

Supplementary Information for

Reverse Diauxie Phenotype in *Pseudomonas aeruginosa* Biofilm Revealed by Exometabolomics and Label-Free Proteomics

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See Methods in primary manuscript for sample sizes, replicates, and error analysis for data reported here.

Supplementary Table 1. NMR exometabolomics data for biofilm and planktonic *P. aeruginosa* cultures grown on CSP medium. <0.1 mM indicates metabolite was detected at concentrations below this level.

Biofilm data					Planktonic data			
Time (h)	0	6	12	18	Time (h)	9	Time (h)	13
Compound	mM	mM	mM	mM	Compound	mM	Compound	mM
Adenine	0.2	0.3	0.1	<0.1	2-Hydroxybutyrate	<0.1	Histidine	<0.1
Alanine	1.0	1.1	0.3	0.1	Acetate	<0.1	Anthranilate	<0.1
Arginine	1.4	1.9	0.9	0.3	Adenine	<0.1	Ethanol	0.1
Asparagine	0.8	0.9	<0.1	<0.1	Alanine	0.6 ± 0.1	DSS	0.1
Aspartate	1.2	1.1	0.3	0.1	Arginine	1.6 ± 0.2	Epicatechin	0.2
Cadaverine	<0.1	<0.1	<0.1	0.2	Asparagine	0.3 ± 0.2		
Citrate	2.6	5.2	1.2	1.1	Aspartate	0.2 ± 0.1		
Cystine	0.6	0.9	0.6	0.3	Cellobiose	0.1 ± 0.05		
Cytosine	0.2	0.3	0.1	0.1	Citrate	3.4 ± 0.05		
DSS	0.3	0.2	0.2	0.2	Cysteine	<0.1		
Ethanol	<0.1	0.1	0.1	0.1	Cystine	0.5 ± 0.05		
Gluconate	<0.1	<0.1	0.3	0.4	Cytosine	0.1 ± 0.05		
Glucose	24.6	31.5	20.0	10.5	DSS	0.3 ± 0.05		
Glutamate	1.0	1.3	0.4	0.2	Dimethylamine	<0.1		
Glycine	1.1	1.4	0.4	0.1	Ethanol	0.1 ± 0.05		
Histidine	0.5	0.7	0.3	0.1	Formate	<0.1		
Isoleucine	1.0	1.4	0.5	0.2	Fumarate	<0.1		
Leucine	0.9	1.1	0.5	0.2	Glucose	27.5 ± 4.5		
Lysine	1.0	1.4	0.6	<0.1	Glutamate	0.5 ± 0.05		
Methionine	0.3	0.4	0.1	0.1	Glutamine	<0.1		
Phenylalanine	0.5	0.6	0.2	0.1	Glycine	1.0 ± 0.05		
Proline	1.1	1.3	0.7	<0.1	Histidine	0.6 ± 0.05		
Serine	1.8	2.6	0.9	0.3	Hypoxanthine	<0.1		
Threonine	1.4	1.4	0.6	0.3	Isoleucine	1.0 ± 0.05		
Tryptophan	0.1	0.2	0.1	<0.1	Isopropanol	<0.1		
Tyrosine	0.6	0.8	0.3	0.1	Lactate	<0.1		
Uracil	0.2	0.3	0.8	<0.1	Leucine	1.3 ± 0.1		
Valine	1.0	1.5	0.5	0.3	Lysine	0.9 ± 0.1		

Methionine	0.2 ± 0.05
Phenylalanine	0.4 ± 0.05
Proline	0.7 ± 0.05
Pyroglutamate	0.1 ± 0.05
Serine	0.8 ± 0.05
Succinate	<0.1
Tartrate	<0.1
Threonine	1.3 ± 0.1
Tryptophan	0.1 ± 0.05
Tyrosine	0.7 ± 0.05
Uracil	0.1 ± 0.05
Valine	1.0 ± 0.1
Xanthine	<0.1

Supplementary Table 2. Inclusive list of metabolically relevant, *P. aeruginosa* proteins with significant increases (blue highlighted, ↑), significant decreases (red highlighted, ↓), and non-significant abundance changes (no highlights) during planktonic growth in LCSP vs. CSP media (see Figure 4a). Proteins in boldface are observed in both planktonic (Figure 4a) and biofilm (Figure 4b) cultures regardless of abundance. ‘*’ indicates homologous protein KEGG ID.

Protein	KEGG ID	Description of Proteins in Figure 4a	Unique Peptides	Sequence Coverage %	$\log_2(x_{LCSP}/x_{CSP})$
LldD	PA4771	L-lactate dehydrogenase	14	44.4	4.88 ↑
MmsA	PA3570	methyilmalonate-semialdehyde dehydrogenase	18	50.1	-5.47 ↓
MmsB	PA3569	3-hydroxyisobutyrate dehydrogenase	9	52.7	-4.53 ↓
HutH	PA5098	histidine ammonia-lyase	8	27.5	-4.02 ↓
Glk	PA3193	glucokinase	2	12.1	-3.82 ↓
OprB	PA3186	porin B	15	44.3	-2.98 ↓
HutU	PA5100	urocanase	23	55.3	-2.89 ↓
Zwf	PA3183	glucose-6-phosphate 1-dehydrogenase	18	53.0	-2.30 ↓
HupA	PA5348	DNA-binding protein	9	73.3	-2.30 ↓
AtoB	PA2001	acetyl-CoA acetyltransferase	15	62.6	-2.28 ↓
Gap	PA3195	glyceraldehyde 3-phosphate dehydrogenase	16	59.0	-2.08 ↓
MaiA	PA2007	maleylacetoacetate isomerase	5	39.2	-1.99 ↓
HutI	PA5092	imidazolonepropionase	3	11.2	-1.78 ↓
Pgl	PA3182	6-phosphogluconolactonase	9	52.1	-1.51 ↓
Hfq	PA4944*	RNA-binding protein	16	51.3	-1.49 ↓
Eda	PA3181	2-dehydro-3-deoxy-phosphogluconate aldolase	8	51.4	-1.44
GlyA2	PA2444	serine hydroxymethyltransferase	5	27.0	-1.35
PhhC	PA0870	aromatic amino acid aminotransferase	18	69.2	-1.18
AstE	PA0901	succinylglutamate desuccinylase	3	16.0	-1.05
AspA	PA5429	aspartate ammonia-lyase	17	48.7	-1.04
SpuD	PA0300	putrescine ABC transporter substrate-binding protein	12	52.6	-0.99
BraF	PA1071	ABC transporter ATP-binding protein	3	18.0	-0.94
GdhB	PA3068	NAD-specific glutamate dehydrogenase	58	48.1	-0.88
PhhB	PA0871	pterin-4-alpha-carbinolamine dehydratase	7	78.8	-0.88
BraG	PA1070	ABC transporter ATP-binding protein	5	29.2	-0.87
DavT	PA0266	5-aminovalerate aminotransferase	18	72.8	-0.79
TyrS	PA4138*	tyrosyl-tRNA synthetase	1	55.3	-0.57
GlhA	PA1580	citrate synthase	21	76.6	-0.55
ProS	PA0956*	prolyl-tRNA synthetase	2	58.0	-0.53
SahH	PA0432	adenosylhomocysteinase	24	68.7	-0.40
NfuA	PA1847	Fe/S biogenesis protein	5	46.9	-0.40
AhpC	PA0139*	alkyl hydroperoxide reductase subunit C	13	78.6	-0.39
PA5475	PA5475	hypothetical protein	8	73.1	-0.39
PA1673	PA1673	bacteriohemerythrin	6	50.3	-0.33
Hom	PA3736	homoserine dehydrogenase	10	38.0	-0.27
ArgB	PA5323	acetylglutamate kinase	11	15.4	-0.24
MexA	PA0425	multidrug resistance protein	14	57.2	-0.22
RnpA	PA5569	ribonuclease P	1	11.9	-0.20

Protein	KEGG ID	Description of Proteins in Figure 4a	Unique Peptides	Sequence Coverage %	$\log_2(x_{LCSP}/x_{CSP})$
CysS	PA1795	cysteine--tRNA ligase	12	32.4	-0.17
LpdG	PA1587	2-oxoglutarate dehydrogenase complex dihydrolipoyl dehydrogenase	23	57.9	-0.13
KatA	PA4236	catalase	9	36.1	-0.07
Pta	PA0835	phosphate acetyltransferase	9	21.2	-0.06
AlgU	PA0762	RNA polymerase sigma factor	3	31.1	0.14
RplV	PA4258	50S ribosomal protein L22	9	54.5	0.15
ArgG	PA3525	argininosuccinate synthase	19	64.9	0.16
PckA	PA5192*	phosphoenolpyruvate carboxykinase (ATP)	2	61.8	0.24
RplP	PA4256	50S ribosomal protein L16	7	48.2	0.28
SpeE1	PA1687	polyamine aminopropyltransferase	8	43.7	0.48
DsbA	PA5489	thiol:disulfide interchange protein	8	44.5	0.49
RapA	PA3308	RNA polymerase-associated protein	16	24.3	0.76

Supplementary Table 3. Inclusive list of metabolically relevant, *P. aeruginosa* proteins with significant increases (blue highlighted, ↑), significant decreases (red highlighted, ↓), and non-significant abundance changes (no highlights) during biofilm growth in LCSP vs. CSP media (see Figure 4b). Proteins in boldface are observed in both planktonic (Figure 4a) and biofilm (Figure 4b) cultures, regardless of abundance. ‘*’ indicates homologous protein KEGG ID.

Protein	KEGG ID	Description of Proteins in Figure 4b	Unique Peptides	Sequence Coverage %	log ₂ (x _{LCSP} /x _{CSP})
FumC1	PA4470	fumarate hydratase	19	59.6	1.51 ↑
PA5217	PA5217	iron ABC transporter substrate-binding protein	12	36.1	1.61 ↑
HisA	PA5141	1-(5-phosphoribosyl)-5-[(5-phosphoribosylamino)methylideneamino] imidazole-4-carboxamide isomerase	6	32.7	1.61 ↑
SodA	PA4468	superoxide dismutase	3	25.1	1.78 ↑
Mqo1	PA3452	malate:quinone oxidoreductase	4	11.1	1.87 ↑
SpeE1	PA1687	polyamine aminopropyltransferase	3	23.8	2.04 ↑
PvdA	PA2386	L-ornithine N5-oxygenase	4	12.4	2.10 ↑
FolE2	PA5539	GTP cyclohydrolase	6	36.2	2.92 ↑
LldD	PA4771	L-lactate dehydrogenase	12	39.6	5.26 ↑
HutI	PA5092	imidazolone-5-propionate hydrolase	4	16.2	-1.37
OprB	PA3186	porin B	14	41.0	-0.85
LeuC	PA3121	3-isopropylmalate dehydratase large subunit	4	14.3	-0.67
TrpS	PA4439	tryptophan--tRNA ligase	10	37.7	0.44
AtpA	PA5556*	ATP synthase F1, alpha subunit	1	39.7	0.63
NqrC	PA2997	Na(+)-translocating NADH-quinone reductase subunit C	4	23.4	0.64
CspA	PA3266	major cold shock protein	3	52.2	0.69
PckA	PA5192*	phosphoenolpyruvate carboxykinase (ATP)	1	53.8	0.76
Hcp1	PA0085	protein secretion apparatus assembly protein	6	38.9	0.78
SecD	PA3821	preprotein translocase subunit	12	23.4	0.79
RdgC	PA3263	putative recombination associated protein	13	64.4	0.81
MurG	PA4412	undecaprenyldiphospho-muramoylpentapeptide beta-N-acetylglucosaminyltransferase	4	23.0	0.82
CysS	PA1795	cysteine--tRNA ligase	8	21.7	0.82
HmuV	PA4706	putative ATP-binding component of ABC transporter	3	25.9	0.84
FtsA	PA4408	cell division protein	14	48.7	0.84
HemC	PA5260*	porphobilinogen deaminase	4	18.8	0.84
ClpP1	PA1801	ATP-dependent Clp protease proteolytic subunit	4	21.1	0.86
PyrH	PA3654	uridylate kinase	8	46.1	0.89
DsbA	PA5489	thiol:disulfide interchange protein	6	28.9	0.90
TrpE	PA0609	anthranilate synthase component I	14	38.8	0.91
Eco	PA2755	ecotin precursor	7	52.6	0.92
SthA	PA2991	soluble pyridine nucleotide transhydrogenase	12	43.1	0.92
ComL	PA4545	competence protein	9	31.1	0.93
TyrS	PA4138	tyrosyl-tRNA synthetase	5	24.5	0.94
LepB	PA0768	signal peptidase I	5	20.8	0.94
TrpC	PA0651	indole-3-glycerol-phosphate synthase	8	30.2	0.96

Protein	KEGG ID	Description of Proteins in Figure 4b	Unique Peptides	Sequence Coverage %	$\log_2(x_{LCSP}/x_{CSP})$
Xpt	PA5298	xanthine phosphoribosyltransferase	2	18.4	0.97
RplL	PA4271	50S ribosomal protein L7/L12	8	86.1	0.98
SecB	PA5128	preprotein translocase subunit	6	58.3	0.99
AceA	PA2634	isocitrate lyase	18	46.7	1.01
HchA	PA1135	molecular chaperone Hsp31/glyoxalase	1	11.7	1.13
ClpV1	PA0090	secretion protein	6	9.3	1.14
PstS	PA5369*	putative phosphate ABC transporter, periplasmic phosphate-binding protein	4	24.1	1.20
PasP	PA0423	hypothetical protein	15	69.6	1.21
ThrB	PA5495	homoserine kinase	2	12.0	1.23
TrmB	PA0382	tRNA (guanine-N(7)-)-methyltransferase	2	9.4	1.23
ArgF	PA3537	ornithine carbamoyltransferase	4	21.6	1.27
PurC	PA1013*	phosphoribosylaminoimidazole-succinocarboxamide synthase	1	34.3	1.32
ThiI	PA5118	thiamine biosynthesis protein	2	12.8	1.35
DnaE	PA3640	DNA polymerase III subunit alpha	6	8.5	1.39
PA1295	PA1295	hypothetical protein	1	24.7	1.39
PA2271	PA2271	acetyltransferase	1	11.7	1.41

Supplementary Table 4. Inclusive list of metabolically relevant, *P. aeruginosa* proteins with significant increases (blue highlighted, ↑), significant decreases (red highlighted, ↓), and non-significant abundance changes (no highlights) during growth in LCSP medium for biofilm vs. planktonic cultures (see Figure 5). Boldface proteins are common to both LCSP (Figure 5) and CSP (Supplementary Figure 2) media, regardless of abundance. ‘*’ indicates homologous protein KEGG ID.

Protein	KEGG ID	Description of Proteins in Figure 5	Unique Peptides	Sequence Coverage %	$\log_2(x_{BF}/x_{Plankt})$
TonB	PA5531	transporter	2	12.0	1.46 ↑
ProS	PA0956*	prolyl-tRNA synthetase	2	44.5	1.53 ↑
PA3922	PA3922	hypothetical protein	16	55.4	1.68 ↑
HemA	PA4666	glutamyl-tRNA reductase	3	15.9	1.83 ↑
LeuA	PA3792*	2-isopropylmalate synthase	1	59.5	1.84 ↑
LeuB	PA3118	3-isopropylmalate dehydrogenase	13	50.8	2.08 ↑
IlvE	PA5013	branched-chain amino acid aminotransferase	9	45.9	2.10 ↑
OprB	PA3186	porin B	12	32.6	2.41 ↑
PiuC	PA4515	hydroxylase	3	16.8	2.42 ↑
PA5217	PA5217	iron ABC transporter substrate-binding protein	13	46.4	2.67 ↑
CysD	PA4443	sulfate adenylyltransferase subunit 2	6	25.9	2.99 ↑
PolA	PA5493	DNA polymerase I	9	14.9	3.07 ↑
OprD	PA0958	porin D	12	37.5	4.19 ↑
FumC1	PA4470	fumarate hydratase	18	58.5	5.32 ↑
FptA	PA4221	Fe(III)-pyochelin outer membrane receptor	40	74.9	5.33 ↑
CcpA	PA4587	cytochrome C551 peroxidase	11	43.4	-7.99 ↓
RpmE	PA5049	50S ribosomal protein L31	4	74.6	-4.28 ↓
KatA	PA4236	catalase	6	25.3	-3.97 ↓
PA1673	PA1673	bacteriohemerythrin	6	50.3	-3.30 ↓
AcnA	PA1562	aconitate hydratase	30	50.9	-3.13 ↓
TatA	PA5068	twin-arginine translocation protein	4	63.4	-3.03 ↓
NirS	PA0519	nitrite reductase	13	30.1	-2.99 ↓
PckA	PA5192*	phosphoenolpyruvate carboxykinase	1	59.3	-2.97 ↓
OprI	PA2853	outer membrane lipoprotein	6	45.8	-2.71 ↓
NuoI	PA2644	NADH-quinone oxidoreductase subunit I	3	17.0	-2.70 ↓
PA2953	PA2953	electron transfer flavoprotein-ubiquinone oxidoreductase	10	27.9	-2.65 ↓
PhhA	PA0872	phenylalanine 4-monooxygenase	3	13.7	-2.64 ↓
AccB	PA4847	acetyl-CoA carboxylase biotin carboxyl carrier protein subunit	5	60.9	-2.55 ↓
PurT	PA3751	phosphoribosylglycinamide formyltransferase	4	15.3	-2.48 ↓
DnaK	PA4761*	chaperone protein	4	19.9	-2.44 ↓
NuoG	PA2642	NADH-quinone oxidoreductase subunit G	21	32.5	-2.42 ↓
NuoA2	PA2637	NADH-quinone oxidoreductase subunit A	1	12.4	-2.40 ↓
OpgH	PA5077	glucosyltransferase	5	12.2	-2.40 ↓
SecY	PA4747	preprotein translocase subunit	2	37.2	-2.34 ↓
RpmD	PA4245	50S ribosomal protein L30	1	24.1	-2.31 ↓
OprF	PA1777	outer membrane porin F	16	49.7	-2.27 ↓

Protein	KEGG ID	Description of Proteins in Figure 5	Unique Peptides	Sequence Coverage %	$\log_2(x_{BF}/x_{Plankt})$
HemN	PA1546	oxygen-independent coproporphyrinogen-III oxidase	9	32.4	-2.20 ↓
NapA	PA1174	nitrate reductase catalytic subunit	5	9.4	-2.17 ↓
Azur	PA4922*	Azurin	1	71.9	-2.13 ↓
UvrA	PA4234	excinuclease ABC subunit A	7	13.2	-2.11 ↓
NuoE	PA2640	NADH-quinone oxidoreductase subunit E	3	25.9	-2.06 ↓
RplT	PA2741	50S ribosomal protein L20	4	28.8	-2.03 ↓
NuoF	PA2641	NADH dehydrogenase I subunit F	12	40.4	-1.93 ↓
DadA1	PA5304	D-amino acid dehydrogenase small subunit	3	8.8	-1.90 ↓
NuoC	PA2639	NADH:-quinone oxidoreductase subunit C/D	13	33.1	-1.90 ↓
IlvD	PA0353	dihydroxy-acid dehydratase	9	24.8	-1.88 ↓
RplL	PA4271	50S ribosomal protein L7/L12	8	86.1	-1.85 ↓
CycH	PA1483	cytochrome c-type biogenesis protein	4	19.9	-1.80 ↓
Dxs	PA4044	1-deoxy-D-xylulose-5-phosphate synthase	5	12.8	-1.80 ↓
PagL	PA4661	lipid A 3-O-deacylase	5	39.9	-1.78 ↓
PyrC	PA3527	dihydroorotase	3	18.4	-1.77 ↓
CoaD	PA0363	phosphopantetheine adenylyltransferase	6	49.7	-1.77 ↓
AotP	PA0892	arginine/ornithine ABC transporter ATP-binding protein	3	20.9	-1.76 ↓
Cc4	PA5490	cytochrome C4	4	33.8	-1.70 ↓
MtnB	PA1683	methylthioribulose-1-phosphate dehydratase	3	24.9	-1.68 ↓
Frr	PA3653*	ribosome recycling factor	1	10.8	-1.67 ↓
Era	PA0771	GTPase	3	15.4	-1.65 ↓
RplM	PA4433	50S ribosomal protein L13	6	54.9	-1.61 ↓
NuoB	PA2638	NADH-quinone oxidoreductase subunit B	4	21.8	-1.61 ↓
Edd	PA3194	phosphogluconate dehydratase	12	32.1	-1.58 ↓
RpsL	PA4268	30S ribosomal protein S12	3	25.2	-1.56 ↓
AcnB	PA1787	aconitate hydratase B	40	69.5	-1.55 ↓
Gmk	PA5336	guanylate kinase	3	18.7	-1.52 ↓
NfuA	PA1847	Fe/S biogenesis protein	5	36.1	-1.51 ↓
SpeA	PA4839	arginine decarboxylase	4	8.8	-1.48 ↓
PA5475	PA5475	hypothetical protein	8	55.9	-1.48 ↓
MutS	PA3620	DNA mismatch repair protein	5	8.7	-1.41
GlmU	PA5552	bifunctional glucosamine-1-phosphate acetyltransferase/N-acetylglucosamine-1-phosphate uridyltransferase	6	24.2	-1.40
PA2652	PA2652	methyl-accepting chemotaxis protein	5	16.0	-1.37
RpmB	PA5316	50S ribosomal protein L28	5	52.6	-1.34
PmpR	PA0964	transcriptional regulator	3	23.0	-1.26
RplB	PA4260	50S ribosomal protein L2	13	53.1	-1.24
Ssb	PA4232	single-stranded DNA-binding protein	5	30.9	-1.23
RpsS	PA4259	30S ribosomal protein S19	6	49.5	-1.19
HemB	PA5243*	delta-aminolevulinic acid dehydratase	8	40.4	-1.18
DapD	PA3666*	tetrahydrodipicolinate succinylase	1	39.2	-1.17
KdsD	PA4457	arabinose-5-phosphate isomerase	5	30.7	-1.15

Protein	KEGG ID	Description of Proteins in Figure 5	Unique Peptides	Sequence Coverage %	$\log_2(x_{BF}/x_{Plankt})$
SodB	PA4366	superoxide dismutase	4	37.8	-1.12
PheS	PA2740	phenylalanine--tRNA ligase subunit alpha	12	45.3	-1.11
RplV	PA4258	50S ribosomal protein L22	9	54.5	-1.09
RpmH	PA5570	50S ribosomal protein L34	2	20.5	-1.09
RplR	PA4247	50S ribosomal protein L18	9	58.6	-1.08
RpsK	PA4240	30S ribosomal protein S11	7	60.5	-1.08
RpsM	PA4241	30S ribosomal protein S13	8	70.3	-1.05
ArcC	PA5173	carbamate kinase	13	45.8	-1.05
AlgU	PA0762	RNA polymerase sigma factor	3	42.0	-1.05
SecY	PA4243	preprotein translocase subunit	6	14.7	-0.99
NagZ	PA3005	beta-hexosaminidase	2	11.7	-0.98
UvrB	PA3138	excinuclease ABC subunit B	3	4.9	-0.97
Mqo1	PA3452	malate:quinone oxidoreductase	7	18.2	-0.90
RplJ	PA4272	50S ribosomal protein L10	7	44.6	-0.89
RplU	PA4568	50S ribosomal protein L21	9	69.9	-0.89
ArgS	PA5051*	arginyl-tRNA synthetase	1	29.6	-0.86
RpsJ	PA4264	30S ribosomal protein S10	9	57.3	-0.86
GroS	PA4386	co-chaperonin	7	60.8	-0.85
MinE	PA3245	cell division topological specificity factor	3	32.1	-0.85
RplC	PA4263	50S ribosomal protein L3	10	55.9	-0.81
NqrF	PA2994	Na(+)-translocating NADH-quinone reductase subunit F	5	21.4	-0.74
PA4753	PA4753	hypothetical protein	3	27.9	-0.69
ClpV1	PA0090	secretion protein	8	12.4	-0.52
HisE	PA5067	phosphoribosyl-ATP pyrophosphatase	2	18.9	1.03
HslO	PA5193	heat shock protein 33	4	24.6	1.11
NqrA	PA2999	Na(+)-translocating NADH-quinone reductase subunit A	2	27.4	1.18
TolQ	PA0969	translocation protein	8	32.0	1.19
AhpF	PA0140	alkyl hydroperoxide reductase	3	8.6	1.20
PilG	PA0408	pilus biosynthesis/twitching motility protein	4	43.7	1.28
ThrB	PA5495	homoserine kinase	2	12.0	1.32
HutU	PA5100	urocanate hydratase	16	42.0	1.38

Supplementary Table 5. Inclusive list of metabolically relevant, *P. aeruginosa* proteins with significant increases (blue highlighted, ↑), significant decreases (red highlighted, ↓), and non-significant abundance changes (no highlights) during growth in CSP medium biofilm vs. planktonic cultures (see Supplementary Figure 2). Boldface proteins are common to both LCSP (Figure 5) and CSP (Supplementary Figure 2) media regardless of abundance. ‘*’ indicates homologous protein KEGG ID.

Protein	KEGG ID	Description of Proteins in Supplementary Figure 2	Unique Peptides	Sequence Coverage %	log ₂ (x _{BF} /x _{Plank})
BetB	PA5373	betaine aldehyde dehydrogenase	4	16.1	1.79 ↑
CysD	PA4443	ATP sulfurylase small subunit	4	15.7	2.22 ↑
HisE	PA5067	phosphoribosyl-ATP pyrophosphohydrolase	2	18.9	2.41 ↑
PolA	PA5493	DNA polymerase I	5	11.3	2.69 ↑
CysA	PA0280	sulfate.thiosulfate ABC transporter ATP-binding protein	4	23.7	2.69 ↑
OprD	PA0958	porin D	12	37.5	3.40 ↑
FptA	PA4221	Fe(III)-pyochelin outer membrane receptor	40	70.4	7.35 ↑
CcpA	PA4587	cytochrome C551 peroxidase	12	54.0	-8.42 ↓
HutH	PA5098	histidine ammonia-lyase	6	24.4	-5.24 ↓
RimO	PA0916	ribosomal protein S12 methylthiotransferase	3	15.7	-4.98 ↓
NfuA	PA1847	Fe/S biogenesis protein	4	38.7	-4.60 ↓
LpdV	PA2250	branched-chain alpha-keto acid dehydrogenase complex dihydrolipoyl dehydrogenase	5	23.7	-4.23 ↓
NosZ	PA3392	nitrous-oxide reductase	5	12.9	-4.12 ↓
RplS	PA3742*	LSU ribosomal protein L19P	1	30.2	-4.00 ↓
PA1673	PA1673	bacteriohemerythrin	5	45.1	-3.95 ↓
TatA	PA5068	twin-arginine translocation protein	4	63.4	-3.80 ↓
CoaD	PA0363	phosphopantetheine adenylyltransferase	3	23.9	-3.79 ↓
OprI	PA2853	outer membrane lipoprotein	7	68.7	-3.72 ↓
AcnA	PA1562	aconitate hydratase	30	51.2	-3.65 ↓
RplL	PA4271	50S ribosomal protein L7/L12	9	86.1	-3.43 ↓
SecY	PA4243	preprotein translocase subunit	4	10.4	-3.29 ↓
MurC	PA4411	UDP-N-acetylmuramate--L-alanine ligase	3	12.7	-3.08 ↓
ProC	PA0393	pyrroline-5-carboxylate reductase	3	16.1	-3.05 ↓
MtnD	PA1684	acireductone dioxygenase	2	23.2	-3.03 ↓
PhhA	PA0872	phenylalanine 4-monooxygenase	4	17.9	-3.02 ↓
OprF	PA1777	outer membrane porin F	14	40.0	-2.99 ↓
SecG	PA4747	preprotein translocase subunit	2	37.2	-2.94 ↓
Mqo1	PA3452	malate:quinone oxidoreductase	8	21.6	-2.91 ↓
RpmD	PA4245	50S ribosomal protein L30	1	24.1	-2.86 ↓
DnaK	PA4761*	chaperone protein	4	19.9	-2.85 ↓
NuoI	PA2644	NADH-quinone oxidoreductase subunit I	4	23.1	-2.77 ↓
PilB	PA4526	type 4 fimbrial biogenesis protein	3	10.6	-2.71 ↓
GcvH2	PA2446	glycine cleavage system protein H	2	15.7	-2.68 ↓
AcsA2	PA4733	acetyl-CoA synthetase	3	9.5	-2.58 ↓
PA2953	PA2953	electron transfer flavoprotein-ubiquinone oxidoreductase	12	32.3	-2.58 ↓

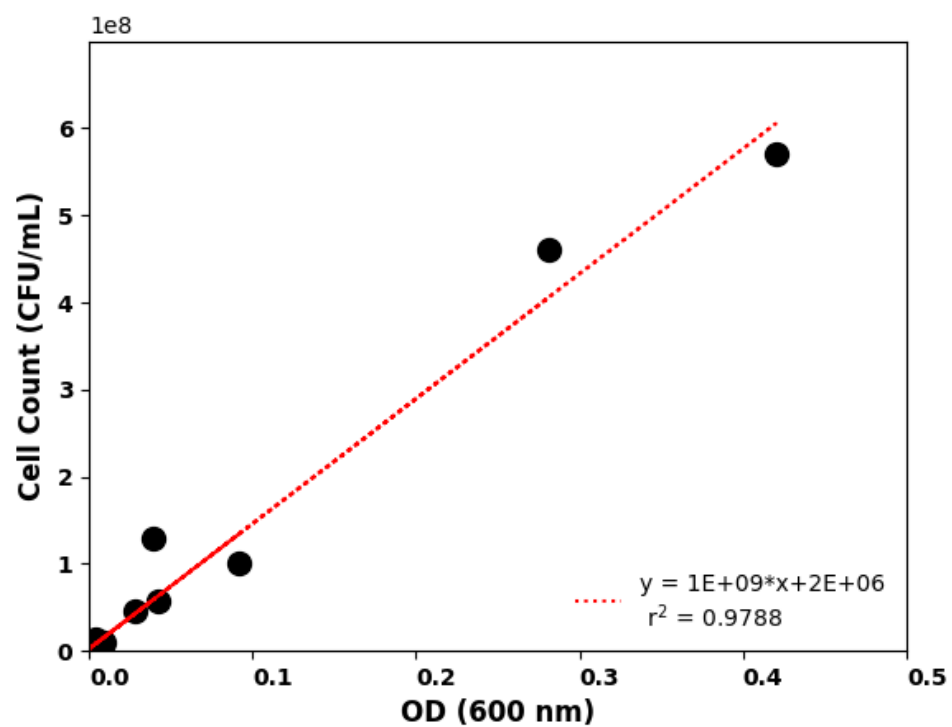
Protein	KEGG ID	Description of Proteins in Supplementary Figure 2	Unique Peptides	Sequence Coverage %	$\log_2(x_{BF}/x_{Plank})$
RpIT	PA2741	50S ribosomal protein L20	3	22.9	-2.52 ↓
PckA	PA5192	phosphoenolpyruvate carboxykinase	0	48.9	-2.51 ↓
NirS	PA0519	nitrite reductase	13	33.1	-2.46 ↓
KatA	PA4236	catalase	5	22.4	-2.46 ↓
HmgA	PA2009	homogentisate 1,2-dioxygenase	18	70.8	-2.43 ↓
GlmM	PA4749	phosphoglucosamine mutase	9	31.0	-2.38 ↓
Ung	PA0750	uracil-DNA glycosylase	6	39.4	-2.34 ↓
HemN	PA1546	oxygen-independent coproporphyrinogen-III oxidase	10	34.3	-2.29 ↓
ArcC	PA5173	carbamate kinase	15	50.6	-2.26 ↓
NuoC	PA2639	NADH:-quinone oxidoreductase subunit C/D	18	39.5	-2.22 ↓
NadA	PA1004	quinolinate synthetase	2	13.9	-2.20 ↓
MurE	PA4417	UDP-N-acetylmuramoylalanine-D-glutamate--2,6- diaminopimelate ligase	3	12.5	-2.19 ↓
GlmU	PA5552	bifunctional glucosamine-1-phosphate acetyltransferase/N-acetylglucosamine-1- phosphate uridyltransferase	5	18.7	-2.19 ↓
RplM	PA4433	50S ribosomal protein L13	6	54.9	-2.12 ↓
CycH	PA1483	cytochrome c-type biogenesis protein	4	19.9	-2.12 ↓
PagL	PA4661	lipid A 3-O-deacylase	3	22.0	-2.12 ↓
NusB	PA4052	transcription antitermination protein	3	39.6	-2.10 ↓
ArcB	PA5172	ornithine carbamoyltransferase	22	80.1	-2.06 ↓
ThrH	PA1757	phosphoserine phosphatase	3	26.3	-2.06 ↓
RpmB	PA5316	50S ribosomal protein L28	5	52.6	-2.05 ↓
Fbp	PA5110	fructose-1,6-bisphosphatase	8	27.4	-2.05 ↓
MtnB	PA1683	methylthioribulose-1-phosphate dehydratase	1	12.2	-2.05 ↓
PA5475	PA5475	hypothetical protein	6	50.0	-2.03 ↓
DnaE	PA3640	DNA polymerase III subunit alpha	5	9.5	-2.03 ↓
ArcA	PA5171	arginine deiminase	29	72.0	-2.02 ↓
MucB	PA0764	sigma factor AlgU regulator	3	16.5	-2.01 ↓
NuoG	PA2642	NADH-quinone oxidoreductase subunit G	24	40.8	-2.00 ↓
DadA1	PA5304	D-amino acid dehydrogenase small subunit	3	8.8	-1.98 ↓
AspA	PA5429	aspartate ammonia-lyase	6	28.5	-1.97 ↓
SpeE1	PA1687	polyamine aminopropyltransferase	4	26.2	-1.94 ↓
NuoE	PA2640	NADH-quinone oxidoreductase subunit E	5	44.6	-1.93 ↓
KdsD	PA4457	arabinose-5-phosphate isomerase	2	14.4	-1.91 ↓
SutA	PA5285*	conserved hypothetical protein	2	31.4	-1.88 ↓
AtoB	PA2001	acetyl-CoA acetyltransferase	12	50.6	-1.88 ↓
Ssb	PA4232	single-stranded DNA-binding protein	5	37.0	-1.88 ↓
IlvD	PA0353	dihydroxy-acid dehydratase	13	35.3	-1.88 ↓
AcnB	PA1787	aconitate hydratase B	41	73.4	-1.87 ↓
GlyQ	PA0009	glycine--tRNA ligase subunit alpha	8	38.7	-1.86 ↓
PurT	PA3751	phosphoribosylglycinamide formyltransferase	4	15.3	-1.81 ↓
Frr	PA3653*	ribosome recycling factor	1	10.3	-1.80 ↓

Protein	KEGG ID	Description of Proteins in Supplementary Figure 2	Unique Peptides	Sequence Coverage %	$\log_2(x_{BF}/x_{Plank})$
RplB	PA4260	50S ribosomal protein L2	12	50.9	-1.80 ↓
RpsS	PA4259	30S ribosomal protein S19	5	46.2	-1.79 ↓
AccB	PA4847	acetyl-CoA carboxylase biotin carboxyl carrier protein subunit	4	53.2	-1.78 ↓
AotP	PA0892	arginine/ornithine ABC transporter ATP-binding protein AotP	5	29.5	-1.75 ↓
PhhB	PA0871	pterin-4-alpha-carbinolamine dehydratase	7	78.8	-1.75 ↓
BauC	PA0130	3-oxopropanoate dehydrogenase	8	31.0	-1.74 ↓
UbiE	PA5063	ubiquinone/menaquinone biosynthesis methyltransferase	4	35.2	-1.72 ↓
Def	PA0019	peptide deformylase	3	21.4	-1.72 ↓
AguA	PA0292	agmatine deiminase	6	22.6	-1.71 ↓
MscL	PA4614	large-conductance mechanosensitive channel	7	51.1	-1.70 ↓
GlyA2	PA2444	serine hydroxymethyltransferase	4	20.3	-1.70 ↓
NuoF	PA2641	NADH dehydrogenase I subunit F	11	40.4	-1.70 ↓
RpmE	PA5049	50S ribosomal protein L31	3	71.8	-1.69 ↓
SodB	PA4366	superoxide dismutase	6	58.0	-1.66 ↓
NuoB	PA2638	NADH-quinone oxidoreductase subunit B	6	32.0	-1.66 ↓
HemB	PA5243	delta-aminolevulinic acid dehydratase	6	37.1	-1.66 ↓
MurD	PA4414	UDP-N-acetylmuramoyl-L-alanyl-D-glutamate synthetase	5	13.6	-1.65 ↓
FumC1	PA4470	fumarate hydratase	10	42.0	-1.65 ↓
AtpF	PA5558	ATP synthase subunit B	9	55.1	-1.63 ↓
GrpE	PA4762	heat shock protein	12	84.4	-1.62 ↓
NagZ	PA3005	beta-hexosaminidase	2	11.7	-1.61 ↓
RpsM	PA4241	30S ribosomal protein S13	9	70.3	-1.57 ↓
DavT	PA0266	5-aminovalerate aminotransferase	19	75.1	-1.54 ↓
AceF	PA5016	dihydrolipoamide acetyltransferase	22	60.0	-1.53 ↓
RplR	PA4247	50S ribosomal protein L18	9	58.6	-1.52 ↓
RplX	PA4252	50S ribosomal protein L24	7	61.5	-1.51 ↓
SucB	PA1586	2-oxoglutarate dehydrogenase complex dihydrolipoyllysine-residue succinyltransferase	21	63.1	-1.49 ↓
RpsL	PA4268	30S ribosomal protein S12	4	32.5	-1.48 ↓
DnaJ	PA4760	molecular chaperone	13	52.8	-1.48 ↓
PmpR	PA0964	transcriptional regulator	3	25.0	-1.47 ↓
PyrC	PA3527	dihydroorotase, homodimeric type	2	6.3	-3.09
Dxs	PA4044	1-deoxy-D-xylulose-5-phosphate synthase	2	8.1	-2.09
GuaA	PA3769	GMP synthase	24	64.6	-1.44
AstB	PA0899	N-succinylarginine dihydrolase	11	39.7	-1.43
DapD	PA3666*	tetrahydrodipicolinate succinylase	1	39.2	-1.43
PdxH	PA1049	pyridoxine/pyridoxamine 5'-phosphate oxidase	6	44.2	-1.43
NadE	PA4920	NAD synthetase	11	60.7	-1.42
MexA	PA0425	multidrug resistance protein	15	58.5	-1.42
MaiA	PA2007	maleylacetoacetate isomerase	5	39.2	-1.41

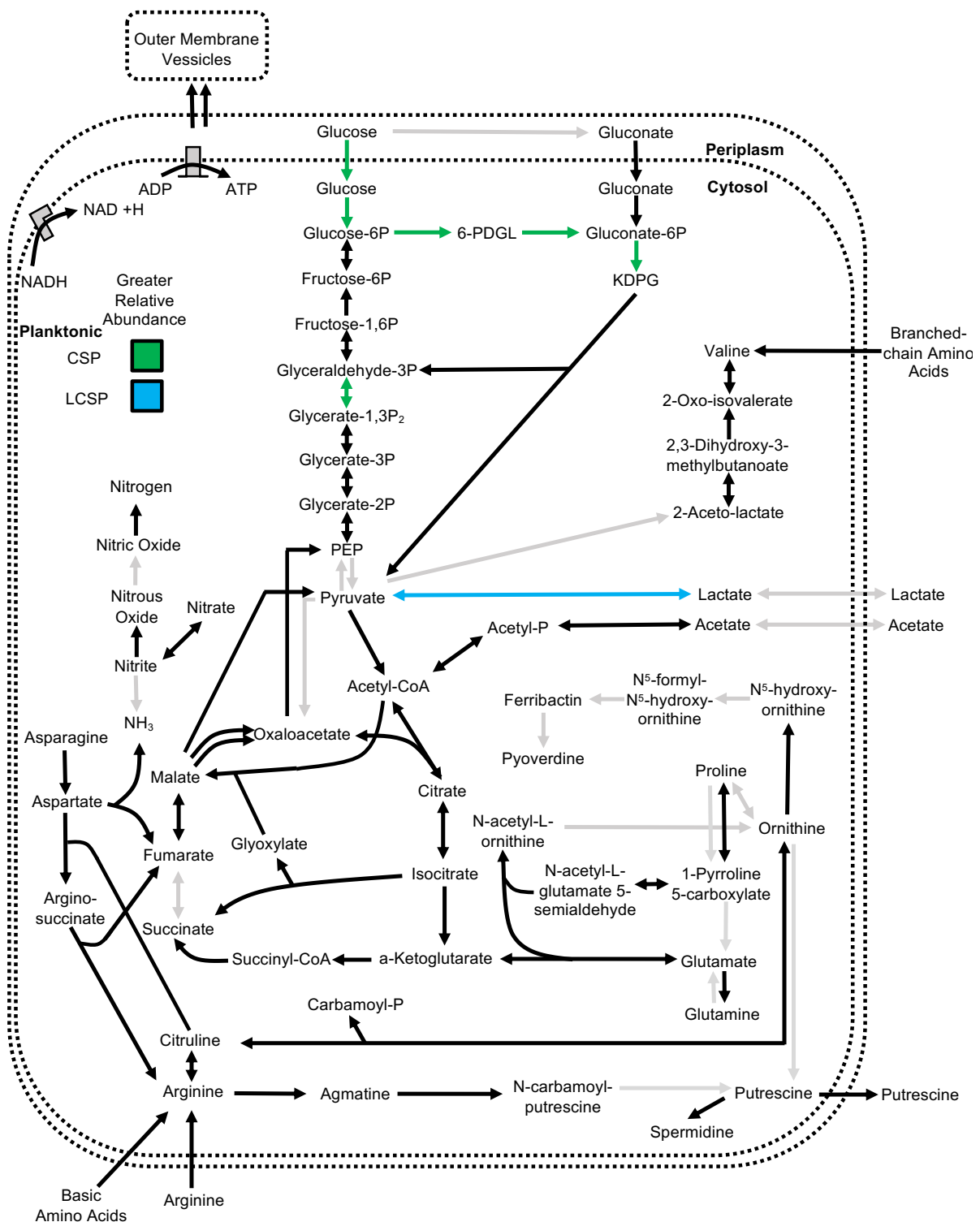
Protein	KEGG ID	Description of Proteins in Supplementary Figure 2	Unique Peptides	Sequence Coverage %	$\log_2(x_{BF}/x_{Plank})$
MurG	PA4412	undecaprenyldiphospho-muramoylpentapeptide beta-N-acetylglucosaminyltransferase	3	19.0	-1.40
EtfA	PA2951	electron transfer flavoprotein subunit alpha	13	80.3	-1.39
GatA	PA4483	Glu-tRNA(Gln) amidotransferase subunit A	18	55.6	-1.39
TyrS	PA4138	tyrosyl-tRNA synthetase	7	33.5	-1.39
PurM	PA0945	phosphoribosylformylglycinamide cyclo-ligase	11	42.2	-1.38
FtsA	PA4408	cell division protein	14	47.0	-1.37
RplP	PA4256	50S ribosomal protein L16	7	48.2	-1.37
PA2652	PA2652	methyl-accepting chemotaxis protein	7	20.1	-1.37
HutU	PA5100	urocanase	24	57.6	-1.37
RpsJ	PA4264	30S ribosomal protein S10	8	57.3	-1.36
RplK	PA4274	50S ribosomal protein L11	7	51.7	-1.35
PanB2	PA4729	3-methyl-2-oxobutanoate hydroxymethyltransferase	7	44.0	-1.35
GroS	PA4386	Hsp10 protein	2	60.8	-1.34
RplJ	PA4272	50S ribosomal protein L10	7	44.6	-1.34
DavD	PA0265	glutarate-semialdehyde dehydrogenase	19	54.5	-1.33
FliC	n/a	A-type flagellin	18	59.9	-1.33
ArgS	PA5051*	arginyl-tRNA synthetase	1	23.7	-1.31
PheS	PA2740	phenylalanyl-tRNA synthetase, alpha-subunit	9	33.4	-1.31
FtsZ	PA4407	cell division protein	13	40.1	-1.30
RplV	PA4258	50S ribosomal protein L22	8	54.5	-1.30
SerS	PA2612	serine--tRNA ligase	14	56.3	-1.29
CarB	PA4756	carbamoyl phosphate synthase large subunit	31	41.5	-1.27
RplC	PA4263	50S ribosomal protein L3	8	41.2	-1.26
GpsA	PA1614	glycerol-3-phosphate dehydrogenase, biosynthetic	5	19.1	-1.26
SthA	PA2991	soluble pyridine nucleotide transhydrogenase	13	45.7	-1.22
AccC	PA4848	acetyl-CoA carboxylase biotin carboxylase subunit	18	52.8	-1.21
RpsI	PA4432	30S ribosomal protein S9	5	36.9	-1.21
RpsQ	PA4254	30S ribosomal protein S17	6	60.2	-1.18
RibH	PA4053	6,7-dimethyl-8-ribityllumazine synthase	7	68.4	-1.17
PctB	PA4310	chemotactic transducer	5	41.3	-1.16
LeuS	PA3987	leucyl-tRNA synthetase	2	26.8	-1.16
ClpB	PA4542	chaperone protein	45	60.8	-1.16
AlaS	PA0903	alanine--tRNA ligase	14	31.7	-1.16
AckA	PA0836	acetate kinase	3	14.0	-1.15
GlnS	PA1794	glutamyl-tRNA synthetase	20	45.0	-1.14
RplU	PA4568	50S ribosomal protein L21	9	69.9	-1.14
CarA	PA4758	carbamoyl phosphate synthase small subunit	6	30.7	-1.13
Adk	PA3686	adenylate kinase	13	78.1	-1.10
RplA	PA4273	50S ribosomal protein L1	13	57.6	-1.08
GltX	PA3134	glutamate--tRNA ligase	1	29.1	-1.08
ArgG	PA3525	argininosuccinate synthase	16	49.6	-1.07

Protein	KEGG ID	Description of Proteins in Supplementary Figure 2	Unique Peptides	Sequence Coverage %	$\log_2(x_{BF}/x_{Plank})$
MutS	PA3620	DNA mismatch repair protein	6	9.8	-1.07
HslV	PA5053	ATP-dependent protease peptidase subunit	3	19.2	-1.07
Cc4	PA5490	cytochrome C4	4	33.8	-1.06
Gap2	PA3001*	putative glyceraldehyde 3-phosphate dehydrogenase	19	51.8	-1.05
DapB	PA4759	dihydrodipicolinate reductase	6	36.9	-1.04
RibB	PA4054	3,4-dihydroxy-2-butanone-4-phosphate synthase	9	38.9	-1.04
AlgR	PA5261	alginate biosynthesis regulatory protein	8	53.6	-1.04
Pgi	PA4732	glucose-6-phosphate isomerase	8	21.1	-1.03
RpsG	PA4267	30S ribosomal protein S7	12	60.3	-1.02
HslU	PA5054	ATP-dependent protease ATP-binding subunit	7	40.3	-1.01
RecA	PA3617	RecA protein	14	59.0	-1.01
Hcp1	PA0085	protein secretion apparatus assembly protein	6	38.9	-0.99
PA0170	PA0170	hypothetical protein	2	28.6	-0.99
HemC	PA5260	prophobilinogen deaminase	6	28.8	-0.99
ClpV1	PA0090	secretion protein	3	5.0	-0.98
Eco	PA2755	ecotin precursor	6	46.2	-0.95
Psd	PA4957	phosphatidylserine decarboxylase	3	20.4	-0.94
GlyS	PA0008	glycyl-tRNA synthetase beta chain	16	38.2	-0.93
PurL	PA3763	phosphoribosylformylglycinamide synthase	19	23.6	-0.91
TolR	PA0970	translocation protein	2	17.1	-0.91
PtsN	PA4464	nitrogen regulatory IIA protein	3	24.0	-0.91
Efp	PA2851	elongation factor P	7	37.8	-0.90
AruC	PA0895	acetylornithine aminotransferase	16	67.0	-0.89
PurH	PA4854	bifunctional phosphoribosylaminoimidazolecarboxamide formyltransferase/IMP cyclohydrolase	16	37.4	-0.89
LysS	PA3700	lysyl-tRNA synthetase	22	56.7	-0.87
IspG	PA3803	4-hydroxy-3-methylbut-2-en-1-yl diphosphate synthase (flavodoxin)	6	22.6	-0.87
ThrC	PA3735	threonine synthase	10	31.3	-0.86
RpsK	PA4240	30S ribosomal protein S11	7	60.5	-0.85
LtaE	PA5413	low specificity l-threonine aldolase	7	26.6	-0.85
PurD	PA4855	phosphoribosylamine--glycine ligase	8	27.3	-0.80
AtpC	PA5553	ATP synthase subunit epsilon	4	30.5	-0.80
GreA	PA4755	transcription elongation factor	9	67.1	-0.79
FabZ	PA3645	3-hydroxyacyl-[acyl-carrier-protein] dehydratase	4	43.8	-0.77
GalU	PA2023	UTP-glucose-1-phosphate uridylyltransferase	11	57.3	-0.76
InfB	PA4744	translation initiation factor IF-2	26	43.2	-0.76
PurC	PA1013	phosphoribosylaminoimidazole-succinocarboxamide synthase	6	51.7	-0.72
ThrS	PA2744	threonine--tRNA ligase	26	48.0	-0.71
CobO	PA1272	cob(II)yrinic acid a,c-diamide adenosyltransferase	2	9.9	-0.70
Dcd	PA3480	deoxycytidine triphosphate deaminase	3	18.6	-0.70
CoaX	PA4279	pantothenate kinase	2	11.3	-0.68
PA3286	PA3286	3-oxoacyl-ACP synthase	9	37.4	-0.66

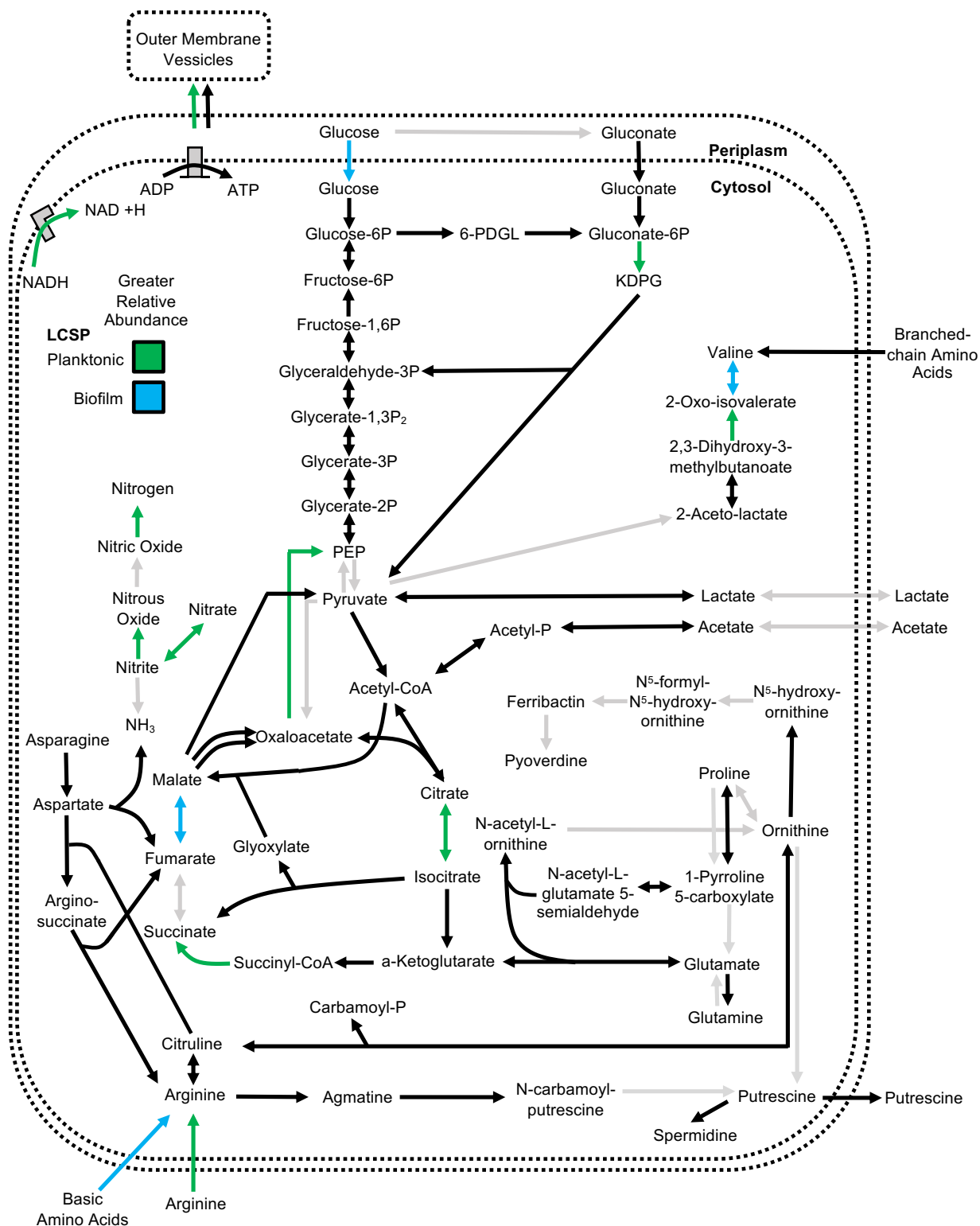
Protein	KEGG ID	Description of Proteins in Supplementary Figure 2	Unique Peptides	Sequence Coverage %	$\log_2(x_{BF}/x_{Plank})$
TolQ	PA0969	translocation protein	7	31.2	0.70
Ggt	PA1338	gamma-glutamyltranspeptidase	11	33.6	0.90
RpmE2	PA3601	50S ribosomal protein L31 type B	1	25.3	1.21



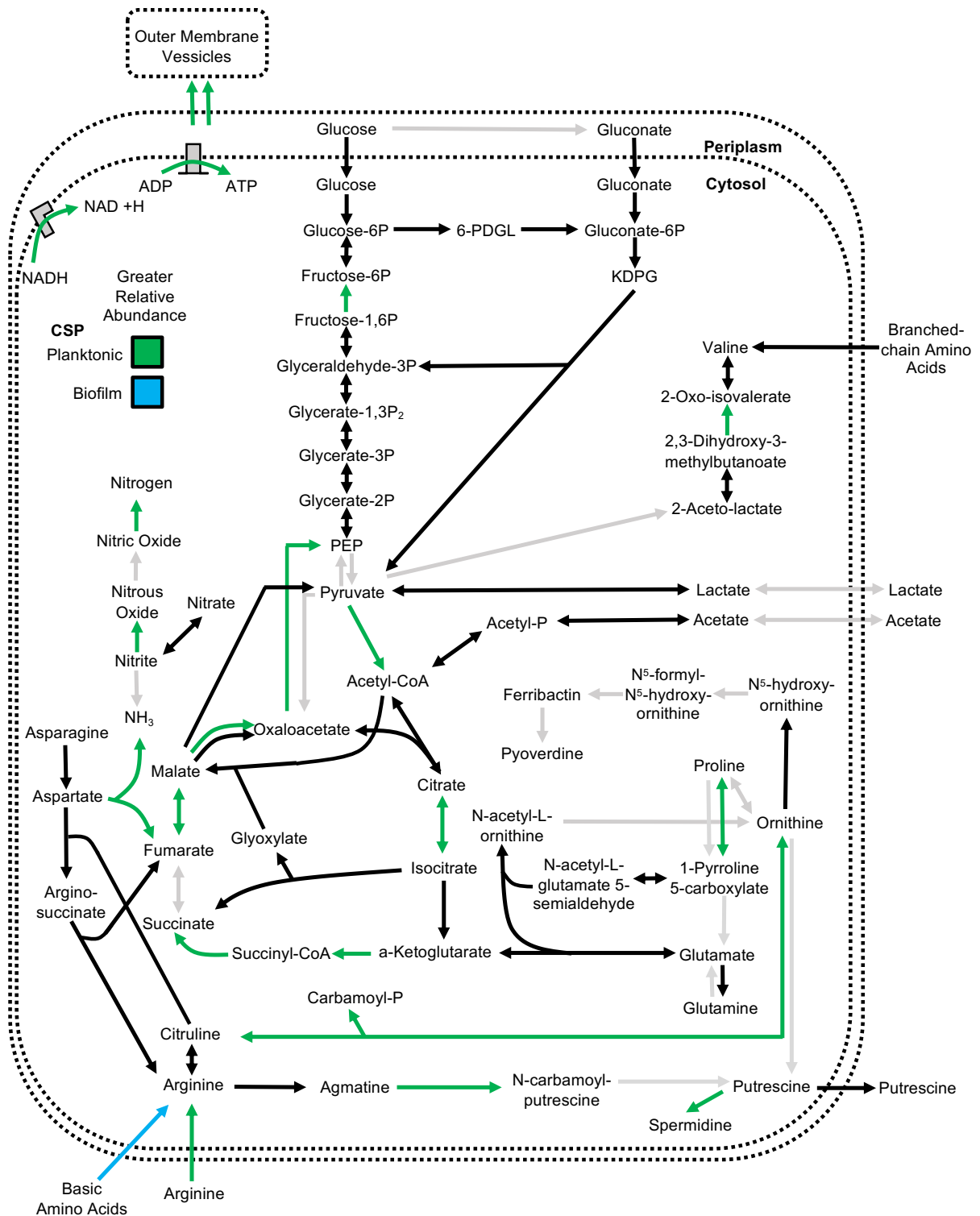
Supplementary Figure 1. Experimental correlation of *P. aeruginosa* isolate 215 optical density (OD 600 nm) and cell count (CFU/ml).



Supplementary Figure 3. Schematic of *P. aeruginosa* planktonic metabolism on CSP vs. LCSP. Dotted lines represent outer and inner cell membranes. Arrows represent proteins responsible for metabolic reactions. Black arrows represent proteins detected which do not have significant expression differentials. Grey arrows represent proteins which are known to exist in *P. aeruginosa* PAO1 but were not detected in this study. Green and blue arrows represent greater relative abundance of proteins from *P. aeruginosa* planktonic culture grown in CSP or LCSP, respectively.



Supplementary Figure 5. Schematic of *P. aeruginosa* biofilm vs. planktonic metabolism in LCSP media. Dotted lines represent outer and inner cell membranes. Arrows represent proteins responsible for metabolic reactions. Black arrows represent proteins detected which do not have significant expression differentials. Grey arrows represent proteins which are known to exist in *P. aeruginosa* PAO1 but were not detected in this study. Green and blue arrows represent greater relative abundance of proteins from *P. aeruginosa* cultures grown in LCSP medium as planktonic or biofilm cultures, respectively.



Supplementary Figure 6. Schematic of *P. aeruginosa* biofilm vs. planktonic metabolism in CSP media. Dotted lines represent outer and inner cell membranes. Arrows represent proteins responsible for metabolic reactions. Black arrows represent proteins detected which do not have significant expression differentials. Grey arrows represent proteins which are known to exist in *P. aeruginosa* PAO1 but were not detected in this study. Green and blue arrows represent greater relative abundance of proteins from *P. aeruginosa* cultures grown in CSP medium as planktonic or biofilm cultures, respectively.