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Permeant Acid-Dependent Gene Expression in Bacillus subtilis

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Permeant Acid-Dependent Gene Expression in *Bacillus subtilis*

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Abstract

Gene expression of *Bacillus subtilis* grown under conditions of 0 mM and 30 mM benzoate was measured using microarray hybridization. An increase in the concentration of benzoate in *B. subtilis* cultures is a means of lowering the internal pH of the organism. The concentrations of benzoate used were determined by a growth curve testing 0, 10, 30, and 60 mM concentrations of benzoate at external pH 7.0. Four biological replicates from 0 mM and 30 mM concentrations were used for the 5006 gene microarray hybridization, performed at the University of Wisconsin-Madison’s Gene Expression Center. Real-time PCR was conducted to verify a few of the genes showing the largest expression ratios and a preliminary analysis of the data obtained from the microarray is underway. Current analyses of these data suggest that 92.6% of the genes that are highly expressed in response to an increase in internal pH are not similar those that were found to be highly expressed as a response to increases in the external pH. These preliminary results are consistent with the original hypothesis that a decrease in the internal pH of *B. subtilis* causes differential gene expression.

Introduction

▪pH, the measure of basicity and acidity, is internally regulated in most organisms. The maintenance of internal pH involves the upregulation and repression of a number of genes.

▪*Bacillus subtilis* is an excellent model organism for studying gram-positive bacteria, such as *Bacillus anthracis* (cause of anthrax). This organism is relatively large and undergoes a sporulation cycle, allowing for the observation of pH throughout different parts of development.

▪Microarray studies conducted last year by Wilks *et al*. indicated certain genes that were upregulated under external acidic conditions. Questions were posed as to what genes are upregulated under internal acidic conditions.

Methods

▪Growth curve experiments were conducted on *B. subtilis* using 2M benzoate, 2M sodium acetate, and 2M methylamine hydrochloride. From these curves it was determined that an RNA extraction would be conducted using 0 mM and 30 mM concentrations of benzoate from a sodium salt of benzoate for real-time PCR and microarray experiments.

▪A benzoate RNA extraction was completed at pH 7.0. Microarrays were sent to the Gene Expression Center at the University of Wisconsin-Madison.

▪*B. subtilis* strain MMB8 were grown in an LBK medium containing 50 mM MOPS buffer and the external pH was adjusted to 7.0 with the addition of 5M KOH. Overnight cultures grew for 18 hours before they were diluted 1:500 for the water bath. There were 4 biological replicates for each benzoate concentration tested (0 mM and 30 mM).

Methods

▪RNA was extracted using a Qiagen RNeasy kit and checked with a NanoDrop spectrophotometer. RNA samples were stored at - 80 C before being sent to the Gene Expression Center at the University of Wisconsin-Madison.

▪cDNA was synthesized from the extracted mRNA with reverse transcriptase using standard procedures for Affymetrix GeneChips. cDNA was then fragmented, N-terminusly labeled with Biotin, and hybridized to the microarray chip using standard protocols. Chips were scanned and then analyzed using the statistical software D-chip.

▪Real-time PCR was conducted using standard procedures for 25 µl samples in 96-well plates with SYBR Green detector. Initial temperature of the thermocycler was 48.0 degrees C and the initial trial was run for 30 minutes. Samples were analyzed using 7500 software.

Results & Discussion

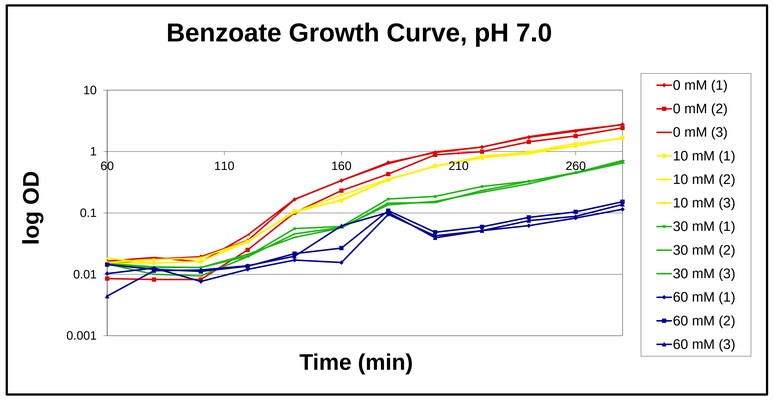


Figure 1. *B. subtilis* growth over time in response to varying concentrations of benzoate.

▪Growth curve experiments using benzoate, sodium acetate, and methylamine hydrochloride allowed for the determination of acid to use as well as possible concentrations. 0 mM and 30 mM were chosen for the microarray study.

▪Real-time PCR was conducted on the following genes: *trkA, katA, alsS, alsD, cadA, yrpC, maeN, yvgW, yqjM,* and *sigB*. These genes were selected because they were upregulated in the presence of external HCl, based on the results of last year’s microarray. These real-time PCR’s were not found to be significantly upregulated under higher concentrations of benzoate.

▪There were 526 genes significant from a 5006 gene microarray.

▪The microarray study comparing 0 mM and 30 mM concentrations off benzoate showed only a 7.046% gene overlap with the microarray study conducted on external acidic conditions using HCl.

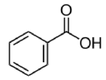
▪Real-time PCR was also started on *padC, citZ, acsA,* and *feuC* based on the findings from my own microarray examining internal acidic conditions.

Results & Discussion Cont.

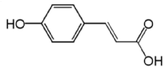
Gene	Mean Log_2 Ratio	Function
padC	**-4.777**	**Phenolic acid decarboxylase**
oppB	-3.872	Oligopeptide ABC transporter, initiation of
ilvN	-3.76	Acetolactate synthesis (small subunit)
citZ	**-2.63**	**Citrate synthase II (major)**
mpr	-2.41	Extracellular metalloprotease
acsA	**-2.225**	**Acetyl-coA synthetase**
purF	-2.182	Purine biosynthesis
ald	-2.105	L-alanine dehydrogenase
leuA	-2.077	Leucine biosynthesis
amhX	-1.868	Amidohydrolase
feuC	**-1.678**	**Iron-uptake system**
sucD	-1.493	Succinyl-coA synthetase (alpha subunit)
serC	-1.493	Serine biosynthesis

Table 1. Most highly induced genes in *B. subtilis* in the presence of 30 mM benzoate.

▪*padC*: encodes a product (phenolic acid decarboxylase) that breaks down coumerate, an acid produced by plants that is found in the soil and is structurally similar to benzoate.



benzoate



coumaric acid

▪*ascA*: codes for acetyl-coA synthetase. This could help remove more acetic acid, it may be that CoA can be used in metabolism without requiring the proton motive force.

▪*feuC*: codes for an iron-uptake system. In the HCl microarray the acid made the plasma membrane more permeable to metals. The results was that a number of metal pumps were highly expressed in response to the influx of toxic metals. In the case of benzoate, however, it is possible that *feuC* is upregulated because more heme rings are needed in he cytoplasm for electron transport for increased ATP production.

▪*citZ*: encodes citrate synthase II (major subunit). This enzyme uses acetic acid and oxaloacetic acid to create citrate, an intermediate within the Krebs cycle.

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