# Predicting bacterial growth conditions from mRNA and protein abundances.

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## Abstract

Cells respond to changing nutrient availability and external stresses by altering the expression of individual genes. Condition-specific gene expression patterns may provide a promising and low-cost route to quantifying the presence of various small molecules, toxins, or species-interactions in natural environments. However, the ability of gene expression signatures alone to predict individual environmental growth conditions remains an open question. Here, we used machine learning to predict 16 closely-related growth conditions using 155 datasets of *E. coli* transcript and protein abundances. We show that models are able to discriminate between different environmental features with a relatively high degree of accuracy. We observed a small but significant increase in model accuracy by combining transcriptome and proteome-level data and show that stationary phase conditions are typically more difficult to distinguish from one another compared with exponential growth. With sufficient training data, gene expression measurements from a single species are capable of distinguishing between environmental conditions that are separated by a single environmental variable. This ability is significantly enhanced when capturing cells during the exponential rather than stationary growth phase, but despite their overall quiescence, stationary phase cells nevertheless retain signatures of the external environment.

## Introduction

Generalist bacterial species must be able to sense outside conditions and alter gene expression patterns according to nutrient availabilities and various environmental features (<https://www.nature.com/articles/s41467-017-01265-1>). For instance, *Escherichia coli* grows inside the comparatively warm, nutrient rich digestive tract of host organisms but spends another portion of its life-cycle exposed to harsh environmental conditions upon being excreted and before finding another host (<https://www.nature.com/articles/nature08112>). Maintenance and reproduction in these vastly different conditions is accomplished by expressing a controlled set of environmentally specific gene products in addition to more ubiquitous “housekeeping” genes (<https://www.nature.com/articles/nbt.3418>).

On top of their native responses, microbial cells can be further engineered to act as sensors for a variety of environmental conditions via rational design of synthetic genetic circuits that may, for instance, cause the cells to fluoresce upon sensing of a particular small molecule (<http://www.pnas.org/content/112/47/14429>). However, individual synthetic biology applications take time to develop and there is still a concern about releasing genetically engineered species into natural environments where they may act as low-cost sensors for pollutants or various environmental phenomena of interest (<https://www.sciencedirect.com/science/article/pii/S0958166916302063>).

To partially alleviate this concern, previous work has shown that the species composition of an environment can serve as a rapid and low-cost biosensor to indicate the presence of various contaminants according to the gene repertoire identified via meta-genomics (<http://mbio.asm.org/content/9/1/e02435-17?related-urls=yes&legid=mbio;9/1/e02435-17>). However, looking at the species composition alone fails to account for the fact that gene *expression* patterns of individual species may be able to provide even higher resolution into the past and current chemical composition of environments. What molecular level data is important, and at what resolution individual environmental differences can be resolved from the gene expression patterns of individual generalist species remains unknown.

Combining different ‘omics’-scale technologies is likely to provide better discriminatory capability compared with only monitoring mRNA abundances, for instance, but integrating datasets is challenging based on the biases of individual methods1 and the inevitability of batch-level effects that occur when datasets are generated across multiple labs and platforms2,3. These problems are further exacerbated when considering the ultimate goal of detecting different environmental conditions *in situ*.

Previous work has looked into the question of predicting external conditions by using the cells internal variables4,5. Other studies have interrogated multi-omic datasets from different growth conditions to understand the function of regulatory networks, individual gene functions, and resource allocation strategies (<https://www.nature.com/articles/nbt.3418>), <http://msb.embopress.org/content/11/2/784.long>). However, the main focus of many of these studies has been to understand differences in gene expression patterns across environmental conditions so as to provide insight into *internal* cellular mechanisms and pathways or to predict cellular level phenotypes such as specific growth rates. By contrast, few studies have focused on using the internal state of cells to predict external environmental conditions across a range of partially-overlapping conditions and cellular growth rates.

Here, we are interested in determining whether gene expression patterns can discriminate between environmental conditions in the absence of prior knowledge about the role and function of individual genes. Our study leverages a large dataset of transcriptomic and proteomic measurements of *E.coli* growth under multiple distinct but closely-related conditions6. We use mRNA and protein composition data to train machine learning models and find that highly similar environmental conditions can be discriminated with a relatively high degree of accuracy. Interestingly, we also investigate which conditions are more- and less-challenging to discriminate and find that prediction accuracies decrease substantially for stationary phase cells, indicating the importance of cellular growth for discriminating between conditions. Finally, we note that our accuracy remains limited by training set size such that our findings present a lower bound on the predictive power that is achievable given a greater availability of training data.

## Results

### Data structure and pipeline design

We used a previously generate dataset of whole-genome *E. coli* mRNA and protein abundances, measured under 34 different conditions6,7. This dataset consists of a total of 155 samples, for which mRNA abundances are available for 152 and protein abundances for 105 (Figure 1). For 102 samples, both mRNA and protein abundances are available. The 34 different experimental conditions were generated by systematically varying four parameters, which we collapsed into 16 conditions by clustering similar conditions together (i.e. 100, 200, 300mm Na+ are labelled as “high Na”). For the remainder of this manuscript, “growth condition” refers to the four-dimensional vector of categorical variables, consisting of i) growth phase (exponential, stationary, late stationary), ii) carbon source (glucose, glycerol, gluconate, lactate), iii) Mg2+ concentrations (base, low, high), and iv) Na+ concentrations (base, high). After labeling these categories, we asked to what extent machine learning models would be capable of discriminating between these growth parameters given only knowledge of mRNA abundances, protein abundances, or both.

We applied a general cross-validation set-up by first splitting samples into training and testing datasets. We next used the training data to fit supervised models to gene-specific composition data so as to maximize predictions of the labeled environmental conditions. Finally, we use these supervised models to predict test set data and report accuracies on this data. We repeated this procedure 60 independent times so as to get a more complete picture of predictive accuracy for statistical purposes. Our pipeline is illustrated graphically in Figure 2 (see Materials and Methods for details).

### Growth conditions can be predicted accurately from both mRNA and protein abundances

After constructing our analysis pipeline, we first asked whether there were major differences in the performance of different machine learning approaches. We tested four different machine learning models, three based on Support Vector Machines (SVMs) with different kernels (radial, sigmoidal, and linear) and the fourth using random forest classification. We trained models to predict the entire four-dimensional condition vector at once for a given sample, and we used the multi-conditional *F*1 score9 to quantify prediction accuracy. The *F*1 score is the harmonic mean of precision and recall. It approaches zero if either quantity approaches zero, and it approaches one if both quantities approach one (representing perfect predictive accuracy). We note that this score is highly conservative as it will classify a prediction as incorrect if a single variable is wrongfully predicted, even if the predictions for the remaining three variables are correct. We assessed model performance during the tuning stage of our pipeline, by recording which model had the best *F*1 score for each tuning run. We found that the SVM model with a radial kernel clearly outcompeted the other models when fit to mRNA data, and the random forest model outcompeted the other models by a comparatively small margin when fit to protein data (Table 1).

We next compared the *F*1 scores for model predictions applied to the test set. When using mRNA abundance data alone, the distribution of *F*1 scores from our 60 independent replications werecentered around a value of 0.7 (Figure 3). The *F*1 score distributions were virtually identical for the three SVM based models and were somewhat lower for the random forest model. Model performance on test data using only protein abundance measurements was slightly worse than those achieved with mRNA abundance data. However, it is important to note that the protein abundance data contains fewer conditions overall, which may partially explain the decreased predictive accuracy of protein only model—a point to which we return to later.

In addition to assessing the overall predictive power using *F*1 scores, we also recorded the percentage of times specific growth conditions were accurately or erroneously predicted in the form of a confusion matrix. Here, the columns headings at the top show the predicted condition from the model on the test set and the rows show the true experimental condition (Figure 4). The large numbers/dark colorings along the diagonal highlight the percentage of true positive predictions whereas any off-diagonal element represents the percentage of incorrect predictions for a given condition. We found that the erroneous off-diagonal predictions are partially driven by the uneven sampling of different conditions in the original dataset. Even though we used sample-number-adjusted class weights in all fitted models, we observed a trend of increasing fractions of correct predictions with increasing number of samples available under training (Supplementary Figure 3).

As we previously noted, the *F*1 score quantifies accuracy by only considering perfect predictions (i.e. when all 4 features are correctly predicted). A sample that is incorrectly classified for all four factors is thus treated the same as one that only differs from the true set of features by a single incorrect factor. In practice, we observed that the majority of incorrect predictions differed from their true condition vector only by a single value (Supplementary Figure 4).

### Joint consideration of mRNA and protein abundances improves model accuracy

We next asked whether predictions could be improved by simultaneously considering mRNA and protein abundances. To address this question, we limited our analysis to the subset of 102 samples for which both mRNA and protein abundances were available, and ran our analysis pipeline for mRNA abundances only, protein abundances only, and for the combined dataset containing both mRNA and protein abundances. For all four machine-learning algorithms, protein abundances yielded significantly better predictions than mRNA abundances (Figure 5, Table 2). This is in contrast to Figure 3, where we saw increased accuracy using mRNA abundance data. However, as previously noted, our dataset contains a larger number mRNA abundance samples, which results in a larger amount of training data. When compared on the same exact conditions—as depicted in Figure 5—protein abundance data appears to be more valuable for discriminating between different growth conditions. Notably, the combined dataset consisting of both mRNA and protein abundance measurements yielded the best overall predictive accuracy, irrespective of machine-learning algorithm used (Figure 5, Table 2).

When considering the confusion matrices for the three scenarios (mRNA abundance, protein abundance, and combined), we found that many of the erroneous predictions arising from mRNA abundances alone were not that common when using protein abundances and vice versa (Supplementary Figures 5,6). For example, when using mRNA abundances, many conditions were erroneously predicted as being exponential phase, glycerol, base Mg2+, base Na+, or as stationary phase, glucose, base Mg2+, high Na+; these particular predictions were rare or absent when using protein abundances. By contrast, when using protein abundances, several conditions were erroneously predicted as being stationary phase, glycerol, base Mg2+, base Na+, and these predictions were virtually absent when using mRNA abundance data. For predictions made from the combined dataset, erroneous predictions unique to either mRNA or protein abundances were generally suppressed, and only those predictions that arose for both mRNA and protein abundances alone remained present in the combined dataset (Supplementary Figure 7).

### Prediction accuracy differs between environmental features

We finally wanted to better assess the sources of inaccuracy in our models. As previously noted, the majority of incorrect predictions differed by only a single factor. The environmental features that accounted for most of these single incorrect predictions were Mg2+ concentration for the protein-only data and carbon sources for mRNA-only data.

Growth phase (e.g. exponential, stationary, late-stationary) is not strictly an environmental variable and using this as a feature may partially skew our results if the goal is to predict *strictly* *external* conditions. We thus trained and tested separate models using only exponential or only stationary phase datasets and asked to what extent these models could predict the remaining 3 environmental features (carbon source, [Mg2+], and [Na+]). We found that prediction accuracy was consistently better for models trained with exponential phase samples compared to models trained on stationary phase samples, irrespective of the machine-learning algorithm or the data source (mRNA, protein abundances, or both) (Figure 6). This observation implies that *E. coli* gene expression patterns during stationary phase are less indicative of the external environment compared to cells experiencing exponential growth. A notable caveat is that we have fewer stationary phase samples and this decrease in accuracy may partially be due to the size of the training dataset. Even despite the lower accuracies, however, predictive accuracy from models trained solely on stationary phase cells was still much higher than random expectation, illustrating that quiescent cells retain a unique signature of the external environment for the conditions studied.

Finally, in order to better understand which conditions were the most problematic to predict, we constructed models to predict only *individual* features rather than the entire set of 4 features. When making predictions based on mRNA abundances only, models were most accurate in predicting growth phase and least accurate for carbon source, with Mg2+ and Na+ concentration falling between these two extremes. By contrast, when making predictions based on protein abundances, the most predictable feature was carbon source, the least predictable was Mg2+ concentration, and Na+ concentration and growth phase fell in-between these two extremes (Figure 7, Supplementary Figure 7). Finally, for the combined mRNA and protein abundance dataset, we found that accuracy for carbon source and Mg2+ concentration generally fell between the accuracies observed using mRNA and protein abundances individually. By contrast, accuracies for the Na+ concentration and growth phase were generally as good as—or better than—the prediction accuracies of the individual datasets (Supplementary Figure 9). Together, these findings highlight that mRNA and protein abundances differ in their ability to discriminate between particular environmental conditions.

### Model validation on external data.

The samples that we studied throughout this manuscript are fairly heterogeneous and were collected by different individuals over a span of several months/years. However, different sample types were still analyzed within the same labs, by the same protocols and thus may be more consistent than one might expect from data collected and analyzed independently by different labs—which would be an ultimate goal of future applications of this methodology. We thus applied our best-fitting protein abundance model to analyze protein data with *similar* conditions that was independently collected and analyzed 10. Since this external dataset did not contain measurements for all of the 4196 proteins that we measured and constructed our model on, we tested two alternative approaches. For the first approach, we filled the missing parts of the external data with the median values of our in-house data before making predictions. In the second approach, we restricted our training data set to only include proteins that appeared in the external validation data set. These two approaches lead to comparable results (Table 3A – 3B). Notably, our model made mostly correct predictions on this dataset. The model was most accurate at distinguishing between different growth phase data, and moderately accurate at distinguishing Na+ concentration and carbon source. The external data did not have variation in Mg2+ levels, however, and our model incorrectly predicted several samples to have high Mg2+.

**Discussion**

Our central goal in this manuscript was to determine whether gene expression measurements from a single species of bacterium are sufficient to predict environmental growth conditions. We analyzed a rich dataset of 152 samples for mRNA data and 105 samples for protein data across 16 distinct laboratory conditions as a proof-of-concept to show that *E.coli* gene expression is responsive to external conditions in a measurable and consistent way that permits identification of external conditions from gene signatures alone using supervised machine learning techniques. While *E. coli* is a well-characterized species, our analysis relies on none of this *a priori* knowledge.

It is thus likely that increasing the number and diversity of training samples and conditions will produce further improvements in accuracy and discrimination between a wider array of conditions.

Interestingly, we found that consideration of mRNA and protein datasets alone are sufficient to produce accurate results, but that joint-consideration of both datasets results in superior predictive accuracy. This finding implies that post-transcriptional regulation is at least partially controlled by external conditions, which has been observed by previous studies that have investigated multi-omics datasets1,11–13. Such regulation may result from post-translational modifications14, stress coping mechanisms15, differential translation of mRNAs, or protein-specific degradation patterns.

An important finding that we discovered was that cellular growth phase places limits on the predictability of external conditions, with stationary phase cells being particularly difficult to distinguish from one-another irrespective of their external conditions. A possible explanation for this behavior might be associated with endogenous metabolism; in which, stationary phase cells start to use the residue of other cells instead of provided carbon source. This new carbon source, which is independent of the externally provided carbon source, may suppress the differences between the cells in different external carbon source environments16,17. Another reason for this behavior might be related to strong coupling between gene expression noise and growth rate. Multiple studies concluded that lower growth rates are associated with higher gene expression noise, which might be a survival strategy in harsh enviroments18. Negative correlations between population average gene expression and noise have been shown for *E. coli* and *Saccharomyces cerevisiae,* lending support for this theory19,20. Finally, we note that stationary phase cells have likely depleted the externally supplied carbon sources after several weeks of growth. The similarity of stationary phase cells to other stationary phase cells may be a consequence of them inhabiting more similar chemical environments to one another than during exponential growth. Nevertheless, discrimination of external environmental factors in stationary phase cells was still much better than random—indicating that these populations continue to retain information about the external environment despite their overall quiescence.

A relevant finding to emerge from our study is that different features of the environment may be more- or less- easy to discriminate from one another and this discrimination may depend on which molecular species is being interrogated. Growth phase, for instance, can be reliably predicted from mRNA concentrations but similar predictions from protein concentrations were less accurate. A possible explanation for this is the fact that mRNAs and proteins have different life-cycles21. Given the comparably slow degradation rates of proteins, a large portion of the stationary phase proteome is likely to have been transcribed during exponential phase growth. As another example, carbon sources can be reliably predicted from protein concentrations, but the accuracy of carbon source predictions from models trained on mRNA concentrations was more limited. Carbon assimilation is known to be regulated by post-translational regulation22–24, which may be a possible reason for this finding (Figure 7, Supplementary Figure 8).

Despite the fact that we investigated over 150 samples spanning 16 unique conditions, a limitation of our work and conclusions is nevertheless sample size (though our study is comparable or larger than similar multi-conditional transcriptomic and/or proteomic studies)10,25–27. The comparison between all of our data with the more limited set that includes only the intersection of samples for which we have both mRNA and protein abundance data (Figure 4 compared to Supplementary Figures 4,5) indicates that prediction accuracy decreases as the size of our training sets gets smaller. This trend indicates that our training set sizes are still ultimately limiting to model accuracy. A second possible issue with our study is associated with sample number bias28–30. We made corrections with weight factors31,32 and display the multi-conditional *F*1 score33 to account for the fact that some conditions contained more data samples, but the predictability of *individual* conditions nevertheless increases with the number of training samples for that particular condition (Supplementary Figure 3). This finding again highlights that increasing training data will likely result in higher prediction accuracies.

Our study is a proof-of-principle towards the goal of using gene expression patterns of natural species as a rapid and low-cost method for assessing environmental conditions. Other research has shown that the gene repertoire, derived from meta-genomic sequencing, may be useful for determining the presence of particular contaminants (<http://mbio.asm.org/content/9/1/e02435-17?related-urls=yes&legid=mbio;9/1/e02435-17>). Our findings suggest that further incorporation of species-specific gene expression patterns can likely improve the accuracy of such methods. While genetically engineered strains may play a similar role as environmental biosensors, our study highlights that—with enough training data—the molecular composition of natural populations may provide sufficient information to accurately resolve past and present environmental conditions.

## Materials and Methods

### Data

We use the same data sets that were used in a previous study [cite]. Except when explicitly noted (i.e. the 102 samples for which we have both mRNA and protein data), we used all of the 155 available datasets.

### Data preparation and training methodology

We used *C-Classification*8for training classification model.

The initial data preparation is similar to a previous study on the same data set 7. After finding suitable subsets of the data for the tests and summing up technical replicate results for proteins, we calculate size factor normalized data with the DeSeq234 package and apply variance stabilizing transformation35 (VST).

Next, we divide the data into two subsets: training&tune set and test set. The division is semi-random such that our algorithm preserves the ratios of different conditions in training&tune and test subsets. We preserve the condition labels for training&tune data (thus our learning is supervised), but we delete the labels of the samples for the test set. We then apply frozen Surrogate Variable Analysis36 (fSVA) to get rid of the batch effects in the sample. This algorithm can correct for batch effects on both training&tune and test data without knowing the labels of the test data. After fSVA, we use principal component analysis37 (PCA) to define the principle axis of training&tune set and rotate the test data set with respect to the principal axis of the training&tune set. We then pick the top 10 most significant axes for both training&tune, and test data sets. Following this step, we train and tune our candidate machine learning algorithms with the dimension reduced training&tune datasetand apply those trained and tuned algorithms on dimension reduced test dataset to make predictions.

The training algorithm first divides the training&tune data into separate training and tuning datasets semi-randomly, trying to preserve the ratios of individual conditions. We repeat this division 10 times and generate 10 training and tuning dataset pairs for each boot strap which ends us up with 600 distinct training pairs (since we perform a total of 60 bootstrap replicates of the entire procedure). Next, we generate a parameter grid for tuning process. We optimized the "cost" parameter for the three SVM models, and the "gamma" parameter for the SVM with radial and sigmoidal kernels in our training&tune set as illustrated in Figure 2. For the random forest algorithm, we optimized three parameters; "mtry", "ntrees", and "nodesize" (Supplementary table 1). Before the model training phase, we applied size factor normalization, normalize batch effects and apply PCA37 for dimension reduction. We use the e1071 package38 for implementing support vector machines with linear, radial, and sigmoid kernels that based on the libSVM8 library and the randomForest package39 for the random forest algorithm.

In the case of combined mRNA and protein data analysis, we calculate size factors with the DeSeq2 algorithm and batch effects with the fSVA algorithm individually, then combine two datasets and apply PCA on combined data. We run this process and call the training and tuning algorithm 600 times with independent divisions between training&tune, and test data sets.

### Prediction methodology and scoring

We apply multiple sets of tests throughout this manuscript: i) we either try to predict an individual parameter such as growth phase, carbon source, Mg2+ concentration or Na+ concentration for each sample, or ii) we try to predict all conditions at once for each sample.

We train each of the four machine learning models, and make predictions on tune set for all variables that we are tuning. For all 10 training and tuning dataset pairs, we apply a class weight normalization during training, where class weights are inversely proportional to number of training samples and calculated independently for each training run. We average the errors calculated by the scoring metric over training-tuning dataset pairs and obtain an average error value estimate for each algorithm and for each parameter combination. Then we find the winning parameter combination for each algorithm and label this parameter combination/algorithm match as winner for each of xx independent divisions between training&tune, and test data sets.

At the end of all procedure we end up with 60\*4=240 winner algorithms with their tuning parameters and their average error values.

### Calculation of the score metric

The metric we use for scoring is the multi-class macro *F*1 score 9,33,40 that normalizes *F*1 scores over individual conditions; i.e. each condition has equal weight instead of each sample. Out of two distinct macro *F*1 score calculation methods in the literature

1. ; where 33
2. 9

we implemented the first.

### Calculation for the external data

The external data consists of five triplets, that we label as samples A, B, C, D, and E. All samples use base Mg2+. Sample A uses glucose as carbon source, does not have any osmotic stress and is in exponential growth phase. Sample B uses glucose as carbon source, does not have any osmotic stress and is in exponential growth phase. Sample C includes 50mM sodium, glucose as carbon source, is in exponential growth phase. With respect to our threshold for classifying osmotic stress, sample C is in-between what we consider base Na+ and high Na+. Samples D and E use glucose as carbon source, do not have any osmotic stress, and were measured after 24 and 72 hours of growth, respectively. Our threshold defines stationary phase as 24-48 hours and late stationary phase as 1 to 2 weeks. So, sample D is in stationary phase, and sample E is in-between the stationary and the late-stationary phases.

### Statistical analysis and data availability

All statistical analyses were performed in R. All processed data and analysis scripts are available on github: <https://github.com/umutcaglar/ecoli_multiple_growth_conditions>

Raw Illumina read data and processed files of read counts per gene and normalized expression levels per gene have been deposited in the NCBI GEO database41 (accession GSE67402 for the glucose time-course previously published7, accession GSE94117 for all other experiments). The mass spectrometry proteomics data have been deposited to the ProteomeXchange Consortium via the PRIDE partner repository42 (accession PXD002140 for the glucose time-course previously published7, accession PXD005721 for all other experiments.

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## Contributions

M.U.C and C.O.W. conceived of the study.

M.U.C. designed the analysis pipeline, analyzed the data, and prepared the figures.

M.U.C., A.J.H. and C.O.W. wrote the manuscript.

## Competing interests

The authors declare no competing financial interests.