Supplemental Material

Chemostat Theory.

Nutrient stress severity was controlled using classic chemostat theory. The theory is based on Monod kinetics (equation 1) which relate culture specific growth rate to growth-limiting substrate concentration:

$$\mu = \frac{\mu_{max} \cdot S}{K_S + S} \quad (1),$$

where μ is the specific growth rate (h^{-1}), μ_{max} is the organism's maximum specific growth rate (h^{-1}), S is the growth limiting nutrient (g/L), and K_s is the Monod (half-saturation constant) for the limiting substrate (g/L). Equation 2 illustrates the relationship between culture specific growth rate and chemostat dilution rate:

$$\mu = D = \frac{F}{V} \quad (2),$$

where D is the chemostat dilution rate (h^{-1}), F is the volumetric medium flow rate (mL/h) and V is the working liquid volume of the chemostat (mL). Substituting the relationship for μ from equation 2 into equation 1 and rearranging for S yields:

$$S = \frac{D \cdot K_S}{\mu_{max} - D} \quad (3)$$

Based on equation 3, a decrease in chemostat dilution rate lowers the steady-state concentration of the growth limiting substrate increasing nutrient limitation stress (52, 53). During chemostat growth, nutrient limitation severity is set by dilution rate not by cell concentration (88). Additionally, the culture specific nutrient uptake rates are related to the

specific growth rates and the respective biomass yields. For example, the specific glucose uptake rate (q_{glc}, g glucose/ g cdw / h) can be defined by:

$$q_{glc} = \frac{\mu}{Y_{biom/glc}} \quad (4)$$

where $Y_{biom/glc}$ is the biomass yield on glucose (g cdw / g glucose).

TABLE S1 Isoelectric focusing protocol

Step	Type	Voltage limit	Time/Volt Hours
1	Step & Hold	500V	825 vh
2	Gradient	1000 V	8 h
3	Gradient	3000 V	1 h
4	Step & Hold	3000 V	4500 vh
5	Gradient	8000 V	1 h
6	Step & Hold	8000 V	12000 vh
7	Gradient	10000 V	1h
8	Step & Hold	10000 V	36000
9	Hold	500V	∞

TABLE S2 Found in Excel Workbook "Supplemental_Data.xlsx" contains yields, specific glucose uptake rates and siderophore (competitive iron-binding capacity) by dilution rate and culturing condition.

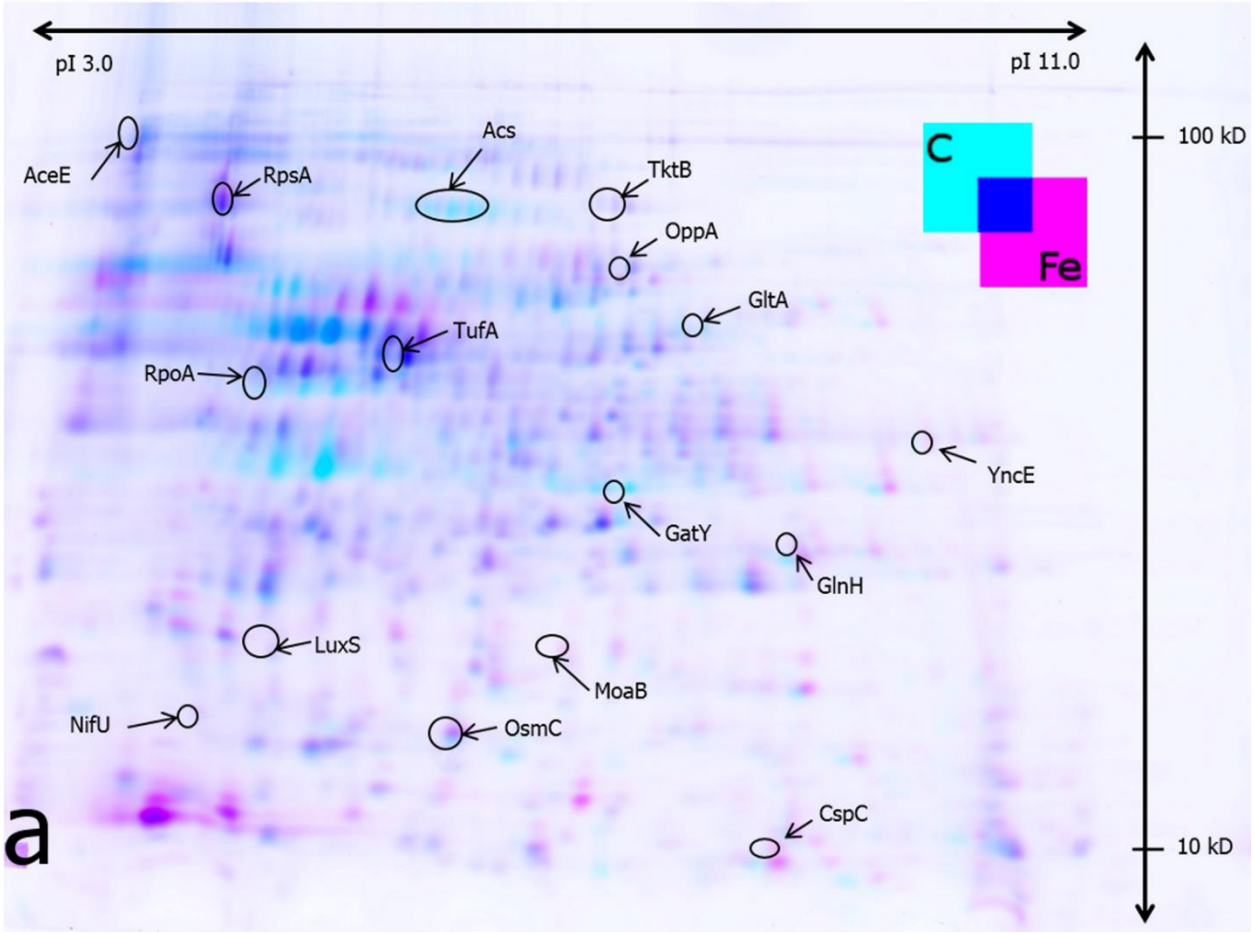
TABLE S3 Found in Excel Workbook "Supplemental_Data.xlsx" contains spot volumes exported from progenesis for the membrane fraction, expression profile plots, statistics from progenesis, brief description of protein, categorized isoform counts, and MASCOT information. Links to MASCOT results (for as long they remain active).

TABLE S4 Found in Excel Workbook "Supplemental_Data.xlsx" contains spot volumes exported from for the membrane fraction, expression profile plots, statistics from Progenesis, brief description of protein, categorized isoform counts, and MASCOT information. Links to MASCOT results (for as long they remain active).

TABLE S5 Proteins observed in the current study that have been associated with Fur or RyhB regulation based on previous transcriptomics studies. Associated gene listed in parentheses after protein name. Transcript data from 1) McHugh et al., 2003 J. Biol. Chem. 278(32) 29478-29486 and 2) Masse et al., 2005 J. Bactriol. 187(20) 6962-6971.

Bfr (bfr), CirA (cirA), CspC (cspB, cspI), ElaB (elaC), FepA (fepE, fepC, fepG, fepD), FhuA (fhuA), FimA (fimE), OmpX (ompX), OppA (oppA), PepB (pepB), PepD (pepB), PflB (pflA), PyrB (pyrL), PyrI (pyrL), RplI (rplL), RplL (rplL), SodA (sodB), SodB (sodB), YbaY (ybaY).

FIG S2. Heatmap of all identified proteins with hierarchical clustering can be found in the Excel Workbook "Supplemental_Data.xlsx".


pI 3.0
pI 11.0
100 kD
10 kD
C
Fe
AceE
RpsA
Acs
TktB
OppA
GltA
TufA
RpoA
YncE
GatY
GlnH
LuxS
MoaB
NifU
OsmC
CspC
a

66

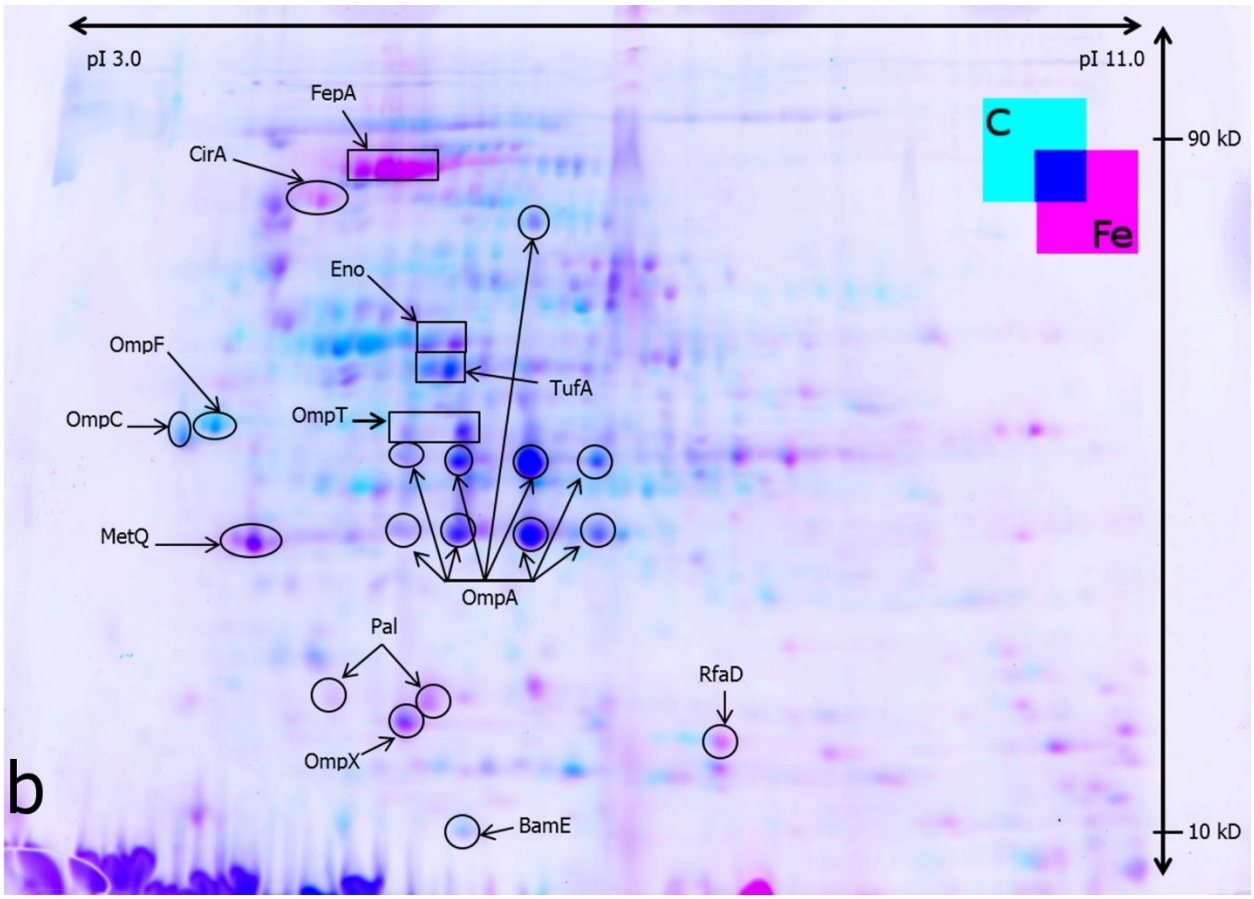


FIG S1. Representative 2-dimensional gels for the *E. coli* cytoplasm protein fraction (a) and membrane protein fraction (b) as a function of iron- or glucose-limited growth. Several proteins are labeled for orientation purposes. Magenta (Fe): iron-limited chemostat data. Cyan (C): glucose-limited chemostat data.