogenase

Continued on facing page

TABLE 1—Continuea					
Open reading frame	Gene name	Fold induction or repression ^a	Gene or protein description ^b		
Ribosomal protein subunits					
YPL131w	RPL1				
YOR063w	RPL3	9.1			
YGL147c	RPL9A	7.6			
YLR325c	RPL38	6.4			
YHR010w	RPL27	5.8			
YLR029c	RPL13A	5.8			
YJL177w	RPL17B	5.7			
YKR094c	RPL40B	5.7			
YOR293w	RPS10A	5.6			
YOR096w	RPS30	5.5			
YKL180w	RPL17	5.5			
YLR048w	RPS0B	5.5			
YHR203c	RPS7A	5.4			
YGL123w	RPS2	5.3			
YLR062c		5.3			
YML073c	RPL6A	5.2			
YBR191w	RPL21A	5.1			
YDR450w	RPS18A	5.1			
YGR027c	RPS31A	5.0			
YIL148w	RPL40A	5.0			

TABLE 1—Continued

^{*a*} Induction factors were calculated by dividing the expression levels in zinc-depleted conditions by those in zinc-replete conditions. Values are means of duplicate experiments for genes with less than 20% variation between the duplicate experiments.

^b Gene annotations were obtained with GeneSpring (A. R. Conway, GeneSpring, version 5.0, Silicon Genetics, Redwood City, Calif., 2002).

(SPII) proteins were used as predictors of stress conditions in industrial fermentations (2, 17) and the maltose genes (MAL)have been used to monitor conditions that affect yeast fermentation activity (19). However, because these genes respond to a broad range of conditions, their usefulness for the identification of the specific cause of a defective fermentation is limited. Here we identify molecular markers useful in monitoring industrial fermentations specifically for conditions of zinc deficiency.

To identify genes useful as molecular markers, genome-wide expression analysis was performed on the *Saccharomyces cerevisiae* industrial Lager 1 strain (6) growing in zinc-depleted and zinc-replete conditions. The low-zinc medium was essentially LZM (23) except that the carbon source was changed to maltose (LZMM), which is the most abundant sugar available to yeast in beer fermentations (21). Lager 1 seed cultures actively growing in LZMM-40 μ M ZnSO₄ were harvested and washed three times with sterile distilled water before inoculation into LZMM with and without 40 μ M ZnSO₄. RNA was isolated from cells at 4 h, when the rates of growth of the two cultures were beginning to diverge (Fig. 1). Radiolabeled (³³P) cDNA produced from isolated RNA was hybridized to Gene-Filters microarrays (Research Genetics) as outlined by Higgins et al. (10). Genes that were induced or repressed more than fivefold in zinc-depleted conditions are listed in Table 1. The majority of the induced genes have been previously reported to be possible targets for the transcriptional activators Zap1p (14) and Msn2p-Msn4p (8) and were therefore subsequently



FIG. 2. Induction of *YOR387c* and *YGL258w* in zinc-depleted conditions is dependent on Zap1p. *YOR387c* and *YGL258w* and *ACT1* expression in wild-type (BY4743) and *zap1* Δ mutant cells grown in Chelex-treated synthetic defined (CSD) medium (14) with (Zn⁺) and without (Zn⁻) 10 μ M ZnSO₄ added was measured.



FIG. 3. YOR387c and YGL258w expression is not induced by oxidative stress or carbon starvation. YOR387c and YGL258w, ZRT1, HSP12, and ACT1 expression was measured in Lager 1 cells grown in modified LZMM with (Zn^+) or without (Zn^-) 40 μ M ZnSO₄ added, LZMM (Zn^+) with 2 mM H₂O₂ added (H₂O₂), and LZMM (Zn^+) with no added maltose (C⁻).

grouped into these categories. The metalloregulatory protein Zap1p is involved in zinc-responsive transcriptional regulation in Saccharomyces cerevisiae (25, 26). The ZRT1 and ZRT2 genes (induced 8.1- and 7.7-fold, respectively) encode highand low-affinity zinc permeases, respectively, and both are Zap1p targets (23, 24). Another Zap1p target induced was the ZRT3 gene (induced 8.3-fold), which encodes the yeast vacuolar permease (15). Up-regulation of these genes would enhance the ability of the Lager 1 strain to maintain intracellular zinc levels. The HSP12 and HSP26 genes, encoding heat shock proteins, were also induced by zinc depletion (Table 1). Both of these are targets of Msn2p-Msn4p and, unlike the Zap1p targets, are induced by a broad range of conditions, including general starvation (1, 18). The presence of a general starvation response is further highlighted by the repression of genes involved in sugar utilization (MAL genes), ethanol production (ADH1), and ribosomal functions (Table 1), all of which are vital for yeast growth and metabolic activity.

Further expression analyses were performed on highly induced candidate genes from the listed Zap1p targets in Table 1. These were chosen for their likely specificity for zinc depletion since those activated by the Msn2p-Msn4p complex are subject to a wide range of stresses (16, 20). The Lager 1 genes most highly induced in response to zinc-depleted conditions were YOR387c and YGL258w (Table 1), both of which have no known cell function (11). They are highly homologous genes, with over 92% DNA identity in their coding regions; this homology probably results in cross-hybridization during expression analyses. Since the promoter regions of these genes are almost identical (99.6% over 1 kb of upstream sequence), both transcripts were regarded as one in subsequent tests. The other genes selected for analysis were ZRT1, based on its specificity to zinc-depleted conditions (23), and the highly induced gene ZPS1 (Table 1), whose product has weak homology to zinc metalloproteinases (11). The kinetics and degrees of induction of the selected genes were measured by Northern analyses (10) during a 10-h incubation in zinc-depleted and zinc-replete medium. By the fourth hour all four genes were induced in zincdepleted conditions (Fig. 1), validating the expression patterns seen in the genome-wide expression analysis (Table 1). YOR387c and YGL258w were rapidly induced, with a very clear increase in transcripts by the second hour of exposure to zinc depletion (Fig. 1). This analysis shows that the highly differential nature of expression of YOR387c and YGL258w and of ZPS1 (Table 1) can be attributed not only to very high levels of expression in zinc-depleted conditions but also to very low expression levels when zinc was present. This is in contrast to what was found for ZRT1, which, although it has increased expression in zinc-depleted conditions, has a relatively high basal level, effectively decreasing its apparent induction. This was surprising since an analysis of ZRT1 expression in a laboratory yeast strain did not show high basal levels (23). This characteristic may be unique to the Lager 1 strain, or other mechanisms may be involved, a possibility supported by observations that changes in nitrogen source affect ZRT1 expression levels (5). Although highly induced by zinc depletion, ZPS1 expression was also found to be affected by changes in pH (13). This is unlikely with YOR387c and YGL258w since an investigation of microarray data using the Yeast Microarray Global Viewer (Laboratoire de Genetique Moleculaire, Ecole Normale Superieure, Paris, France [http://transcriptome.ens.fr /ymgv/who.php]) did not identify any other conditions that significantly affected its expression.

YOR387c and YGL258w were identified in a genome-wide characterization of the zinc-responsive regulon (14) as possible Zap1p targets. To verify this, YOR387c and YGL258w expression in a zap1 Δ mutant was measured. The YOR387c and YGL258w transcripts were not evident in the zap1 Δ mutant when the mutant was grown in zinc-depleted conditions, whereas they were characteristically present at high levels in the wild type (Fig. 2). This confirmed that YOR387c and



FIG. 4. YOR387c and YGL258w expression is not induced by iron depletion. Shown are YOR387c and YGL258w, ZRT1, ARN2, and ACT1 expression patterns from GeneFilters microarray analysis of Lager 1 cells grown for 4 h in low-iron medium with (Fe⁺) and without (Fe⁻) 25 μ M FeCl₃ added.

YGL258w expression is induced through the Zap1p transcriptional activator in response to zinc deficiency.

To further analyze the specificity of *YOR387c* and *YGL258w* induction, their expression in a Lager 1 strain exposed to oxidative stress and carbon starvation, two conditions known to impact yeast during industrial fermentations (3), was measured. Figure 3 shows that *YOR387c* and *YGL258w* expression was not induced in these conditions. A similar pattern of induction was observed with the *ZRT1* transcript; however, the basal level of expression in unstressed conditions was higher. The integrity of the stress conditions was confirmed by the induction of the Msn2p-Msn4p-activated gene, *HSP12*, in all stress conditions compared to induction in the control medium (Fig. 3).

Iron, another trace element in yeast, is, like zinc, essential for yeast growth and metabolic activity (22). To determine the effect of iron depletion on *YOR387c* and *YGL258w* expression, transcript levels in the Lager 1 strain growing in low-iron medium (7) were measured by genome-wide expression analysis. From Fig. 4 it is clear that *YOR387c* and *YGL258w* were not induced in response to iron-depleted conditions and therefore are not part of a general response to depletion of divalent cations. *ZRT1* on the other hand showed some induction. The induction of the *ARN2* gene, which encodes a siderochrome iron transporter (9), confirmed the iron-depleted condition (Fig. 4).

Genome-wide expression analysis was useful in identifying *YOR387c* and *YGL258w* as molecular markers of zinc-deficient conditions. The expression of these genes was highly inducible from a virtually undetectable basal level, making them easily discernible targets for detection of differential expression by rapid methods of gene transcript analysis such as real-time PCR. These experiments and publicly available microarray information confirmed the specificity of *YOR387c* and *YGL258w* induction for zinc-depleted conditions and show that they are

induced 2 h before zinc depletion is seen to affect growth of the culture to a significant level. They are therefore not only effective predictors of defective fermentation but also identifiers of the actual cause so that subsequent preventative measures can be carried out.

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