Evidence supporting predicted metabolic pathways for *Vibrio cholerae*

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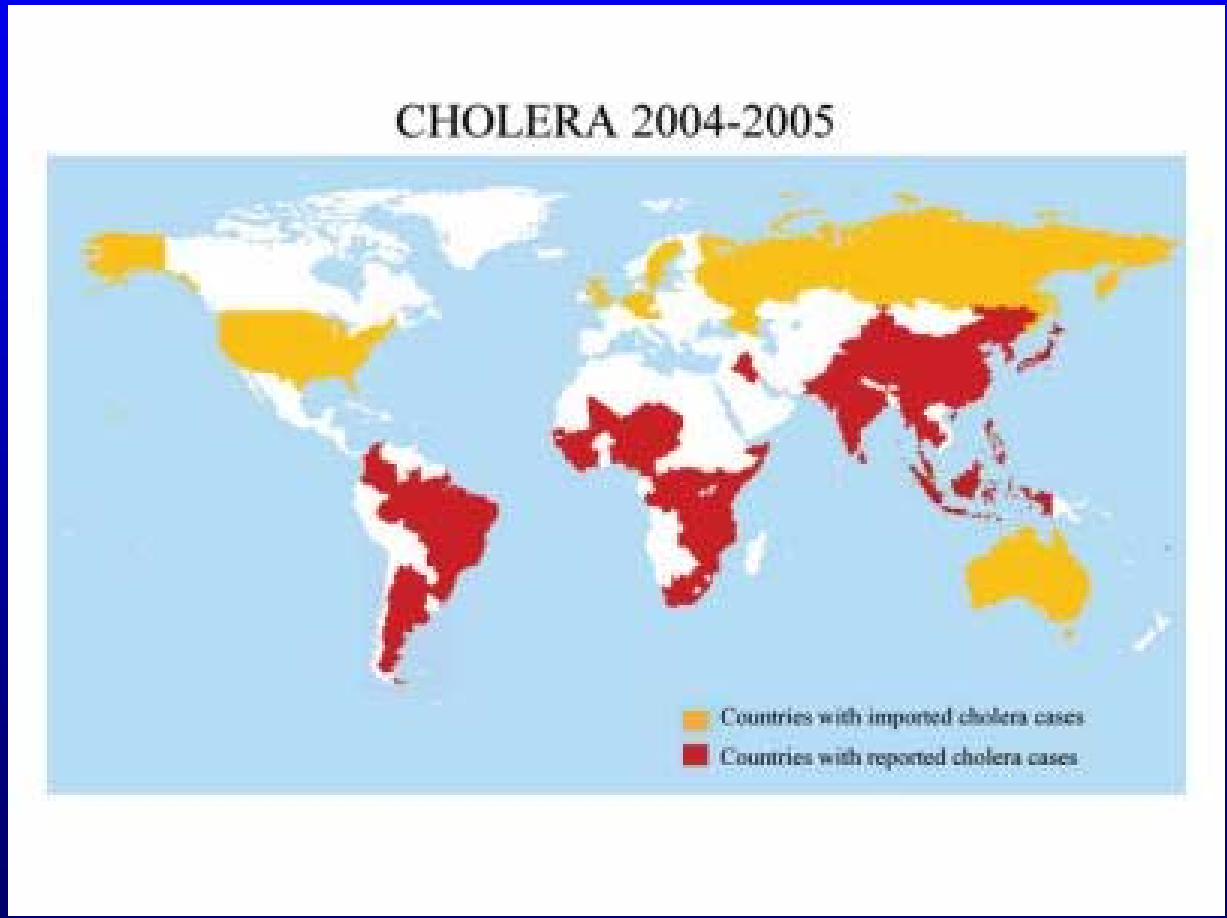
Vibrio cholerae causes cholera

- Untreated patients can be killed in 24 h.

7 pandemics worldwide since 1817



Vibrio cholerae

- Two distinctive lifestyles
  - a bacterium well adapted to aquatic habitats
  - a pathogen living in human intestine

Objectives

- Predict the metabolic pathways for *V. cholerae*
- Validate the predicted pathways

VchoCyc

PGDB	Quantity
Pathways	171
Compounds	656
Enzymes	639
Enzymatic Reactions	912
With enzymes in VchoCyc	654

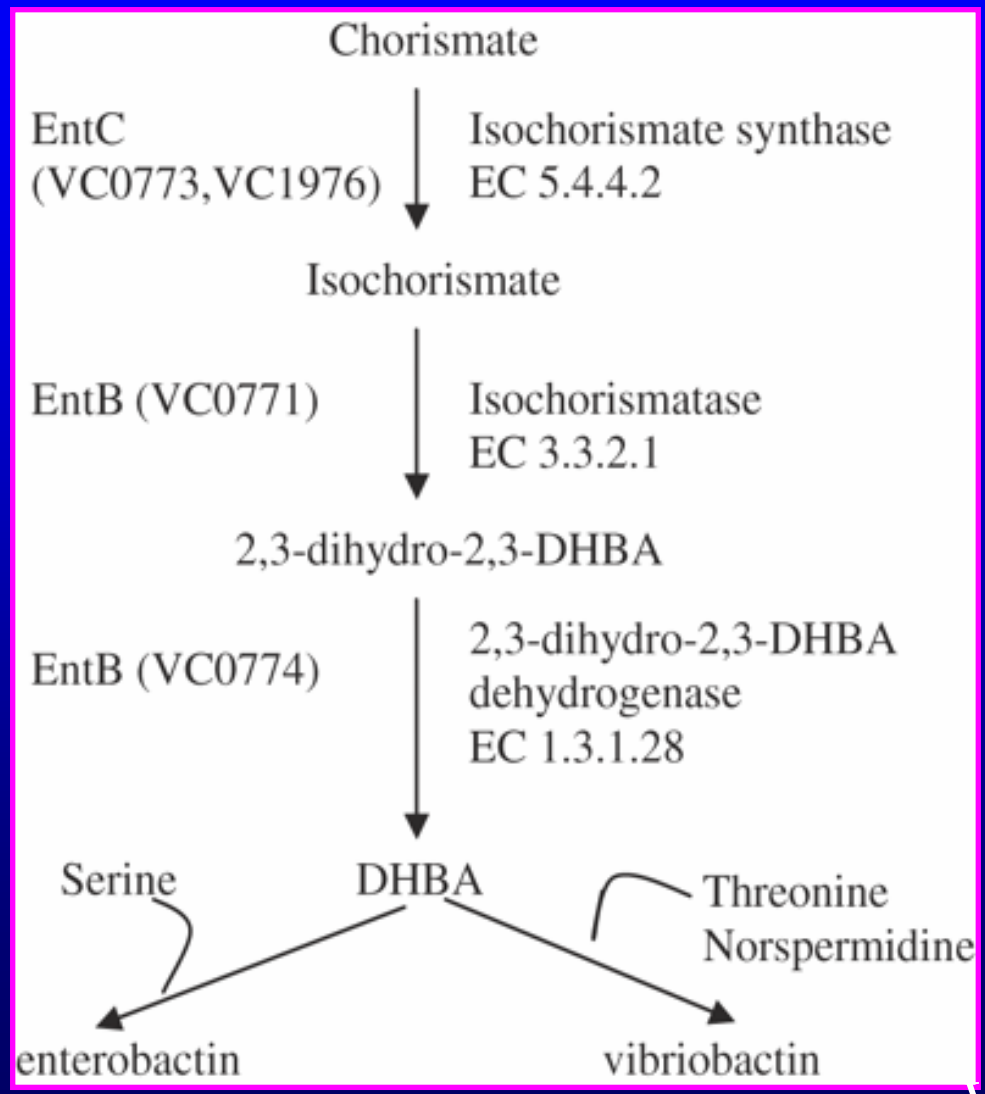
How do we validate the predicted pathways in VchoCyc?

Three types of evidence

- Previous literature
- Clinical tests
- Microarray expression data

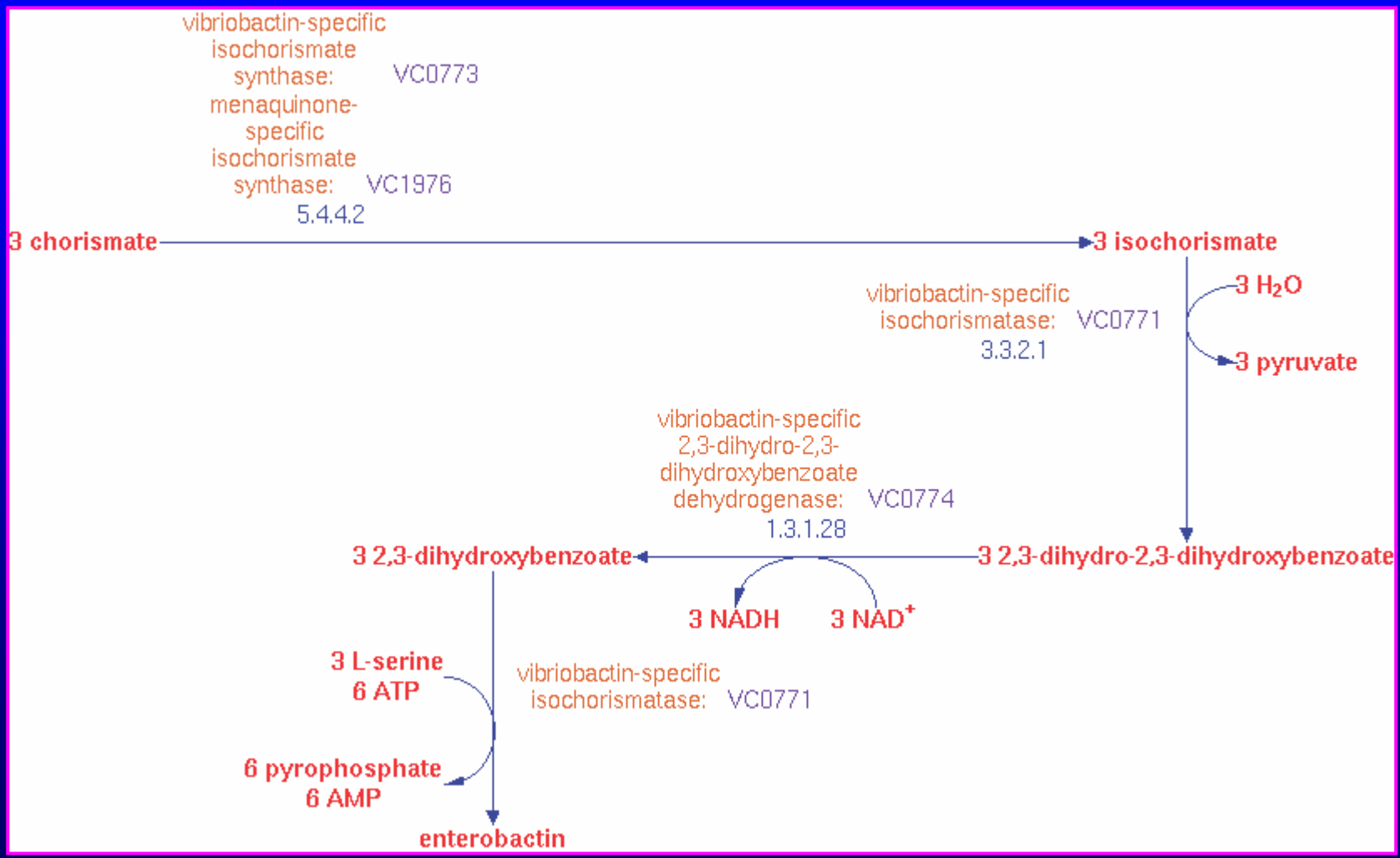
Using evidence from previous literature to validate predicted pathways

Known biosynthetic pathways of enterobactin and vibriobactin



Wyckoff et al, 2001

Predicted biosynthetic pathways of enterobactin

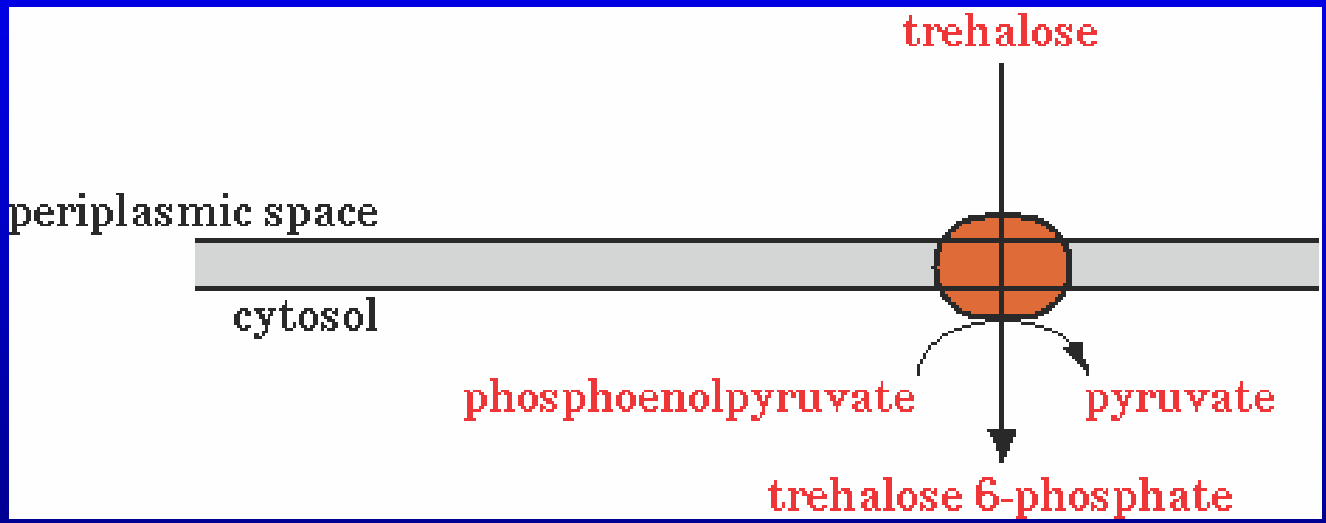


Using evidence from clinical tests to validate predicted pathways

Evaluating the pathways using clinical tests

- If a nutrient can support V. cholerae growth, the corresponding transporters and metabolic pathways/reactions should exist.
- Else, either no transporters or no metabolic pathways/reactions.

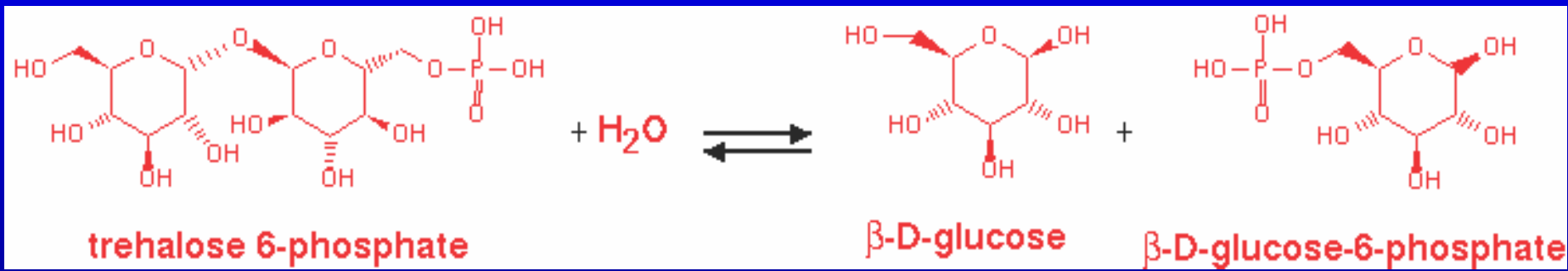
Trehalose acid production clinical test for V. cholerae



If predicted pathway exists, we expect to find a transporter and enzymes for this test

Trehalose, acid production (+)

EC 3.2.1.93



Found both enzyme and transporter: evidence supports the predicted pathway

D-sorbitol acid production clinical test for V. cholerae

Clinical test for V. cholerae is negative for D-sorbitol acid production.

Should not find transporter and enzymes for this test

Neither a transporter nor a reaction was found.

VchoCyc predictions are consistent with clinical tests

- 16 out of the total 17 clinical tests were consistent.
- 1 out of 17 was inconsistent: D-mannose with acid production (+).
  - the reaction to catalyze mannose was found but not the transporter for it.

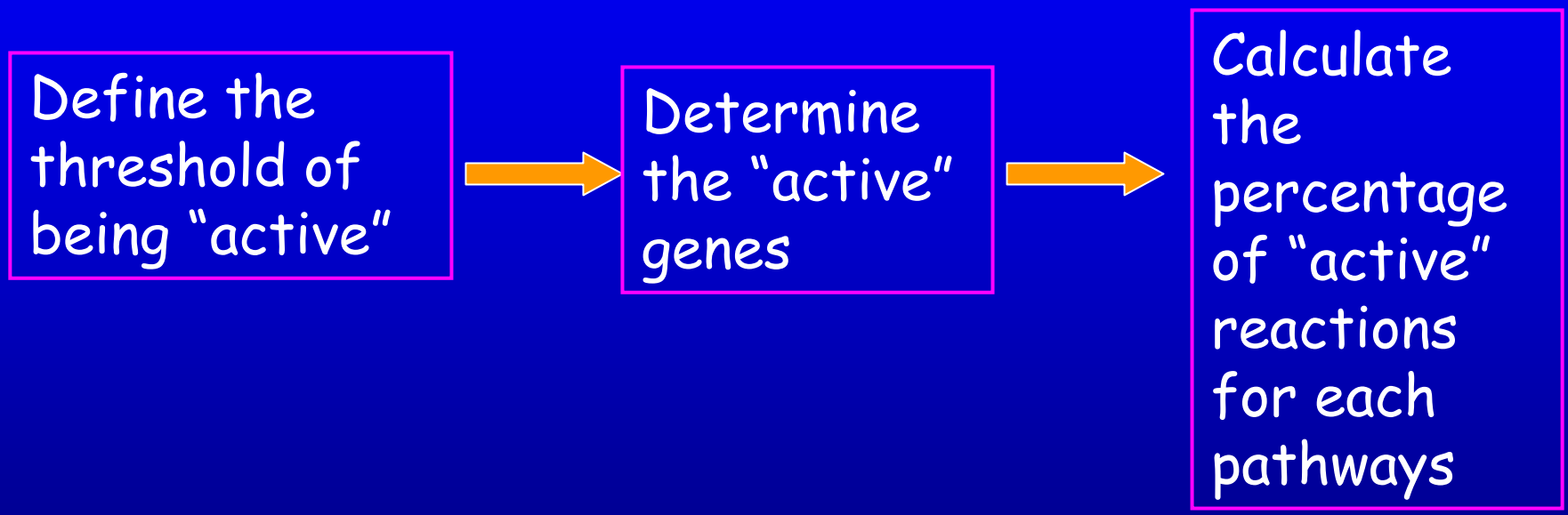
Using evidence from microarray expression data to validate predicted pathways

When a "large" percentage of the reactions in a pathway are "active" simultaneously, we have expression evidence supporting this pathway.

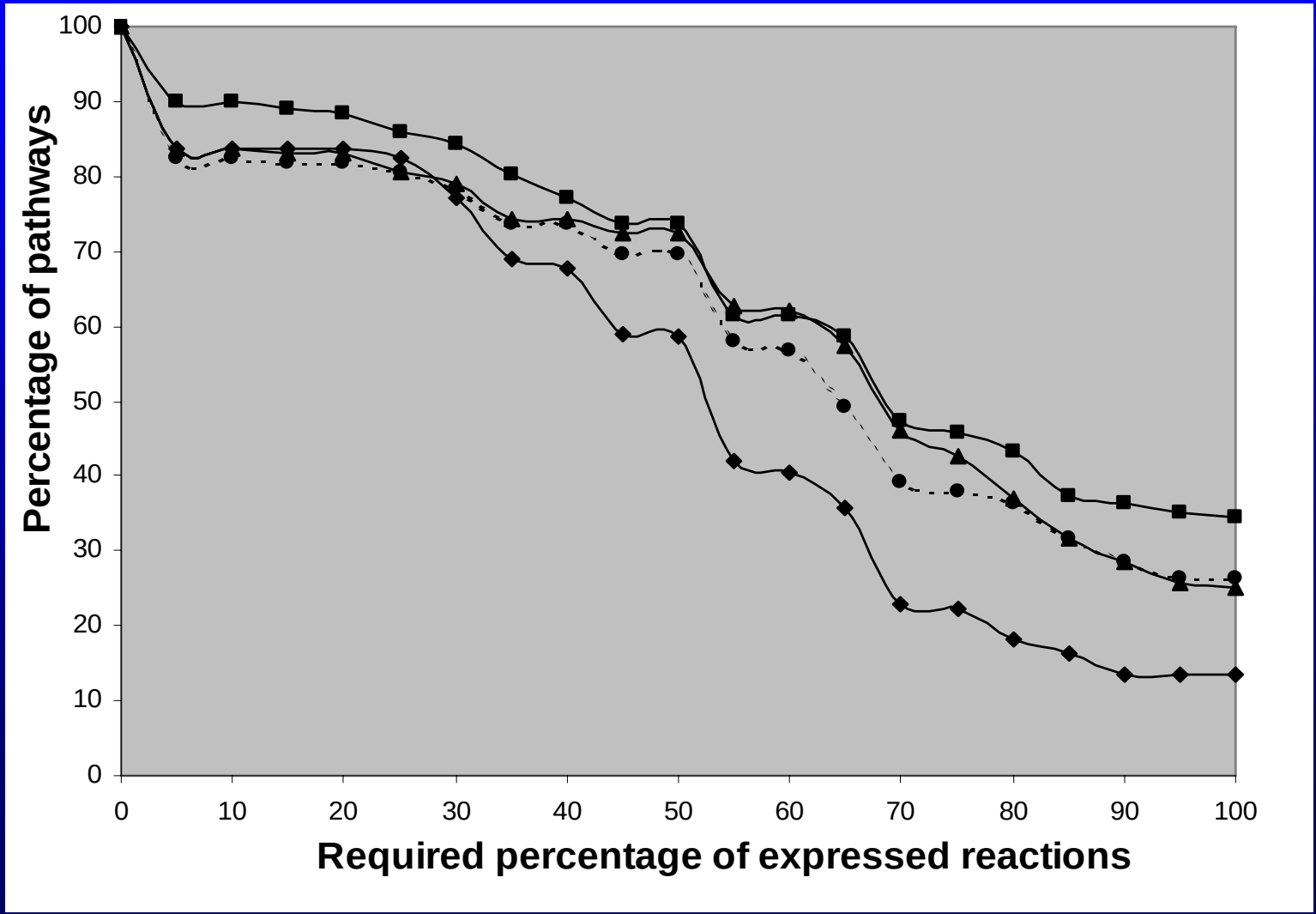
Microarray data

- Minimal media with Maltose or Lactate
- LB rich medium
- Patient stool samples

Approach


Define the threshold of being "active"
Determine the "active" genes
Calculate the percentage of "active" reactions for each pathways

For a given proportion of expressed reactions in a pathway, how much percent of pathways have supporting evidence?



Example pathways with strong evidence from microarray data

Pathway name	Minimal medium + lactate	Minimal medium + maltose	LB	Patient
aspartate biosynthesis and degradation	100	100	100	100
fatty acid elongation -- saturated	100	100	100	100
fatty acid elongation -- unsaturated	100	100	100	100
glycine biosynthesis I	100	100	100	100
glycine cleavage	100	100	100	100

Example pathways with no evidence from microarray data

Pathway name	Minimal medium + lactate	Minimal medium + maltose	LB	Patient
4-hydroxyproline degradation	0	0	0	0
Entner-Doudoroff pathway	0	0	0	0
L-idonate degradation	0	0	0	0
tyrosine biosynthesis II	0	0	0	0

Different media provide evidence for different pathways

- In patient data, there is evidence for the amino acid biosynthesis pathways. Consistent with Merrell et al, 2002.
- In LB medium, there is evidence for degradation pathways of amino acids.

Summary

- We constructed VchoCyc
- We used three sources of evidence to support the predicted pathways in VchoCyc
  - Previously known pathways in literature
  - Consistency with clinical tests
  - Consistency with microarray data

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