ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, June 2005, p. 2246–2259 Vol. 49, No. 6
0066-4804/05/$08.00+0 doi:10.1128/AAC.49.6.2246–2259.2005

Transcriptional Response of *Enterococcus faecalis* V583 to Erythromycin

Ågot Aakra,[1]* Heidi Vebø,[1] Lars Snipen,[2] Helmut Hirt,[3] Are Aastveit,[2] Vivek Kapur,[4] Gary Dunny,[3] Barbara Murray,[5] and Ingolf F. Nes[1]*

Laboratory of Microbial Gene Technology[1] and Section for Bioinformatics and Data Analysis,[2] Department of Chemistry, Biotechnology and Food Science, Norwegian University of Life Sciences, Ås, Norway; Department of Microbiology, University of Minnesota Medical School, Minneapolis, Minnesota 55455[3]; Department of Veterinary Pathobiology, Biomedical Genomics Center, University of Minnesota, St. Paul, Minnesota 55108[4]; and Center for the Study of Emerging and Re-Emerging Pathogens, The University of Texas Medical School, Houston, Texas 77030[5]

Received 1 November 2004/Returned for modification 16 January 2005/Accepted 27 February 2005

A transcriptional profile of *Enterococcus faecalis* V583 (V583) treated with erythromycin is presented. This is the first study describing a complete transcriptional profile of *Enterococcus*. *E. faecalis* is a common and nonvirulent bacterium in many natural environments, but also an important cause of nosocomial infections. We have used a genome-wide microarray based on the genome sequence of V583 to study gene expression in cells exposed to erythromycin. V583 is resistant to relatively high concentrations of erythromycin, but growth is retarded by the treatment. The effect of erythromycin treatment on V583 was studied by a time course experiment; samples were extracted at five time points over a period of 90 min. A drastic change in gene transcription was seen with the erythromycin-treated cells compared to the untreated cells. Altogether, 260 genes were down-regulated at one or more time points, while 340 genes were up-regulated. Genes encoding hypothetical proteins and genes encoding transport and binding proteins were the two most dominating groups of differentially expressed genes. The gene encoding *ermB* (EFA0007) was expressed, but not differentially, which indicated that other genes are important for the survival and growth maintenance of V583 treated with erythromycin. One of these genes is a putative MsrC-like protein, which was up-regulated at all time points studied. Other specific genes that were found to be up-regulated were genes encoding ABC transporters and two-component regulatory systems, and these may be genes that are important for the specific response of V583 to erythromycin.

Enterococci are commonly found in the gastrointestinal tracts of humans and animals. Most enterococci are not virulent, and some are frequently found in artisan fermented foods, in which they contribute to longer shelf life, flavor, and texture. However, enterococci are best known as antibiotic-resistant opportunistic pathogens that are commonly recovered from patients who have received multiple courses of antibiotics and have been hospitalized for prolonged periods. Innate resistance to many antibiotics is a common trait among enterococci (see, e.g., reference 7 and the references therein). They also acquire antibiotic resistance determinants, including resistance to vancomycin, very rapidly from the environment (see, e.g., references 16–18). The two species best known to be involved in infections in human and animal bodies are *Enterococcus faecalis* and *E. faecium* (6).

The complete genome sequence of the vancomycin-resistant *E. faecalis* V583 (V583) is now available (20). Access to the genome sequence opens new possibilities to gain basic information on the molecular biology of the organism, and one of the tools that can be used to exploit the genome sequence experimentally is the DNA microarray technology. Microarrays give us the opportunity to study all transcriptional events going on in a cell and identify which genes are involved in certain cell processes in one experiment. The main advantage of the microarray technology is the ability to study the transcription of thousands of genes in one experiment. By nature, the microarray technology is explorative and hypothesis generating. The results of microarray experiments are, in principle, snapshots of the transcriptional activities of the cell. Moreover, since transcription and translation are coupled processes in prokaryotes, the transcriptome should reflect the proteome well. Indeed, it has been shown that regulation of the majority of genes parallels the level of proteins produced (2). On a genome-wide scale, it is difficult to speculate about which bacterial genes are regulated or not during certain conditions, and it is also hard to gain such information through the use of traditional low-throughput methods. By the use of microarrays, one can relatively quickly obtain information about gene expression levels and thereby explore the responses of the cells to changing growth conditions.

V583 survives and grows in media containing relatively high levels of the commonly used macrolide antibiotic erythromycin, but the addition of erythromycin to the culture retards cell growth. In sensitive cells, erythromycin inhibits protein synthesis by binding to the large ribosomal subunit close to the peptidyl transfer center. Protein synthesis is thereby aborted during early rounds of translation, since access to the nascent peptide channel is prevented (29). In gram-negative bacteria,

* Corresponding author. Mailing address: Laboratory of Microbial Gene Technology, Norwegian University of Life Sciences, P.O. Box 5003, N-1432 Ås, Norway. Phone: 47 64965894. Fax: 47 64941465. E-mail for Ågot Aakra: agot.aakra@umb.no.

intrinsic resistance to erythromycin is due to the impermeability of the cellular outer membrane to this hydrophobic macrolide. Two main mechanisms of erythromycin resistance that have been identified in gram-positive bacteria are as follows: (i) Target modification by the *erm* (erythromycin resistant methylase) genes that encode enzymes which methylate rRNA has been described. The rRNA methylation causes conformational changes in the P site of the rRNA and prevention of macrolide binding. (*erm*-mediated resistance in enterococci has been described. The pAD1-like plasmid pTEF1 of V583 encodes ErmB, an rRNA adenine dimethylase family protein.) (ii) A macrolide efflux resistance mechanism, which is an energy-dependent pump, has been described for both gram-positive and gram-negative bacteria (22).

This paper describes the use of a genome-wide amplicon-based microarray based on the genome sequence of V583 to obtain a profile of the transcriptional events in V583 cells treated with erythromycin. The aim of the work was to obtain an overview of how erythromycin affects transcriptional events and bacterial growth, aside from described resistance mechanisms, and how this bacterium tolerates stress. Taking into account the observed phenotypic effects of erythromycin on V583, it was expected that genes involved in general stress responses, genes involved in protein synthesis, and genes encoding (multi)drug resistance would be among the genes affected by the antibiotic treatment.

MATERIALS AND METHODS

Bacterial strain and growth conditions. The bacterium studied was *E. faecalis* V583 (V583), which was the first vancomycin-resistant clinical isolate reported in the United States (24) and whose genome sequence has been completed (20). V583 samples were grown overnight in flasks with brain heart infusion (BHI) medium (Difco) at 37°C on a rotary shaker (300 rpm). Cultures were then diluted 50× and grown in BHI, as described above, for 1 h. Cultures were split in two, and erythromycin (Sigma) was added to one of the cultures. The final concentration of erythromycin was 50 μg/ml. The two cultures (BHI and BHI plus erythromycin) were then incubated further, and 3-ml samples of each culture were collected immediately after the addition of erythromycin (*t*0), and after 15 (*t*15), 30 (*t*30), 60 (*t*60), and 90 (*t*90) min. Growth of the cultures was monitored spectrophotometrically (optical density at 600 nm [OD_{600}]). Samples were centrifuged for 5 min at 4°C, and pellets were flash frozen in liquid N_2 prior to RNA extraction.

Construction of V583 microarrays. PCR primers for all V583 open reading frames (The Institute for Genomic Research, May 2002 update) were designed using Primer3 (freeware; http://frodo.wi.mit.edu/cgi-bin/primer3/primer3_www.cgi), which has commonly been used for primer design during microarray construction (3, 13). Primers were designed to amplify full-length or ~500-bp amplicons from genomic DNA. PCRs (50-μl reaction volume) were run with 50 mM KCl; 10 mM Tris-HCl (pH 9.0); 0.1% Triton X-100; 2.5 mM $MgCl_2$; 2 μM of each primer; 0.2 mM of each dATP, dCTP, dGTP, and dTTP; 10 to 20 ng genomic DNA; and 1.25 U *Taq* polymerase (Promega). Amplifications were performed with Perkin-Elmer 9600 thermocyclers with an initial denaturation at 94°C for 3 min and then 35 cycles of denaturation at 94°C for 1 min, annealing at 54 to 56°C for 1 min (the annealing temperature varied depending on the melting temperatures of the primers), and an extension at 74°C for 1 min. The PCR was finalized with incubation at 74°C for 10 min. The quality of the PCR products was assessed by agarose gel electrophoresis. The PCR products were cleaned up using a QIAquick 96 PCR purification kit (QIAGEN). Of a total of 3,337 predicted open reading frames in the V583 genome, 3,160 PCR products were obtained. The purified PCR products were eluted in 3× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate)-0.01% sodium dodecyl sulfate (SDS) and printed on Corning UltraGaps slides. All PCR products, representing the predicted V583 open reading frames, were printed in five copies on the slides. In addition, negative controls (three genes from *Arabidopsis thaliana*, buffer [3× SSC, 0.01% SDS], and empty spots) and digested genomic DNA from V583 (positive control) were spotted on the arrays. Altogether, 3,502 samples were spotted on the slides.

RNA isolation. Total RNA extractions from the samples collected at the time points described above were performed with RNeasy Mini columns (QIAGEN), with DNA digestions being done on the columns by the addition of 82 Kunitz units of RNase-free DNase (QIAGEN) and incubation at room temperature for 15 min. The integrity of and concentration of RNA samples were measured using a RNA 600 Nano LabChip kit and a Bioanalyzer 2100 (Agilent Technologies). For both strains, 10 μg of each RNA sample was used in separate hybridization experiments on identical arrays.

cDNA synthesis, fluorescent labeling, hybridization, and microarray data analysis. For reverse transcription and cDNA synthesis, 30 μg of random hexamers (Amersham) and 10 μg of total RNA was initially preheated at 70°C for 10 min and was incubated for 10 min at 4°C and then for 2 h at 42°C in an Eppendorf Mastercycler. The reverse transcription (RT)-PCR mix was 1× first-strand buffer, 10 mM dithiothreitol; 380 units of Superscript II RT; 500 μM concentrations of dATP, dCTP, and dGTP; 300 μM dTTP (all from Invitrogen), and 200 μM amino-allyl-labeled dUTP (Sigma). After hydrolysis with 10 μl of 1 M NaOH and 10 μl of 0.5 M EDTA for 15 min at 65°C, the samples were neutralized with the addition of 25 μl of 1 M Tris-HCl (pH 7.4) and cleanup was performed with Microcon 30 filters (Millipore). The fluorescent monofunctional *N*-hydroxysuccinimide-ester dyes cyanine-3 and cyanine-5 (Amersham) were coupled to the cDNAs originating from cultures grown without erythromycin and with erythromycin, respectively, for 1 h, quenched with 1.5 M hydroxylamine (Sigma), mixed, and finally cleaned with a QIAquick PCR purification kit (QIAGEN). The samples (30 μl) were then dried and used for hybridization within 12 h. Hybridizations to the microarrays were conducted as follows. Slides were prehybridized by incubation at 50°C for 30 min in a solution containing 1% bovine serum albumin (Calbiochem), 3.5× SSC, and 0.1% (wt/vol) SDS. The dried fluorescently labeled cDNA samples were resuspended in the following hybridization solution (40 μl): 5× SSC, 0.1% (wt/vol) SDS, 1.0% (wt/vol) bovine serum albumin, 50% (vol/vol) formamide, and 0.01% (wt/vol) single-stranded salmon sperm DNA. The resuspended probes were added to the arrays and incubated, in darkness, at 42°C for 6 h. After hybridization, excess hybridization solution and unspecific bound probe were washed away by four washing steps and gentle shaking, in darkness, for 2 min in 2× SSC-0.1% SDS, 1 min in 1× SSC, 1 min in 0.2× SSC, and 30 seconds in 0.05× SSC. Immediately after washing, the arrays were dried by centrifugation at 600 rpm for 5 min in an Eppendorf 5810R tabletop centrifuge. Three replicate hybridizations were performed with two separate batches of RNA. The two batches of RNA were obtained in two separate growth experiments.

Hybridized arrays were scanned at wavelengths of 532 nm (cyanine-3) and 635 nm (cyanine-5) at a 10-μm resolution to obtain two TIFF images with a ScanArrayExpress Microarray scanner (Packard Bioscience). Fluorescent intensities and spot morphologies were analyzed using the QuantArray program ver. 3.0 (Packard BioScience), and spots were excluded based on slide or morphology abnormalities.

Raw data from each array was preprocessed independently. A Lowess smoothed background was subtracted from all foreground intensities, and a cross-validated Lowess method was used in an intensity-dependent normalization of every array. The $\log_2$ ratios for each spot were further analyzed using a mixed model (30) to detect differentially expressed genes. A mixed model was fitted to the data for each of the five sample times (0, 15, 30, 60, and 90 min) separately. Data for the three arrays at every sample time were described by $y_{ijk} = \mu_i + u_{ij} + e_{ijk}$ where y_{ijk} is the observed $\log_2$ ratio of gene i (1, . . ., 3,502) on array j (1, 2, 3) and in spot k (1, . . ., 5) on that array, μ_i is the expected $\log_2$ ratio for gene i, u_{ij} is a random effect of gene i on array j, and e_{ijk} is the remaining noise. The variance components were estimated under the assumption of Gaussian errors using a restricted maximum likelihood approach coping with the unbalanced data due to missing spots. Differentially expressed genes were identified by testing the hypothesis H_0, defined as $\mu_i = 0$, against H_1, defined as $\mu_i \neq 0$. A chi-square test for every gene resolves this for the model in the y_{ijk} equation (4), and a Bonferroni-corrected rejection level of a P value of <0.01 was used throughout. If H_0 ($\mu_I = 0$) was rejected, and μ_i is >0, genes were considered to be up-regulated in the erythromycin-treated cells. If H_0 was rejected, and μ_i is <0, genes were considered to be down-regulated. All data analysis algorithms were programmed by using Matlab (MathWorks, Inc.), but a subset of the data was also analyzed by the SAS system (SAS Institute, Inc.) to check the validity of the code.

Confirmation of expression levels of specific genes by real-time RT-PCR. To confirm independently the differential gene expression observed by microarray experiments, four genes were selected for analysis by real-time quantitative RT-PCR (RTQ). Primers and probes for the RTQ were designed using the Assays-

By-Design file builder (ver. 2.0; http://www.appliedbiosystems.com/support/software/assaysbydesign/installs.cfm?prod_id=1541; Applied Biosystems). Primers and probes were synthesized by and purchased from Applied Biosystems. The genes selected for RTQ analyses were EF0633 (*tryS-1*, encoding tyrosyl-tRNA synthetases), EF2653 (encoding a transcriptional regulator of the Cro/CI family), and EF0105 (*argF-1*, encoding ornithine carbamoyltransferase). EF1964 (*gap-2*, encoding glyceraldehyde-3-phosphate dehydrogenase), which is constitutively expressed, was used to normalize the *Taq*Man data. The RTQ analyses were run on an ABI PRISM 7700 sequence detector (Applied Biosystems). cDNA was synthesized as above, with 15 ng RNA as template. Real-time PCR was performed using the *Taq*Man Universal PCR Master Mix (Applied Biosystems), according to the manufacturer's instructions. To ensure that the cDNA was not contaminated by genomic DNA, reactions without reverse transcriptase were also included. Differential expression was determined by calculating the change in threshold cycle (ΔC_t) for each gene, with RNA isolated from cells grown with and without erythromycin and harvested at the five time points mentioned above.

RESULTS

We have studied the transcriptional responses of *E. faecalis* V583 to treatment with the macrolide antibiotic erythromycin through a time course experiment utilizing a genome-wide microarray based on the V583 genome sequence. By applying a time course experiment in this manner, we were able to investigate both an immediate response to this stress and the cells' adaptation to a more permanent presence of erythromycin. In the following, we focus on genes that were differentially expressed in the untreated and the erythromycin-treated cells.

We applied a stringent confidence level (a P value of <0.01 plus Bonferroni correction for multiple comparisons) for the determination of significant differential transcription. Genes were "scored" as significantly up- or down-regulated as described above, with a threshold P value of <0.01 and the conservative Bonferroni correction for multiple comparisons, ensuring a very low number of false negatives.

Circa 600 (18%) of the predicted V583 genes were found to be differentially transcribed at one or more of the five time points examined during the 90-min time course experiment. Obviously, the erythromycin exposure seriously affected the transcriptional events in these enterococci: 260 genes were found to be significantly up-regulated (induced; $\log_2$ ratios of stressed cells/nonstressed cells were different from and higher than 0) and 340 genes down-regulated (repressed; $\log_2$ ratios of stressed cells/nonstressed cells were different from and lower than 0) at one or more time points. Among the up-regulated genes, four were plasmid encoded, while 27 plasmid-encoded genes were down-regulated. The numbers of differentially expressed genes, sorted by their cellular roles, are shown in Table 1. The total number of V583 genes represented on the array was 3,160 (out of total of 3,337 genes in the V583 genome), which means that ca. 8% of all V583 genes were up-regulated and ca. 10% down-regulated in the erythromycin-exposed cells. This shows that transcriptional events in these enterococcal cells are strongly altered by erythromycin exposure. During the 90-min time course, the number of down-regulated genes is higher than the number of induced genes at all time points except *t*90 (the last time point) (Tables 2 and 3), which indicates a general decrease in transcriptional activity by exposure to erythromycin.

Growth of erythromycin-treated *E. faecalis* V583. Cell samples were collected at five time points after the addition of erythromycin (50 μg/ml) to one of the cultures. We chose this level of erythromycin in the cultures on the basis of preliminary experiments (results not shown), where we found that growth of V583 was retarded but still significant. (V583 does grow in medium with >400 μg erythromycin/ml.) Our time course experiment was performed over a period of 90 min, and in this first part of the growth, the erythromycin-treated cells grow more slowly than the untreated cells. Thus, this work describes

TABLE 1. Number of genes that were differentially expressed at one or more time points, sorted by cellular role[a]

Cellular role	No. of differentially expressed genes	
	Down-regulated	Up-regulated
Amino acid biosynthesis	5	2
Biosynthesis of cofactors/prosthetic groups/carriers	6	1
Cell envelope	24	12
Cellular processes	28	8
Central intermediary metabolism	5	3
DNA metabolism	5	16
Energy metabolism	30	11
Fatty acid and phospholipid metabolism	1	6
Hypothetical proteins	118	83
Other categories	8	1
Protein fate	9	8
Protein synthesis	14	13
Purine/pyrimidine/nucleoside/nucleotide	11	8
Regulatory functions	17	14
Signal transduction	17	9
Transcription	3	3
Transport and binding protein	40	49
Unknown function	23	17
Viral functions	1	0

[a] Differentially expressed plasmid-encoded genes are also included. Genes that were up-regulated at some time points and down-regulated at other time points are counted twice. Likewise, genes that have been assigned more than one cellular role were counted twice.

TABLE 2. Number of up-regulated genes in each functional category at all time points studied

Cellular role	No. of up-regulated genes at time point indicated				
	*t*0	*t*15	*t*30	*t*60	*t*90
Amino acid biosynthesis	2	0	0	0	0
Biosynthesis of cofactors/prosthetic groups/carriers	0	1	1	0	0
Cell envelope	1	4	5	5	5
Cellular processes	0	2	6	5	7
Central intermediary metabolism	2	2	3	1	0
DNA metabolism	1	7	6	11	6
Energy metabolism	0	1	3	2	7
Fatty acid and phospholipid metabolism	0	4	5	2	3
Hypothetical protein	4	25	45	36	48
Other categories	0	0	1	0	0
Protein fate	0	2	4	5	5
Protein synthesis	0	8	9	5	3
Purine/pyrimidine/nucleoside/nucleotide	0	3	4	1	4
Regulatory functions	0	4	7	7	9
Signal transduction	0	2	4	2	5
Transcription	0	1	1	1	1
Transport and binding protein	6	18	24	21	28
Unknown function	2	5	9	11	9
Viral functions	0	0	0	0	0
Total	18	89	137	115	140

TABLE 3. Number of down-regulated genes in each functional category at all time points studied

Cellular role	No. of down-regulated genes at time point indicated				
	*t*0	*t*15	*t*30	*t*60	*t*90
Amino acid biosynthesis	1	1	2	3	4
Biosynthesis of cofactors/prosthetic groups/carriers	1	4	4	0	1
Cell envelope	5	10	16	10	9
Cellular processes	1	10	10	7	10
Central intermediary metabolism	1	2	2	1	1
DNA metabolism	0	4	4	0	1
Energy metabolism	3	4	12	21	11
Fatty acid and phospholipid metabolism	0	0	0	0	1
Hypothetical protein	29	59	47	35	43
Other categories	0	2	3	2	3
Protein fate	0	2	2	4	4
Protein synthesis	0	6	6	5	6
Purine/pyrimidine/nucleoside/nucleotide	0	4	3	6	5
Regulatory functions	2	7	6	3	4
Signal transduction	1	2	4	11	3
Transcription	0	1	2	0	0
Transport and binding protein	6	11	18	16	12
Unknown function	6	8	9	6	9
Viral functions	0	1	1	0	0
Total	56	138	151	130	127

the response of V583 to erythromycin during the first cycles of growth. We chose a relatively low level of erythromycin for the experiments, according to the observation by Hutter et al. (11) that lower concentrations are better for obtaining good microarray results. During the experiments, growth was measured spectrophotometrically. After 15 min, the OD of the erythromycin-treated culture was approximately 82% of the OD of the untreated (control) culture; after 30 min, the OD of the erythromycin-treated culture was approximately 69% of that of the untreated culture; after 60 min, the OD of the erythromycin-treated culture was approximately 54% of that of the untreated culture; and after 90 min, the OD of the treated culture was 40% of the OD of the untreated culture. The growth curves of erythromycin-treated and untreated V583 are shown in Fig. 1.

EFA0007, the *ermB* gene. EFA0007, which was referred to as an rRNA adenine dimethylase family protein encoded on pTEF1 (20) is, in fact, ErmB (>99% identity with ErmB from *Bacillus cereus*, *Streptococcus pyogenes*, *E. faecium* [BlastP]). This gene was expressed in cultures both with and without erythromycin, but the gene was not differentially expressed at any time point. This is in accordance with earlier observations that the *ermB* is constitutively expressed in enterococci (B. Murray, unpublished results). The retarded cell growth in the presence of erythromycin confirms that EFA0007 does not fully protect the bacterium against this antimicrobial compound and suggests that additional genes are involved in the cells' protection against this stress factor. Alternatively, the apparent lack of differential expression may be explained by posttranscriptional regulation of this gene; *ermB* may be posttranscriptionally regulated by a stalling on the ribosome during translation.

Up-regulated (induced) genes. The number of up-regulated genes in each functional category at various time points is shown in Table 2. Among the up-regulated genes, the genes encoding hypothetical proteins represented the highest percentage (85 genes; around 32% of all up-regulated genes), while genes related to transport and binding functions represented around 17% (44 genes) of the genes induced by the erythromycin stress. Moreover, genes encoding proteins involved in energy metabolism (10 genes), protein synthesis (13 genes), synthesis of cell envelope components (12 genes), and regulatory functions (13 genes) were up-regulated. The majority of up-regulated genes related to protein synthesis are genes encoding ribosomal proteins. In addition, genes classified in the category of genes of unknown function represent a considerable part of the induced genes (17 genes).

Among the up-regulated genes, three genes encoded drug

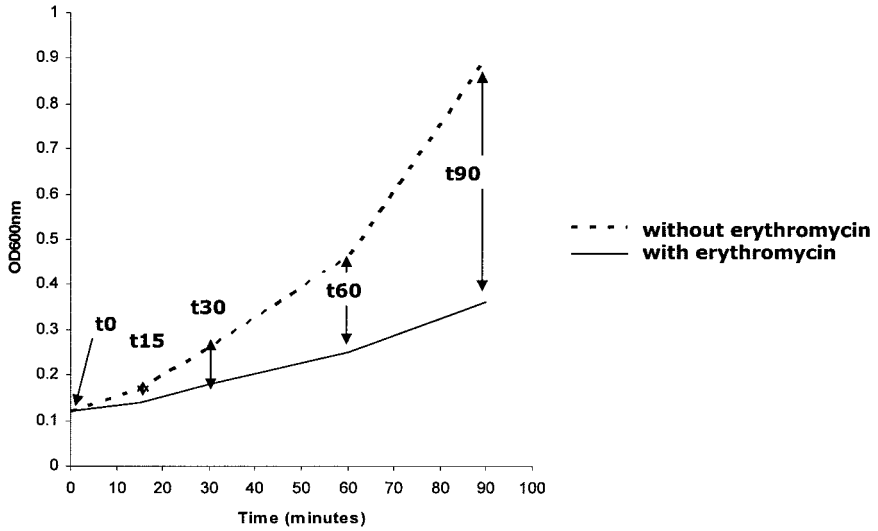

FIG. 1. Growth of *E. faecalis* V583 treated with erythromycin (50 μg/ml; solid line) and of untreated *E. faecalis* V583 (broken line). Growth was measured spectrophotometrically at a λ of 600 nm. Arrows indicate time points at which samples were collected for RNA extraction and microarray experiments.

resistance proteins. These genes were EF0420 (up-regulated at *t*30 and *t*60), EF1370 (up-regulated at *t*30, *t*60, and *t*90), and EF1078 (up-regulated at *t*15, *t*30, *t*60, and *t*90). Four genes only were induced at all time points. These genes were EF1400 (predicted to encode a cadmium-translocating ATPase), EF1413, EF1916 (encoding a GTP-binding protein), and EF2720 (encoding an ABC transporter). The gene encoding a putative MsrC-like protein (EF1413) is of particular interest. MsrC is widely spread among *Enterococcus faecium*, and MsrC is believed to be an efflux pump involved in low-level macrolide resistance in this species (23, 25). However, the EF1413 is only 40% identical (60% similar) to the *E. faecium* MsrC, and it is therefore not clear whether the EF1413 encodes a functional MsrC-like protein. Table 4 gives the full list of genes found to be induced (up-regulated), with $\log_2$ ratios. Genes predicted to encode hypothetical proteins and plasmid encoded genes are excluded from this list.

Down-regulated (repressed) genes. The numbers of down-regulated genes in each functional category at various time points are shown in Table 3. Among the down-regulated genes, those encoding hypothetical proteins are the dominant category (115 genes; 35%), followed by genes encoding transport and binding proteins (38 genes; 11%), genes with unknown function (23 genes), genes encoding cell envelope-related proteins (24 genes), and genes involved in energy metabolism (29 genes), regulatory function (13 genes), protein synthesis (14 genes), and cellular processes (23 genes). The majority of down-regulated genes related to protein synthesis are eight tRNA synthetases (cysteinyl-tRNA synthetase, tyrosyl-tRNA synthetase, alanyl-tRNA synthetase, aspartyl-tRNA synthetase, tryptophanyl-tRNA synthetase [EF2228], threonyl-tRNA synthetase, valyl-tRNA synthetase, and seryl-tRNA synthetase). The tryptophanyl-tRNA synthetase gene encoded by EF2679 (*trpS*) was significantly up-regulated at all time points except *t*0. Transcription was found to be repressed at all five time points examined for 10 genes. Table 5 lists all of the down-regulated genes, with $\log_2$ ratios. Genes encoding hypothetical proteins and plasmid genes are not included.

Validation of microarray data by real-time quantitative RT-PCR analyses of selected genes. Verifications of the expression levels of four genes observed by microarrays were performed by real-time quantitative RT-PCR analyses (*Taq*Man assays). The genes selected for these RTQ analyses represented various functional categories and different patterns of expression. The transcription profiles of the genes examined by RTQ correlated well with the transcription profiles observed by the use of microarrays ($r = 0.83$).

DISCUSSION

The present study focuses on functional genomics of *Enterococcus faecalis* V583, applying a genome-wide microarray based on the genome sequence of V583. In general, the knowledge of gene expression in enterococci is scarce. This is the first paper describing a global transcriptional profile of *Enterococcus*, and more studies are definitely needed to obtain a deeper understanding of gene expression in this species. We used a microarray to study the transcriptional responses of V583 to the macrolide antibiotic erythromycin. V583 is resistant to relatively high levels of erythromycin, but exposure to erythromycin retards its growth. The work was performed to (i) gain insight into the mechanisms involved in resistance to erythromycin by V583, (ii) obtain clues about transcriptional responses to general stress in bacteria, and (iii) generate hypotheses for subsequently gaining deeper knowledge on antibiotic resistance and general stress in this bacterium.

In the experiments, V583 was treated with erythromycin (50 μg/ml), which decreased the growth of the cells significantly compared to the untreated control during the 90-min time course experiment. The concentration of antibiotic to apply in such experiments is, of course, a matter of discussion. Hutter et al. (11) claimed that compound concentrations should be at concentrations that are just low enough not to affect the growth of the organism. This probably varies with the strains and compounds being studied.

We chose a stringent confidence level plus Bonferroni correction to score for significant differential expression, in the data analysis. Consequently, the number of false positives is, per definition, very low. We preferred this method, although some information might have been lost. Moreover, we decided to score for significant up- and down-regulation instead of using the common fold change cutoff values. Thus, the conclusions are based on experimental data precision (noise levels and number of replicates); this is appropriate since microarrays probably underestimate actual mRNA induction ratios (2). Also, treating each array as a random sample from a population of all possible arrays makes all conclusions more general. Even our simple mixed model gives a quite accurate description of the variance structure of normalized microarray data from repeated experiments and allows for an extended information extraction from every experiment. Our analysis differs from that by Wernisch et al. (30) in that we have excluded the array main effect. Our argument for this is that all arrays have been normalized separately, and thus any array main effects will be ignorable, which is also the conclusion drawn by Wernisch et al. (30) in analyzing similar data. Another variant of our mixed model approach is taken by Wolfinger et al. (31). They use one variance component per gene, which makes the model much more complex. More research must be done in this area of bioinformatics before we can come to a conclusion as to which strategy is appropriate. For now, we have chosen to use a simple model, in line with the principle of parsimony.

The target of erythromycin and other macrolides is protein synthesis, more specifically the 50S (large) ribosomal subunit. These antibiotics block the ribosome exit tunnel, which prevents the movement and release of the nascent peptide chain. The *ermB* gene (EFA0007) was not found to be significantly up- or down-regulated at any time point. Hence, this gene is probably constitutively expressed and contributes to the erythromycin resistance of V583. However, based on the high level of differential transcription and slow growth observed for erythromycin-exposed cells compared to untreated cells, it appears that a battery of other genes respond expressionally to the presence of erythromycin. These responses are not necessarily all due to resistance, but rather an adaptation to changes in growth conditions. Thus, these genes contribute to maintenance of the growth during erythromycin exposure. Three genes encoding drug resistance proteins (EF0420, EF1078, and EF1370), on the other hand, appear to play a role

TABLE 4. Log_2 ratios of genes whose transcription increased at one or more of the five time points (altogether 90 min) at which the effect of erythromycin treatment was studied, sorted by functional category (cellular role)

Protein encoded by gene	Cellular role(s)	ORF	Log_2 ratio at:				
			*t*0	*t*15	*t*30	*t*60	*t*90
Cystathionine beta-lyase (*metC*)[a]	Amino acid biosynthesis	EF0290	0.71	0	0	0	−1.05
Prephenate dehydrogenase	Amino acid biosynthesis	EF1565	0.71	0	0	0	0
Cobyric acid synthase, putative	Biosynthesis of cofactors/prosthetic groups/carriers	EF2586	0	0.87	0.86	0	0
Alanine racemase (*alr*)	Cell envelope	EF0849	0	0	0.78	0.87	1.11
Membrane protein, putative	Cell envelope	EF0860	0	0	0	0	0.77
Glycosyl transferase, group 2 family protein	Cell envelope	EF0887	0	0.61	0	0.98	0
Extracellular protein, putative	Cell envelope	EF0944	0	0.72	0	0	0
UDP-*N*-acetylglucosamine–*N*-acetylmuramyl-(pentapeptide) pyrophosphoryl-undecaprenol *N*-acetylglucosamine transferase (*murG*)	Cell envelope	EF0994	0	0.64	0	0	0
Membrane protein, putative	Cell envelope	EF1384	0	0	0	0.79	0.81
Bacterial sugar transferase	Cell envelope	EF2177	0	0.75	0.71	0	0
Glycosyl transferase, group 1 family protein	Cell envelope	EF2890	0	0	0.60	0	0
Glycosyl transferase, group 1 family protein	Cell envelope	EF2891	0	0	0.69	0.83	0
Membrane protein	Cell envelope	EF3176	0.74	0	0	0	0
Adhesion lipoprotein	Cell envelope/cellular processes	EF3206	0	0	0	0.96	0.70
NAD-dependent epimerase/dehydratase family protein	Cell envelope/energy metabolism	EF2165	0	0	0.74	0	0.71
Cell division protein, FtsW/RodA/SpovE family	Cellular processes	EF1300	0	0	0	0.86	1.23
MsrC protein, putative	Cellular processes	EF1413	1.88	2.96	3.24	3.36	3.44
Beta-lactamase, putative	Cellular processes	EF1502	0	0	0.62	0	0
Competence protein (*comG3*)	Cellular processes	EF2044	0	0	0.65	0	0.76
Competence protein (*comG2*)	Cellular processes	EF2045	0	0	0	0.73	0.85
Competence protein (*comG1*)	Cellular processes	EF2046	0	0	0.73	0.90	0
Chromosome partitioning protein ParB	Cellular processes	EF3298	0	0	0	0	0.69
ATPase, ParA family	Cellular processes	EF3299	0	0	0	0	0.89
Drug resistance transporter, EmrB/QacA family protein	Cellular processes/transport and binding protein	EF0420	0	0	0.69	0.98	0
Multidrug resistance protein, putative	Cellular processes/transport and binding protein	EF1078	0	0.78	1.24	1.18	0.93
Drug resistance transporter, EmrB/QacA family protein	Cellular processes/transport and binding protein	EF1370	0	0	0.83	0.97	0.68
Cell division ABC transporter, permease protein FtsX, putative	Cellular processes/transport and binding protein	EF1760	0	0.80	1.13	0.72	0.69
Glycerol dehydrogenase, putative	Central intermediary metabolism	EF1358	0	0	0.66	0	0
Alkaline phosphatase (*phoZ*)	Central intermediary metabolism	EF2973	0.79	0.85	0.62	0.70	0
Heptaprenyl diphosphate synthase, component II, putative	Central intermediary metabolism	EF3260	0.83	0.84	0.84	0	0
Chromosomal replication initiator protein DnaA	DNA metabolism	EF0001	0	0	0	0	0.65
MutT/nudix family protein	DNA metabolism	EF0780	0	0.70	0	0.95	0
DNA repair exonuclease family protein	DNA metabolism	EF0972	0	0	0	1.01	0
DNA polymerase III, alpha subunit (*dnaE*)	DNA metabolism	EF1044	0	0.65	0	0.71	0
Endonuclease III (*nth*)	DNA metabolism	EF1155	0	0	0.66	0	0
ATP-dependent DNA helicase RecQ	DNA metabolism	EF1545	0	0.76	0.88	1.00	1.08
MutT/nudix family protein	DNA metabolism	EF1587	0	0	0	0	0.78
Site-specific recombinase, phage integrase family	DNA metabolism	EF1648	0	0	0	0.73	0.81
Endonuclease IV (*nfo*)	DNA metabolism	EF1736	0	0.61	0.75	0.67	0
Site-specific recombinase, phage integrase family	DNA metabolism	EF2043	0	0	0	0.66	0
Toprim domain protein	DNA metabolism	EF2305	0	0	0	0.76	0.76
A/G-specific adenine glycosylase (*mutY*)	DNA metabolism	EF2704	0	0.65	0.68	0	0
Regulatory protein RecX, putative	DNA metabolism	EF2705	0	0.65	0	0.94	0
Exonuclease	DNA metabolism	EF2736	0.91	0	0.97	0	0
ATP-dependent DNA helicase RecG	DNA metabolism	EF3113	0	0.60	0	0.74	0.69
Beta-phosphoglucomutase (*pgmB*)	Energy metabolism	EF0956	0	0	0	−0.90	1.27
Glycosyl hydrolase, family 65	Energy metabolism	EF0957	0	0	0	−1.12	1.36
Galactose-1-phosphate uridylyltransferase (*galT*)	Energy metabolism	EF1071	0	0	0.60	0	0
Glycosyl hydrolase, family 13	Energy metabolism	EF1347	0	0	0	−0.99	0.80
Glucan 1,6-alpha-glucosidase, putative	Energy metabolism	EF1348	0	0	0	−1.01	0.77
Glycosyl hydrolase, family 13	Energy metabolism	EF1349	0	0	0	−0.99	0.93
Glycosyl hydrolase, family 4	Energy metabolism	EF1411	0	0	0	0	0.87
Ferredoxin (*fer*)	Energy metabolism	EF1543	0	0	0.60	0.79	0
Glycosyl hydrolase, family 1	Energy metabolism	EF1606	0	0.69	0.70	0.70	0
Glycerate kinase, putative	Energy metabolism	EF2646	0	0	0	−1.65	0.70
3-Oxoacyl-(acyl-carrier-protein) synthase II (*fabF-1*)	Fatty acid and phospholipid metabolism	EF0283	0	0.83	0	0	0
Holo-(acyl-carrier-protein) synthase (*acpS*)	Fatty acid and phospholipid metabolism	EF0848	0	0	0.69	0.67	0.91
Cardiolipin synthetase, putative	Fatty acid and phospholipid metabolism	EF1608	0	0.71	0.63	0	0
1-Acyl-sn-glycerol-3-phosphate acyltransferase, putative	Fatty acid and phospholipid metabolism	EF2691	0	1.06	0.65	0.74	1.09
Enoyl-(acyl-carrier-protein) reductase II (*fabK*)	Fatty acid and phospholipid metabolism	EF2883	0	0.67	0.72	0	0.89
Fatty acid/phospholipid synthesis protein PlsX	Fatty acid and phospholipid metabolism	EF3112	0	0	0.64	0	0
TraE protein, putative	Other categories	EF2320	0	0	0.61	0.69	0

Continued on following page

TABLE 4—*Continued*

Protein encoded by gene	Cellular role(s)	ORF	Log_2 ratio at:				
			*t*0	*t*15	*t*30	*t*60	*t*90
ATP-dependent Clp protease, ATP-binding subunit ClpE	Protein fate	EF0706	0	0	0.79	0	0
DnaJ protein	Protein fate	EF1310	0	0	0	0	0.79
Heat shock protein HslV	Protein fate	EF1647	0	0	0	0.74	0
Signal recognition particle protein (*ffh*)	Protein fate	EF1700	0	0.74	0.80	0.81	1.05
Preprotein translocase, SecE subunit	Protein fate	EF2730	0	0	0.75	1.17	0.71
Signal peptidase I	Protein fate	EF3073	0	0.64	0	0	0.72
Ribosomal protein L18 (*rplR*)	Protein synthesis	EF0223	0	0.66	0.75	0	0
Ribosomal protein S5 (*rpsE*)	Protein synthesis	EF0224	0	0.58	0.64	0	0
Ribosomal protein L30 (*rpmD*)	Protein synthesis	EF0225	0	0.60	0.61	0	0
Ribosomal protein L15 (*rplO*)	Protein synthesis	EF0226	0	0.66	0.92	0	0
Ribosomal protein L27 (*rpmA*)	Protein synthesis	EF0970	0	0.73	0	0	0
Ribosomal protein S16 (*rpsP*)	Protein synthesis	EF1694	0	0	0.73	0	0
Ribosome recycling factor (*frr*)	Protein synthesis	EF2395	0	0	0.81	0.77	0
Translation elongation factor Ts (*tsf*)	Protein synthesis	EF2397	0	0.72	0.82	0	0
Ribosomal-protein-alanine acetyltransferase, putative	Protein synthesis	EF2473	0	0.63	0	0.75	0
Ribosomal-protein-alanine acetyltransferase, putative	Protein synthesis	EF2474	0	0	0	0	0.66
Tryptophanyl-tRNA synthetase (*trpS*)	Protein synthesis	EF2679	0	0.86	1.23	1.01	0.82
Ribosomal protein L33 (*rpmG-2*)	Protein synthesis	EF2731	0	0	0.62	0.99	1.17
Ribosomal protein S4 (*rpsD*)	Protein synthesis	EF3070	0	0	0	0.66	0
Dihydroorotate dehydrogenase (*pyrD-1*)	Purine/pyrimidine/nucleoside/nucleotide	EF0285	0	0.82	0.67	0	0
Dihydroorotate dehydrogenase (*pyrD-2*)	Purine/pyrimidine/nucleoside/nucleotide	EF1714	0	0.71	0	0	0
Dihydroorotase (*pyrC*)	Purine/pyrimidine/nucleoside/nucleotide	EF1718	0	0	0	0	0.66
Phosphoribosylaminoimidazole carboxylase, ATPase subunit (*purK-2*)	Purine/pyrimidine/nucleoside/nucleotide	EF2362	0	0	0	0	0.68
Xanthine phosphoribosyltransferase (*xpt*)	Purine/pyrimidine/nucleoside/nucleotide	EF2365	0	0	0.79	1.04	0
Uridylate kinase (*pyrH*)	Purine/pyrimidine/nucleoside/nucleotide	EF2396	0	0.76	0.68	0	0
Inosine-uridine preferring nucleoside hydrolase	Purine/pyrimidine/nucleoside/nucleotide	EF2587	0	0	0.79	0	0.90
2′,3′-Cyclic-nucleotide 2′-phosphodiesterase, putative	Purine/pyrimidine/nucleoside/nucleotide	EF2902	0	0	0	0	0.80
Transcriptional regulator, DeoR family	Regulatory functions	EF0719	0	0	0	1.78	0
Transcriptional regulator, PemK family	Regulatory functions	EF0850	0	0	0.67	0.84	0.95
DNA-binding response regulator	Regulatory functions	EF1050	0	0	0.76	0	0
Transcriptional regulator, Cro/CI family	Regulatory functions	EF1369	0	0.68	1.18	1.40	1.13
Transcription antiterminator BglG family protein	Regulatory functions	EF1515	0	0.69	0.62	0	0.89
Protease synthase and sporulation negative regulatory protein pai 1	Regulatory functions	EF1590	0	0.70	0.65	0.66	0.87
Sucrose operon repressor ScrR (*scrR-1*)	Regulatory functions	EF1604	0	0	0.65	1.05	0.75
Transcriptional regulator (*codY*)	Regulatory functions	EF1645	0	0	0.69	0.73	0.78
Transcriptional regulator, TetR family	Regulatory functions	EF2066	0	0	0	0.68	0
Phosphosugar-binding transcriptional regulator, putative	Regulatory functions	EF2259	0	0	0	0	0.70
Transcriptional regulator, Cro/CI family	Regulatory functions	EF2653	0	0	0	0	0.94
Transcriptional regulator, Cro/CI family	Regulatory functions	EF2852	0	0	0	0	0.93
Zinc-binding transcriptional regulator, Cro/CI family	Regulatory functions	EF3272	0	0.84	0	0	0
PTS system component, mannose-specific IIC component	Signal transduction	EF0021	0	0	0	−1.22	1.04
PTS system, mannose-specific IID component	Signal transduction	EF0022	0	0	0	−1.34	1.04
PTS system, fructose-specific family, IIABC component	Signal transduction	EF0717	0	0	0	0.90	0
PTS system, IIABC component	Signal transduction	EF0958	0	0	0	−1.27	0
Sensor histidine kinase	Signal transduction	EF1051	0	0	0.69	0	0
Sensor histidine kinase	Signal transduction	EF1261	0	0	0.65	0	0
PTS system, IIABC component	Signal transduction	EF1516	0	1.13	1.44	1.06	1.06
PTS system, IIC component	Signal transduction	EF2978	0	0	0	0	0.66
PTS system, IIB component	Signal transduction	EF2979	0	0.69	0.72	0	0.94
ATP-dependent RNA helicase, DEAD/DEAH box family	Transcription	EF0846	0	0	0	0.68	0
Transcription termination factor Rho	Transcription	EF1170	0	0	0.68	0	0.84
ATP-dependent helicase, DEAH-box family, putative	Transcription	EF3214	0	0.62	0	0	0
Iron compound ABC transporter, substrate-binding protein	Transport and binding protein	EF0188	0	0	0	0	0.82
Na^+/H^+ antiporter (*nhaC-2*)	Transport and binding protein	EF0636	0	0.64	0	1.01	0
Sodium/dicarboxylate symporter family protein	Transport and binding protein	EF0744	0	1.44	1.20	0.87	0.71
Amino acid ABC transporter, ATP-binding protein	Transport and binding protein	EF0760	0	0	0.63	0	0
ABC transporter, ATP-binding/permease protein	Transport and binding protein	EF0789	0	0.70	1.08	1.09	0
ABC transporter, ATP-binding/permease protein	Transport and binding protein	EF0790	0	0	0.75	0.96	0
Potassium uptake protein	Transport and binding protein	EF0872	0	0.65	0	0	0
ABC transporter, ATP-binding/permease protein	Transport and binding protein	EF0941	0	0	0	0.72	0
Amino acid permease family protein	Transport and binding protein	EF1103	0	0	0.84	0.93	0.66
ABC transporter, ATP-binding protein	Transport and binding protein	EF1255	0.94	0.75	0	0	0
ABC transporter, ATP-binding/permease protein	Transport and binding protein	EF1341	0	0.69	0	0	0
Sugar ABC transporter, permease protein	Transport and binding protein	EF1344	0	0.62	0	−0.93	1.25
Cadmium-translocating P-type ATPase	Transport and binding protein	EF1400	0.65	0.71	0.86	0.78	1.09
V-type ATPase, subunit B	Transport and binding protein	EF1499	0	0	0	0	0.79
Iron compound ABC transporter, ATP-binding protein	Transport and binding protein	EF1639	0	0	0.62	0	0.95
Iron compound ABC transporter, permease protein	Transport and binding protein	EF1640	0	0.60	0.74	0.67	1.04

Continued on facing page

TABLE 4—*Continued*

Protein encoded by gene	Cellular role(s)	ORF	Log_2 ratio at:				
			*t*0	*t*15	*t*30	*t*60	*t*90
ABC transporter, ATP-binding/permease protein, MDR family	Transport and binding protein	EF1732	0	1.18	0.86	0.78	1.69
ABC transporter, ATP-binding/permease protein, MDR family	Transport and binding protein	EF1733	0	1.13	0.95	0.81	1.54
Amino acid permease family protein	Transport and binding protein	EF2047	0	0	0.90	1.21	0
Transport ATP-binding protein CydD, putative	Transport and binding protein	EF2058	0	0	0.68	0	0
Transport ATP-binding protein CydC, putative	Transport and binding protein	EF2059	0	0	0.61	0	0.83
ABC transporter, ATP-binding/permease protein	Transport and binding protein	EF2226	0	0	0	1.00	0.70
Xanthine permease	Transport and binding protein	EF2364	0.73	0	0.97	1.07	0
Amino acid permease	Transport and binding protein	EF2377	0	0.63	0.74	0.92	0
ABC transporter, permease protein	Transport and binding protein	EF2485	0	0	0.62	0	0
AzlC protein	Transport and binding protein	EF2509	0	0	0	0.75	0
Glycine betaine/L-proline transport ATP binding subunit	Transport and binding protein	EF2641	0	0	0	1.30	0
Glycine betaine/L-proline ABC transporter, glycine betaine/L-proline-binding/permease protein	Transport and binding protein	EF2642	0	0	0.82	1.75	0.86
Permease, GntP family	Transport and binding protein	EF2647	0	0	0	−2.31	1.32
Spermidine/putrescine ABC transporter	Transport and binding protein	EF2649	0	0	0	0	1.05
Spermidine/putrescine ABC transporter	Transport and binding protein	EF2650	0	0	0	0	0.92
Spermidine/putrescine ABC transporter	Transport and binding protein	EF2651	0	0	0	0	0.94
Spermidine/putrescine ABC transporter	Transport and binding protein	EF2652	0	0	0	0	0.67
ABC transporter, ATP-binding protein	Transport and binding protein	EF2720	0.93	0.69	1.12	0.86	0.80
ABC transporter, ATP-binding protein	Transport and binding protein	EF2769	0	0	−0.60	0	0.72
Ion transporter, putative	Transport and binding protein	EF2854	0	0	0	0	0.72
Potassium uptake protein TrkA	Transport and binding protein	EF2910	0	0.65	0.84	0.86	1.15
ABC transporter, ATP-binding protein	Transport and binding protein	EF2986	0	0	0.62	0	0
Cytosine/purines, uracil, thiamine, allantoin permease family protein	Transport and binding protein	EF3000	0	0	0	0	0.82
Formate/nitrite transporter family protein	Transport and binding protein	EF3069	0.82	0.87	0	0.73	0.66
Iron compound ABC transporter, permease protein	Transport and binding protein	EF3085	0	0	0	0	0.69
ABC transporter, permease protein	Transport and binding protein	EF3199	0	0	0.98	0	1.26
ABC transporter, ATP-binding protein	Transport and binding protein	EF3200	0	0.70	0.86	0	0.94
ABC transporter, permease protein	Transport and binding protein	EF3208	0	0.71	0.98	0	0
Oxidoreductase, DadA family	Unknown function	EF0414	0	0	0	0.72	0
LysM domain protein	Unknown function	EF0443	0.85	1.21	1.30	1.03	0
Glyoxalase family protein	Unknown function	EF0666	0	0.71	0	0	0
Methyltransferase, putative	Unknown function	EF0691	0	0	0	0.75	0
Type 2 phosphatidic acid phosphatase family protein	Unknown function	EF0796	0	0	0	0.69	0
DegV family protein	Unknown function	EF1191	0	0	0	0	0.77
Metallo-beta-lactamase YycJ	Unknown function	EF1197	0	0	0.90	0.93	0.92
GTP-binding protein	Unknown function	EF1527	0	0	0.77	0.68	0.74
GTPase, putative	Unknown function	EF1549	0	0	0.71	0	0
Acetyltransferase, GNAT family	Unknown function	EF1589	0	0	0	0	1.00
LacX protein, putative	Unknown function	EF1644	0	0.64	0.70	0.81	0
Lipase/acylhydrolase, putative	Unknown function	EF1683	0	0	0.69	0	0.67
GTP-binding protein	Unknown function	EF1916	0.99	1.02	1.39	1.09	1.18
GTP-binding protein LepA	Unknown function	EF2352	0	0	0.63	1.04	0
Diacylglycerol kinase catalytic domain protein	Unknown function	EF2661	0	0	0	0.86	0.92
Oxidoreductase, pyridine nucleotide-disulfide family	Unknown function	EF2899	0	0.75	0.73	1.06	1.02
Glucose-inhibited division protein B (*gidB*)	Unknown function	EF3300	0	0	0	0	0.67

[a] Gene names in parentheses.

for the ability of V583 to survive erythromycin treatment. Their influence must, however, be limited, since the growth of V583 is so retarded by erythromycin.

It was expected that the exposure of V583 to erythromycin would affect genes involved in protein synthesis, despite the expression of *ermB*. The induction of eight genes encoding ribosomal proteins (r-proteins), along with two r-protein-Ala-acetyltransferases, might be an indication that the overproduction of ribosomal proteins is one way to evade the effect of erythromycin. To balance the number and function of the ribosomes, the cells may have to compensate by up-regulation of these genes. Five of the induced r-protein genes and one of the r-protein-Ala-acetyltransferase genes are induced after 15 min of growth, i.e., their induction is a quick response. Closely related to the known target of erythromycin in sensitive cells is the fact that eight tRNA synthetase genes are repressed. The repression of tRNA synthetase genes may be seen as a logical consequence of the reduced ability of a cell's capacity to synthesize proteins, when an antibiotic binds a ribosomal subunit. The up-regulation of genes encoding r-proteins and two translation elongation factors, as well as down-regulation of tRNA synthetase genes, in response to translation inhibitors, has been noticed also by other authors (5, 19, 28).

Although the V583 cells are defined as resistant to erythromycin and will survive in the presence of relatively high levels of erythromycin, the cell growth is impaired, and, as shown above, the transcriptional activities are greatly altered. The differential transcription reflects the effect of erythromycin as well as the adaptation of V583 to the general stress. The retarded growth follows as a consequence of a slow-down of

TABLE 5. Log_2 ratios of genes whose transcription decreased at one or more of the five time points (altogether 90 min) at which the effect of erythromycin treatment was studied, sorted by functional category

Protein encoded by gene	Cellular role(s)	ORF	Log_2 ratio at:				
			*t*0	*t*15	*t*30	*t*60	*t*90
Ornithine carbamoyltransferase (*argF-1*)[a]	Amino acid biosynthesis	EF0105	0	0	−1.37	−2.81	−2.19
Cysteine synthase B, putative	Amino acid biosynthesis	EF0289	0	0	0	0	−0.75
Cystathionine beta-lyase (*metC*)	Amino acid biosynthesis	EF0290	0.71	0	0	0	−1.05
Arginine repressor (*argR*)	Amino acid biosynthesis	EF0676	0	0	0	−0.94	0
Aspartate aminotransferase, putative	Amino acid biosynthesis	EF0891	−1.00	−1.08	−0.96	−0.74	−1.03
Phosphomevalonate kinase	Biosynthesis of cofactors/prosthetic groups/carriers	EF0902	−0.75	−0.73	0	0	0
Thiamin biosynthesis ApbE, putative	Biosynthesis of cofactors/prosthetic groups/carriers	EF1225	0	0	−0.92	0	0
NH_3-dependent NAD^+ synthetase (*nadE*)	Biosynthesis of cofactors/prosthetic groups/carriers	EF2625	0	0	0	0	−0.88
Phosphomethylpyrimidine kinase (*thiD*)	Biosynthesis of cofactors/prosthetic groups/carriers	EF2775	0	−0.83	−0.95	0	0
Thiamine-phosphate pyrophosphorylase (*thiE*)	Biosynthesis of cofactors/prosthetic groups/carriers	EF2776	0	−0.75	−0.76	0	0
Hydroxyethylthiazole kinase, putative	Biosynthesis of cofactors/prosthetic groups/carriers	EF2777	0	−0.63	−0.72	0	0
Adhesion lipoprotein	Cell envelope	EF0055	0	0	−0.84	0	0
Glycosyl hydrolase, family 20	Cell envelope	EF0114	0	0	0	−0.87	0
Surface exclusion protein, putative	Cell envelope	EF0146	−0.84	−0.85	−0.72	0	0
Chitinase, family 2	Cell envelope	EF0361	0	0	0	−0.86	−0.86
Chitin binding protein, putative	Cell envelope	EF0362	0	0	0	−1.09	−0.96
LemA protein	Cell envelope	EF0468	0	0	−1.11	0	0
Penicillin-binding protein, putative	Cell envelope	EF0746	0	−0.73	−1.39	0	−0.87
Gram-positive anchor protein, putative	Cell envelope	EF0775	−0.95	−1.10	−0.81	−0.67	−0.91
Membrane protein, putative	Cell envelope	EF1027	0	0	−0.60	0	0
Pheromone cAM373 precursor lipoprotein	Cell envelope	EF1340	0	−0.79	0	0	0
Coccolysin	Cell envelope	EF1818	0	0	0	−0.85	0
Endocarditis specific antigen	Cell envelope	EF2076	0	−0.76	−0.77	−1.12	−1.51
DltD protein	Cell envelope	EF2746	0	0	−0.65	0	0
D-Alanyl carrier protein (*dltC*)	Cell envelope	EF2747	−0.86	−0.79	−1.22	0	0
Basic membrane protein DtlB	Cell envelope	EF2748	0	0	−0.65	0	0
D-Alanine-activating enzyme, putative	Cell envelope	EF2749	0	0	−0.74	0	0
Sortase family protein	Cell envelope	EF3056	0	0	−0.77	−1.56	−1.32
Rod shape-determining protein MreD	Cell envelope	EF3061	0	0	0	−0.93	−1.28
Rod shape-determining protein MreC	Cell envelope	EF3062	0	0	0	−1.01	−1.21
Pheromone cAD1 precursor lipoprotein	Cell envelope	EF3256	0	−0.68	−0.73	0	0
Gls24 protein	Cellular processes	EF0079	0	−1.04	−0.97	−1.16	−1.39
Gls24 protein	Cellular processes	EF0080	0	−0.98	−1.20	−1.28	−1.87
Regulatory protein, putative (*pfoR*)	Cellular processes	EF0097	0	0	−0.78	0	0
Superoxide dismutase, Mn (*sodA*)	Cellular processes	EF0463	0	−0.65	0	0	0
Low-temperature requirement C protein, putative	Cellular processes	EF0639	0	0	0	0.96	−1.21
Polysaccharide lyase, family 8	Cellular processes	EF0818	0	−0.70	−0.89	0	−1.24
Universal stress protein family	Cellular processes	EF1058	0	−1.20	−1.28	−1.44	−2.27
Streptomycin 3-adenylyltransferase, putative	Cellular processes	EF1076	−0.71	−0.97	−0.64	−0.88	−0.77
Autoinducer-2 production protein LuxS	Cellular processes	EF1182	0	−0.68	−0.66	0	−0.99
Cold shock protein CspC	Cellular processes	EF1991	0	−0.98	0	0	0
Negative regulator of genetic competence MecA, putative	Cellular processes	EF2677	0	0	0	0	−0.66
Alkyl hydroperoxide reductase, C subunit (*ahpC*)	Cellular processes	EF2739	0	−0.70	−0.68	−0.68	−0.68
Dps family protein	Cellular processes	EF3233	0	0	−0.70	0	0
Nucleotidyltransferase family protein	Central intermediary metabolism	EF0137	0	−0.69	−0.87	0	−0.77
Hydrolase, alpha/beta hydrolase fold family	Central intermediary metabolism	EF1028	0	0	−0.70	0	0
6-Aminohexanoate-cyclic-dimer hydrolase, putative	Central intermediary metabolism	EF1033	0	0	0	−0.95	0
Microcompartment protein	Central intermediary metabolism	EF1623	−0.66	−0.76	0	0	0
Chromosomal replication initiator protein DnaA	DNA metabolism	EF0001	0	0	0	0	0.65
Thermonuclease precursor (*nuc-1*)	DNA metabolism	EF0511	0	0	0	0	−0.70
Exonuclease RexB	DNA metabolism	EF1112	0	−0.67	−0.64	0	0
MutT/nudix family protein	DNA metabolism	EF1141	0	−0.58	−0.59	0	0
L-Serine dehydratase, iron-sulfur-dependent, beta subunit (*sdhB-1*)	Energy metabolism	EF0098	0	0	−1.08	0	0
L-Serine dehydratase, iron-sulfur-dependent, alphasubunit (*sdhA-1*)	Energy metabolism	EF0099	0	0	−0.67	0	0
Arginine deiminase (*arcA*)	Energy metabolism	EF0104	0	−0.65	−1.73	−3.16	−2.55
Carbamate kinase (*arcC-1*)	Energy metabolism	EF0106	0	0	−0.92	−1.99	−1.26
Phosphoglycerate mutase 1 (*gpm*)	Energy metabolism	EF0195	0	0	−0.62	0	−0.65
Aldehyde dehydrogenase	Energy metabolism	EF0253	−0.74	−0.82	−0.90	−0.72	−0.91
L-Lactate dehydrogenase (*ldh-2*)	Energy metabolism	EF0641	0	0	0	−0.78	−0.75
Phosphoglucomutase/phosphomannomutase family protein	Energy metabolism	EF0677	0	0	0	−1.16	0

Continued on facing page

TABLE 5—*Continued*

Protein encoded by gene	Cellular role(s)	ORF	Log_2 ratio at:				
			*t*0	*t*15	*t*30	*t*60	*t*90
Aldehyde-alcohol dehydrogenase (*adhE*)	Energy metabolism	EF0900	0	0	−0.77	0	0
Methylglyoxal synthase (*mgsA*)	Energy metabolism	EF0939	−0.88	−1.10	−0.96	−0.85	−0.96
Beta-phosphoglucomutase (*pgmB*)	Energy metabolism	EF0956	0	0	0	−0.90	1.27
Glycosyl hydrolase, family 65	Energy metabolism	EF0957	0	0	0	−1.12	1.36
Glycosyl hydrolase, family 1	Energy metabolism	EF1020	0	0	0	−1.93	0
Aldose 1-epimerase (*galM*)	Energy metabolism	EF1068	−0.84	−1.07	−0.72	0	−0.75
Iron-sulfur cluster binding protein	Energy metabolism	EF1109	0	0	−0.78	0	0
NADH peroxidase (*npr*)	Energy metabolism	EF1211	0	0	−0.59	0	−0.73
Glycosyl hydrolase, family 13	Energy metabolism	EF1347	0	0	0	−0.99	0.80
Glucan 1,6-alpha-glucosidase, putative	Energy metabolism	EF1348	0	0	0	−1.01	0.77
Glycosyl hydrolase, family 13	Energy metabolism	EF1349	0	0	0	−0.99	0.93
Glyceraldehyde 3-phosphate dehydrogenase (*gap-1*)	Energy metabolism	EF1526	0	0	0	0	−0.98
Pyruvate formate-lyase activating enzyme (*pflA*)	Energy metabolism	EF1612	0	0	0	−0.73	0
Formate acetyltransferase (*pflB*)	Energy metabolism	EF1613	0	0	0	−0.86	0
Alcohol dehydrogenase, zinc containing	Energy metabolism	EF1826	0	0	−0.84	−0.70	−1.01
Mannose-6-phosphate isomerase, class I (*manA*)	Energy metabolism	EF2589	0	0	0	−0.69	0
Glycerate kinase, putative	Energy metabolism	EF2646	0	0	0	−1.65	0.70
Endo-beta-*N*-acetylglucosaminidase	Energy metabolism	EF2863	0	0	0	−1.39	0
Ribokinase (*rbsK*)	Energy metabolism	EF2961	0	0	0	−1.32	−1.16
Glycosyl hydrolase, family 65	Energy metabolism	EF3157	0	0	0	−1.34	0
Lipase/acylhydrolase	Fatty acid and phospholipid metabolism	EF0169	0	0	0	0	−1.00
IS*1216*, transposase	Other categories	EF0514	0	0	−0.63	−0.81	−0.81
Transposase, putative	Other categories	EF0913	0	−0.69	0	0	0
Aminopeptidase C (*pepC*)	Protein fate	EF0302	0	0	0	0	−0.82
Rotamase family protein	Protein fate	EF0685	0	−0.60	−0.75	−0.80	−1.01
Preprotein translocase, YajC subunit, putative	Protein fate	EF0897	0	0	0	0	−0.83
Signal peptidase I	Protein fate	EF1111	0	−0.66	0	0	0
Serine proteinase, V8 family	Protein fate	EF1817	0	0	0	−0.86	0
Serine protease DO (*htrA*)	Protein fate	EF3027	0	0	0	−0.67	0
Peptidase, M20/M25/M40 family	Protein fate	EF3178	0	0	−0.62	−0.80	−1.32
Cysteinyl-tRNA synthetase (*cysS*)	Protein synthesis	EF0045	0	0	−0.59	0	0
RNA methyltransferase, TrmH family	Protein synthesis	EF0047	0	−0.60	−0.61	0	0
Tyrosyl-tRNA synthetase (*tryS-1*)	Protein synthesis	EF0633	0	0	0	−0.67	−1.61
Glutamyl-tRNA (Gln) amidotransferase, C subunit (*gatC*)	Protein synthesis	EF0724	0	−0.65	−0.68	0	0
Ribosomal protein L25 (*rplY*)	Protein synthesis	EF0820	0	0	0.68	−1.01	−2.07
Alanyl-tRNA synthetase (*alaS*)	Protein synthesis	EF1379	0	0	0	0	−0.69
Ribosomal subunit interface protein (*yfiA*)	Protein synthesis	EF1764	0	−0.78	0	0	0
Aspartyl-tRNA synthetase (*aspS*)	Protein synthesis	EF1970	0	0	0	0	−0.77
Tryptophanyl-tRNA synthetase	Protein synthesis	EF2228	0	0	−0.62	0	0
Ribosomal protein L33 (*rpmG-3*)	Protein synthesis	EF2856	0	−0.98	0	0	0
Threonyl-tRNA synthetase (*thrS*)	Protein synthesis	EF2858	0	−0.72	−0.64	−0.90	−1.20
Valyl-tRNA synthetase (*valS*)	Protein synthesis	EF2931	0	0	0	−0.67	0
Seryl-tRNA synthetase (*serS-2*)	Protein synthesis	EF3292	0	0	0	−0.67	−0.83
Ribosomal protein L34 (*rpmH*)	Protein synthesis	EF3333	0	−0.61	0	0	0
Adenylosuccinate synthetase (*purA*)	Purine/pyrimidine/nucleoside/nucleotide	EF0014	0	−0.73	−0.73	0	−0.70
Phosphopentomutase (*deoB*)	Purine/pyrimidine/nucleoside/nucleotide	EF0185	0	0	−0.69	0	0
Ribonucleoside-diphosphate reductase 2, beta subunit (*nrdF*)	Purine/pyrimidine/nucleoside/nucleotide	EF0470	0	0	0	−0.80	0
Ribonucleoside-diphosphate reductase 2, alpha subunit (*nrdE*)	Purine/pyrimidine/nucleoside/nucleotide	EF0471	0	0	0	−0.69	−0.79
Ribonucleoside-diphosphate reductase 2, NrdH-redoxin (*nrdH*)	Purine/pyrimidine/nucleoside/nucleotide	EF0473	0	−0.59	0	−0.80	−0.83
Aspartate carbamoyltransferase (*pyrB*)	Purine/pyrimidine/nucleoside/nucleotide	EF1719	0	−0.63	0	0	0
Pyrimidine operon regulatory protein PyrR (*pyrR*)	Purine/pyrimidine/nucleoside/nucleotide	EF1721	0	−0.82	0	0	0
Inosine-uridine-preferring nucleoside hydrolase	Purine/pyrimidine/nucleoside/nucleotide	EF1921	0	0	0	−0.92	0
Anaerobic ribonucleoside-triphosphate reductase (*hrdD*)	Purine/pyrimidine/nucleoside/nucleotide	EF2754	0	0	0	−0.96	−0.83
Anaerobic ribonucleoside-triphosphate reductase activating protein (*nrdG*)	Purine/pyrimidine/nucleoside/nucleotide	EF2755	0	0	0	−0.96	−1.14
Transcriptional regulator, Cro/CI family	Regulatory functions	EF0129	0	−0.69	−0.73	0	0
Transcriptional regulator, Cro/CI family	Regulatory functions	EF0869	0	−0.64	0	0	0
Transcriptional regulator, DeoR family	Regulatory functions	EF1124	−0.79	−0.84	−0.68	0	−0.71
Transcriptional regulator, LysR family	Regulatory functions	EF1303	0	0	−0.87	0	0
Transcriptional regulator, Cro/CI family	Regulatory functions	EF2142	−0.67	0	−0.67	0	0
Transcriptional regulator, Cro/CI family	Regulatory functions	EF2338	0	−0.79	−0.60	0	−0.88
Transcriptional regulator, Cro/CI family	Regulatory functions	EF2544	0	−0.75	0	0	0
Transcriptional regulator	Regulatory functions	EF2703	0	−0.65	−0.84	0	0
Transcriptional regulator, AraC family	Regulatory functions	EF2711	0	0	0	−1.00	0
Transcriptional antiterminator, bglG family	Regulatory functions	EF2966	0	0	0	−1.08	−1.28
DNA-binding response regulator	Regulatory functions	EF3289	0	0	0	0	−0.84

Continued on following page

TABLE 5—*Continued*

Protein encoded by gene	Cellular role(s)	ORF	Log_2 ratio at:				
			*t*0	*t*15	*t*30	*t*60	*t*90
PTS system, mannose-specific IIAB component	Signal transduction	EF0020	0	0	0	−0.88	0
PTS system, mannose-specific IIC component	Signal transduction	EF0021	0	0	0	−1.22	1.04
PTS system, mannose-specific IID component	Signal transduction	EF0022	0	0	0	−1.34	1.04
PTS system, mannitol-specific IIBC component	Signal transduction	EF0411	0	0	0	0	−0.73
PTS system, IID component	Signal transduction	EF0553	0	0	0	−0.73	0
PTS system, IIABC component	Signal transduction	EF0958	0	0	0	−1.27	0
PTS system, IIB component	Signal transduction	EF1012	0	0	0	−2.91	0
PTS system, IIB component	Signal transduction	EF1017	0	0	0	−2.75	0
PTS system, IIA component	Signal transduction	EF1018	0	0	0	−2.68	0
PTS system, IIC component	Signal transduction	EF1019	0	0	0	−3.28	0
Response regulator	Signal transduction	EF1336	0	−0.79	−0.60	0	0
Response regulator	Signal transduction	EF1633	−0.80	−1.06	−0.92	−0.80	−0.86
PTS system, IIA component	Signal transduction	EF1801	0	0	−0.69	0	0
PTS system, IIBC component	Signal transduction	EF2213	0	0	0	−1.83	0
PTS system, IID component	Signal transduction	EF3029	0	0	−0.66	0	0
Sensor histidine kinase	Signal transduction	EF3290	0	0	0	0	−0.97
RNA polymerase sigma-54 factor (*rpoN*)	Transcription	EF0782	0	−0.76	−0.64	0	0
RNA polymerase sigma-70 factor, ECF subfamily	Transcription	EF3180	0	0	−1.12	0	0
ABC transporter, ATP-binding protein	Transport and binding protein	EF0056	0	0	−0.75	0	0
ABC transporter, permease protein	Transport and binding protein	EF0179	0	−0.59	0	0	0
Amino acid ABC transporter, amino acid-binding/ permease protein	Transport and binding protein	EF0247	0	0	−0.68	0	0
Na^+/H^+ antiporter (*nhaC-1*)	Transport and binding protein	EF0402	0	0	0	−1.10	−0.98
Cation ABC transporter, permease protein	Transport and binding protein	EF0576	0	0	−0.62	0	0
ABC transporter, ATP-binding protein	Transport and binding protein	EF0793	−0.85	−0.92	−0.86	0	−0.75
Amino acid ABC transporter, ATP-binding protein	Transport and binding protein	EF0892	0	−0.61	0	0	0
Peptide ABC transporter, peptide-binding protein	Transport and binding protein	EF0907	0	0	0	−0.67	−1.39
ABC transporter, ATP-binding/TOBE domain protein	Transport and binding protein	EF0938	0	0	0	−1.39	0
$Mn2^+/Fe2^+$ transporter, NRAMP family	Transport and binding protein	EF1057	0	−1.13	−1.12	−1.23	−1.78
ABC transporter, ATP-binding/permease protein	Transport and binding protein	EF1100	−0.76	−0.86	−0.70	0	0
Phosphotransferase enzyme II, B component SgaB (*sgaB*)	Transport and binding protein	EF1128	−0.86	−0.74	0	0	0
ABC transporter, permease protein	Transport and binding protein	EF1254	0	0	0	0	−0.88
Cation-transporting ATPase, E1-E2 family	Transport and binding protein	EF1268	0	−0.64	−0.72	0	0
Magnesium-translocating P-type ATPase	Transport and binding protein	EF1304	−0.84	−1.01	−1.31	0	0
ABC transporter, ATP-binding protein	Transport and binding protein	EF1331	0	−0.79	−0.69	0	0
Sugar ABC transporter, permease protein	Transport and binding protein	EF1344	0	0.62	0	−0.93	1.25
Sugar ABC transporter, sugar-binding protein	Transport and binding protein	EF1345	0	0	0	−1.39	0
Magnesium-translocating P-type ATPase	Transport and binding protein	EF1352	0	0	−0.62	0	0
V-type ATPase, subunit F	Transport and binding protein	EF1492	0	0	−0.86	0	0
ABC transporter, ATP-binding protein	Transport and binding protein	EF1673	−0.65	0	−0.82	0	0
Uracil permease	Transport and binding protein	EF1720	0	−0.60	0	0	0
Phosphate ABC transporter, phosphate-binding protein	Transport and binding protein	EF1759	0	0	−0.61	0	0
ABC transporter, permease protein, putative	Transport and binding protein	EF2049	0	0	−1.18	0	0
ABC transporter, ATP-binding protein	Transport and binding protein	EF2050	0	0	−1.16	0	0
ABC transporter, ATP-binding protein	Transport and binding protein	EF2074	0	0	0	−0.70	−0.76
ABC transporter, permease protein	Transport and binding protein	EF2081	0	0	0	−0.68	0
ABC transporter, ATP-binding protein	Transport and binding protein	EF2394	0	0	−0.61	0	0
Permease, GntP family	Transport and binding protein	EF2647	0	0	0	−2.31	1.32
ABC transporter, ATP-binding protein	Transport and binding protein	EF2769	0	0	−0.60	0	0.72
Ribose uptake protein, putative	Transport and binding protein	EF2959	0	0	0	−1.19	−0.77
Ribose transporter protein RbsD	Transport and binding protein	EF2960	0	0	−0.70	−1.48	−1.30
Peptide ABC transporter, peptide-binding protein	Transport and binding protein	EF3106	0	0	0	−1.88	−1.13
Peptide ABC transporter, permease protein	Transport and binding protein	EF3107	0	0	0	−1.75	−0.82
Peptide ABC transporter, permease protein	Transport and binding protein	EF3108	0	0	0	−2.19	−0.87
Peptide ABC transporter, ATP-binding protein	Transport and binding protein	EF3109	0	0	0	−1.33	0
Peptide ABC transporter, ATP-binding protein	Transport and binding protein	EF3110	0	0	0	−1.65	−0.86
Cytosine permease, putative	Transport and binding protein	EF3277	−0.80	−0.74	0	0	0
AMP-binding family protein	Unknown function	EF0452	0	0	−0.61	0	0
OsmC/Ohr family protein	Unknown function	EF0453	0	0.95	−1.05	−1.16	−1.67
Acetyltransferase, GNAT family	Unknown function	EF0678	0	0	0	−1.32	0
Oxidoreductase, aldo/keto reductase 2 family	Unknown function	EF0877	0	0	0	−0.67	0
Pentapeptide repeat family protein	Unknown function	EF0905	−0.81	−1.04	−0.92	0	−0.75
N-acyl-D-amino-acid deacylase family protein	Unknown function	EF1062	−0.92	−1.02	0	0	0
Acetyltransferase, GNAT family	Unknown function	EF1075	−0.87	−0.97	−0.79	0	−0.78
Acetyltransferase, GNAT family	Unknown function	EF1077	−0.64	−0.92	−0.64	0	0
YkgG family protein	Unknown function	EF1110	−0.67	−0.71	0	0	0
Oxidoreductase, aldo/keto reductase family	Unknown function	EF1138	0	0	0	0	−0.77
HesA/MoeB/ThiF family protein	Unknown function	EF1329	0	−0.58	−0.64	0	0
CelC-related protein	Unknown function	EF2440	0	0	0	−1.31	0
PDZ domain protein	Unknown function	EF2450	0	0	0	0	−0.68
HD domain protein	Unknown function	EF2470	0	0	0	0	−0.80

Continued on facing page

TABLE 5—*Continued*

Protein encoded by gene	Cellular role(s)	ORF	Log_2 ratio at:				
			*t*0	*t*15	*t*30	*t*60	*t*90
Aldehyde oxldoreductase, putative	Unknown function	EF2570	0	0	−0.80	0	0
Hydrolase, haloacid dehalogenase-like family	Unknown function	EF2681	0	−0.86	0	0	0
Oxidoreductase, Gfo/Idh/MocA family	Unknown function	EF2734	0	0	0	0	−0.76
Thioredoxin reductase/glutathione-related protein	Unknown function	EF2738	0	0	−0.62	0	0
DNA-binding protein, putative	Unknown function	EF2933	0	0	−0.69	0	0
Glyoxalase family protein	Unknown function	EF3092	0	0	0	−0.71	0
Hydrolase, haloacid dehalogenase-like family	Unknown function	EF3158	0	0	0	−1.16	−0.72
Cell-envelope-associated acid phosphatase	Unknown function	EF3245	0	0	−0.78	0	0
Gp35	Viral functions	EF2143	0	−0.59	−0.59	0	0

[a]Gene names in parentheses.

many cellular activities when important processes, such as protein synthesis, apparently are repressed. In our experiments, we found that a lower number of genes was induced than repressed at all time points except for *t*90 (see Tables 1 to 5). At *t*90, it is tempting to look upon the altered transcriptional activity in the stressed cells as a consequence of an adaptation to the presence of erythromycin. At this point, the cells may have adapted to the stressing conditions and may have overcome the critical effects of the drug.

Ng et al. (19) report the induction of several genes related to purine biosynthesis in *Streptococcus pneumoniae* R6 exposed to various translation inhibitors, including erythromycin. This effect on purine synthesis is not clear for V583; only *purK* is up-regulated, and *purA* is down-regulated. Three genes related to pyrimidine biosynthesis (*pyrD-1*, *pyrD-2*, and *pyrC*) are induced, however. Similarly to what was found with *S. pneumoniae* R6 (19), the purine salvage gene *xpt* had a relative increase in transcription in V583 in response to the erythromycin treatment. Therefore, it looks as if the responses of the two relatively closely related bacteria *E. faecalis* and *S. pneumoniae* to erythromycin have common themes, but most of the transcriptional responses appear distinct.

Transport and binding proteins represent the second most dominant group of differentially expressed genes (Tables 1 to 5). This group of genes appears to be heavily influenced by various stressors in many bacteria (see, e.g., references 19, 21, and 26). The altered transcription of transport and binding proteins indicates that altered transport is an important part of general stress response mechanisms. The large number of differentially expressed genes encoding ABC transporters and permeases emphasizes the importance of such genes in stress responses.

Another interesting group of induced genes are those related to fatty acid and phospholipid metabolism (six genes). As mentioned briefly above, erythromycin resistance in gram-negative bacteria is usually mediated by a low permeability of the outer membrane to the hydrophobic macrolide. One may speculate as to whether a slightly altered cell membrane may have contributed also to the erythromycin adaptation in the gram-positive V583 cells.

In time course experiments like ours, one should draw attention to genes that are differentially expressed at all time points examined. Our results showed that 14 genes were regulated, 4 up and 10 down, at all five time points. Among these, the most interesting gene is EF1413, which encodes a putative MsrC protein, although the sequence identity with the described MsrC is not more than ca. 40%. EF1413 was up-regulated at all time points and showed the strongest differential expression of all genes on the microarray (Table 4). EF1413 was not differentially expressed in experiments with chloramphenicol treatment of V583 (Å. Aakra, unpublished results). The *msrC* is distributed in many isolates of *E. faecium* and has even been suggested to be specific for this species (23, 25). Comparative genomic hybridizations with five *E. faecalis* strains indicate the presence of the putative *msrC* in these genomes, as well (Aakra, unpublished). Singh et al. (25) found that the *msrC* conferred an increased resistance to macrolides among *E. faecium* isolates. Our observation of strong induction of EF1413 (log_2 ratio of 1.88 at *t*0; log_2 ratio of 3.44 at *t*90) in erythromycin exposed *E. faecalis* V583 support the involvement of an MrsC homologue in macrolide resistance in this species as well.

EF1732 and EF1733 encode two ABC transporters belonging to the MDR family. These two genes were up-regulated at all time points except *t*0. (In a similar study, the same two genes were also strongly up-regulated in response to chloramphenicol treatment [Aakra, unpublished].) Taken together, these results make it tempting to speculate that the efflux of erythromycin by proteins encoded by EF1413, EF1732, and EF1733 is an important part of the survival mechanism for V583 when exposed to this antibiotic.

At *t*0, i.e., immediately after the addition of erythromycin, 18 genes were up-regulated and 56 genes were down-regulated, which were considerably fewer genes than those regulated at the other time points under study. It must be assumed that the genes that show an immediate response also are crucial for the growth and survival of erythromycin-exposed V583 cells.

Several studies on stress responses (and genes involved therein) of *E. faecalis* have been published (e.g., see references 1, 8, 9, 14, and 15). Some papers focus on two-component signal transduction pathways, which commonly are related to bacterial stress (10, 15). A few of the genes found to have a significantly differential expression in our work have been discussed in these papers, e.g., genes encoding the Gls24 proteins (EF0079 and EF0080), two histidine kinases and their cognate response regulators (EF3290 and EF3289, EF1051 and EF1050), L-lactate dehydrogenase (EF0641), L-serine dehydratase (EF0098, EF0099), carbamate kinase (EF0106), and superoxide dismutase (EF0463). In this work, we found that the *gls24* genes (EF0079, EF0080) were repressed at all time points except *t*0, which is in contrast with the hypotheses of Giard et al. (9) that these genes are induced under stress. The

repression of the *gls24* genes was also found in our study of the transcriptional responses of V583 to chloramphenicol treatment (Aakra, unpublished). Similarly to Giard et al. (9), the EF0604 which encodes another Gls24 protein was not found to be differentially expressed at any time point of our study. Therefore, the role of Gls24 in stress responses in *E. faecalis* is not obvious. Regarding the two-component signal transduction pathways, the histidine kinase (HK) EF3290 and the cognate response regulator (RR) EF3289 were both down-regulated at *t*90. The function of this HK-RR pair is not known (10), and it remains to be seen whether the repression of EF3290/EF3289 at *t*90 in this study is stress related. The other pair of differentially expressed HK-RR (EF1051/EF1050) is similar to known HK-RR systems in *Listeria monocytogenes* and *S. pneumoniae* (10). EF1051 and EF1050 were both induced at *t*30. Recently, the EF1051/EF1050 pair was described by Teng et al. (27), who named this pair of genes *etaRS* (enterococcal two-component system a). Teng et al. (27) showed the involvement of EtaRS in both stress responses and virulence, and our results support their hypothesis on the function of *etaRS*. EF0641 encoding L-lactate dehydrogenase (*ldh*) was down-regulated at *t*60 and *t*90. Previously, it was shown that *ldh* was induced under stress (8), and it was speculated that also this gene could be involved in stress responses (8), but the function is unclear. The repression of *ldh* in this study indicates that the possible involvement of this gene in stress is not general. Likewise, *sodA* (encoding superoxide dismutase), *arcC* (encoding carbamate kinase), and *sdhA* and *sdhB* (encoding L-serine dehydratase) were down-regulated in our work, while they were induced in the study by Giard et al. (8). Based on these comparisons, it appears that a considerable number of the genes affected by environmental stress in *E. faecalis* are not general stress genes but, rather, are specified by certain conditions.

Interestingly, EF1078, which was induced at all time points except *t*0, is identical to *emeA* (enterococcal multidrug resistance efflux), which was characterized by Jonas et al. (12). *emeA* is probably a homolog of the *norA* found in *Staphylococcus aureus* (12). Jonas et al. (12), showed that *emeA* is involved in resistance to many toxic compounds, among them erythromycin (12). Thus, our study also supports the results of Jonas et al. on the function of *emeA*.

We have presented the transcriptional profile of erythromycin-exposed *E. faecalis* V583. The addition of erythromycin to this bacterium causes numerous events of differential transcription. Efflux of the macrolide molecules from the cells may be an important part of the survival mechanism, in addition to the resistance conferred by ErmB. This work adds information to the growing archive of condition-specific bacterial transcription signatures, which, in the future, will aid the elucidation of microbial physiology, metabolism, ecology, pathogenesis, etc. With a comprehensive archive of such transcription signatures, it will also be possible to pay more attention to all genes encoding hypothetical proteins or putative genes. This group represents a significant part of prokaryotic genomes and a dominant group of regulated genes in many microarray studies published so far. Profiling of transcriptional events in V583 under other stress conditions is in progress. Hopefully, this will lead to a deeper understanding of the biology of this bacterium.

ACKNOWLEDGMENTS

This work was financially supported by grant 142656/140 from the Research Council of Norway to I.F.N.

We thank Linda Godager and Merete Lunde for assistance with the RTQ analyses. Bjørn E. Kristiansen, of the Norwegian Microarray Consortium, Oslo, is acknowledged for printing of the microarray slides.

REFERENCES

1. **Breton, Y. L., A. Maze, A. Hartke, S. Lemarinier, Y. Auffray, and A. Rince.** 2002. Isolation and characterization of bile salts-sensitive mutants of *Enterococcus faecalis*. Curr. Microbiol. **45:**434–439.
2. **Conway, T., and G. K. Schoolnik.** 2003. Microarray expression profiling: capturing a genome-wide portrait of the transcriptome. Mol. Microbiol. **47:**879–889.
3. **Cummings, C. A., M. M. Brinig, P. W. Lepp, S. van de Pas, and D. A. Relman.** 2004. *Bordetella* species are distinguished by patterns of substantial gene loss and host adaptation. J. Bacteriol. **186:**1484–1492.
4. **Diggle, P. J., K. Liang, and S. L. Zeger.** 1994. Analysis of longitudinal data. Clarendon Press, Oxford, United Kingdom.
5. **Evers, S., K. Di Padova, M. Meyer, H. Langen, M. Fountoulakis, W. Keck, and C. P. Gray.** 2001. Mechanism-related changes in the gene transcription and protein synthesis patterns of *Haemophilus influenzae* after treatment with transcriptional and translational inhibitors. Proteomics **1:**522–544.
6. **Facklam, R. R., M. G. S. Carvalho, and L. M. Teixeira.** 2002. History, taxonomy, biochemical characteristics and antibiotic susceptibility testing of enterococci, p. 1–54. *In* M. S. Gilmore, D. B. Clewell, P. Courvalin, G. M. Dunny, B. E. Murray, and L. B. Rice (ed.), The enterococci: pathogenesis, molecular biology, and antibiotic resistance. ASM Press, Washington, D.C.
7. **Franz, C. M., W. H. Holzapfel, and M. E. Stiles.** 1999. Enterococci at the crossroads of food safety? Int. J. Food Microbiol. **47:**1–24.
8. **Giard, J. C., J. M. Laplace, A. Rince, V. Pichereau, A. Benachour, C. Leboeuf, S. Flahaut, Y. Auffray, and A. Hartke.** 2001. The stress proteome of *Enterococcus faecalis*. Electrophoresis **22:**2947–2954.
9. **Giard, J. C., N. Verneuil, Y. Auffray, and A. Hartke.** 2002. Characterization of genes homologous to the general stress-inducible gene *gls24* in *Enterococcus faecalis* and *Lactococcus lactis*. FEMS Microbiol. Lett. **206:**235–239.
10. **Hancock, L., and M. Perego.** 2002. Two-component signal transduction in *Enterococcus faecalis*. J. Bacteriol. **184:**5819–5825.
11. **Hutter, B., C. Schaab, S. Albrecht, M. Borgmann, N. A. Brunner, C. Freiberg, K. Ziegelbauer, C. O. Rock, I. Ivanov, and H. Loferer.** 2004. Prediction of mechanisms of action of antibacterial compounds by gene expression profiling. Antimicrob. Agents Chemother. **48:**2838–2844.
12. **Jonas, B. M., B. E. Murray, and G. M. Weinstock.** 2001. Characterization of *emeA*, a *norA* homolog and multidrug resistance efflux pump, in *Enterococcus faecalis*. Antimicrob. Agents Chemother. **45:**3574–3579.
13. **Koide, T., P. A. Zaini, L. M. Moreira, R. Z. Vencio, A. Y. Matsukuma, A. M. Durham, D. C. Teixeira, H. El-Dorry, P. B. Monteiro, A. C. da Silva, S. Verjovski-Almeida, A. M. da Silva, and S. L. Gomes.** 2004. DNA microarray-based genome comparison of a pathogenic and a nonpathogenic strain of *Xylella fastidiosa* delineates genes important for bacterial virulence. J. Bacteriol. **186:**5442–5449.
14. **Laport, M. S., J. A. C. Lemos, M. D. C. F. Bastos, R. A. Burne, and M. Giambiagi-de Marval.** 2004. Transcriptional analysis of the *groE* and *dnaK* heat-shock operons of *Enterococcus faecalis*. Res. Microbiol. **155:**252–258.
15. **Le Breton, Y., G. Boel, A. Benachour, H. Prevost, Y. Auffray, and A. Rince.** 2003. Molecular characterization of *Enterococcus faecalis* two-component signal transduction pathways related to environmental stresses. Environ. Microbiol. **5:**329–337.
16. **Murray, B. E.** 1990. The life and times of the enterococcus. Clin. Microbiol. Rev. **3:**46–65.
17. **Murray, B. E.** 2000. Vancomycin-resistant enterococcal infections. N. Engl. J. Med. **342:**710–721.
18. **Murray, B. E.** 1997. Vancomycin-resistant enterococci. Am. J. Med. **102:**284–293.
19. **Ng, W. L., K. M. Kazmierczak, G. T. Robertson, R. Gilmour, and M. E. Winkler.** 2003. Transcriptional regulation and signature patterns revealed by microarray analyses of *Streptococcus pneumoniae* R6 challenged with sublethal concentrations of translation inhibitors. J. Bacteriol. **185:**359–370.
20. **Paulsen, I. T., L. Banerjei, G. S. Myers, K. E. Nelson, R. Seshadri, T. D. Read, D. E. Fouts, J. A. Eisen, S. R. Gill, J. F. Heidelberg, H. Tettelin, R. J. Dodson, L. Umayam, L. Brinkac, M. Beanan, S. Daugherty, R. T. DeBoy, S. Durkin, J. Kolonay, R. Madupu, W. Nelson, J. Vamathevan, B. Tran, J. Upton, T. Hansen, J. Shetty, H. Khouri, T. Utterback, D. Radune, K. A. Ketchum, B. A. Dougherty, and C. M. Fraser.** 2003. Role of mobile DNA in the evolution of vancomycin-resistant *Enterococcus faecalis*. Science **299:**2071–2074.
21. **Paustian, M. L., B. J. May, and V. Kapur.** 2001. *Pasteurella multocida* gene expression in response to iron limitation. Infect. Immun. **69:**4109–4115.

22. **Pechere, J. C.** 2001. Macrolide resistance mechanisms in Gram-positive cocci. Int. J. Antimicrob. Agents **18**(Suppl. 1)**:**S25–S28.
23. **Portillo, A., F. Ruiz-Larrea, M. Zarazaga, A. Alonso, J. L. Martinez, and C. Torres.** 2000. Macrolide resistance genes in *Enterococcus* spp. Antimicrob. Agents Chemother. **44:**967–971.
24. **Sahm, D. F., J. Kissinger, M. S. Gilmore, P. R. Murray, R. Mulder, J. Solliday, and B. Clarke.** 1989. In vitro susceptibility studies of vancomycin-resistant *Enterococcus faecalis*. Antimicrob. Agents Chemother. **33:**1588–1591.
25. **Singh, K. V., K. Malathum, and B. E. Murray.** 2001. Disruption of an *Enterococcus faecium* species-specific gene, a homologue of acquired macrolide resistance genes of staphylococci, is associated with an increase in macrolide susceptibility. Antimicrob. Agents Chemother. **45:**263–266.
26. **Smoot, L. M., J. C. Smoot, M. R. Graham, G. A. Somerville, D. E. Sturdevant, C. A. Migliaccio, G. L. Sylva, and J. M. Musser.** 2001. Global differential gene expression in response to growth temperature alteration in group A *Streptococcus*. Proc. Natl. Acad. Sci. USA **98:**10416–10421.
27. **Teng, F., L. Wang, K. V. Singh, B. E. Murray, and G. M. Weinstock.** 2002. Involvement of PhoP-PhoS homologs in *Enterococcus faecalis* virulence. Infect. Immun. **70:**1991–1996.
28. **VanBogelen, R. A., and F. C. Neidhardt.** 1990. Ribosomes as sensors of heat and cold shock in *Escherichia coli*. Proc. Natl. Acad. Sci. USA **87:**5589–5593.
29. **Weisblum, B.** 1995. Erythromycin resistance by ribosome modification. Antimicrob. Agents Chemother. **39:**577–585.
30. **Wernisch, L., S. L. Kendall, S. Soneji, A. Wietzorrek, T. Parish, J. Hinds, P. D. Butcher, and N. G. Stoker.** 2003. Analysis of whole-genome microarray replicates using mixed models. Bioinformatics **19:**53–61.
31. **Wolfinger, R. D., G. Gibson, E. D. Wolfinger, L. Bennett, H. Hamadeh, P. Bushel, C. Afshari, and R. S. Paules.** 2001. Assessing gene significance from cDNA microarray expression data via mixed models. J. Comput. Biol. **8:**625–637.

ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Sept. 2004, p. 3989 Vol. 49, No. 9
0066-4804/04/$08.00+0 doi:10.1128/AAC.49.9.3989.2005

ERRATUM

Transcriptional Response of *Enterococcus faecalis* V583 to Erythromycin

Ågot Aakra,[1]* Heidi Vebø,[1] Lars Snipen,[2] Helmut Hirt,[3] Are Aastveit,[2] Vivek Kapur,[4] Gary Dunny,[3] Barbara E. Murray,[5] and Ingolf F. Nes[1]*

Laboratory of Microbial Gene Technology[1] and Section for Bioinformatics and Data Analysis,[2] Department of Chemistry, Biotechnology and Food Science, Norwegian University of Life Sciences, Ås, Norway; Department of Microbiology, University of Minnesota Medical School, Minneapolis, Minnesota 55455[3]; Department of Veterinary Pathobiology, Biomedical Genomics Center, University of Minnesota, St. Paul, Minnesota 55108[4]; and Center for the Study of Emerging and Re-Emerging Pathogens, The University of Texas Medical School, Houston, Texas 77030[5]

Volume 49, no. 6, p. 2246–2259, 2005. Page 2246: The article byline should read as given above.