1. Bacterial Cultural Media

1.1 Artificial sputum media recipes for *Rothia mucilaginosa* culture

Pig mucin 2%

KCl 0.22mg/mL

NaCl 5mg/mL

Egg Yolk Emulsion 0.25%

Salmon sperm DNA 1.4mg/mL

Ferritin 0.004mg/mL

Essential amino acid mix 0.375x

Non-essential amino acid mix 0.5x

$MgSO_4$ 1mM

BME vitamin stock 1x

Trace metals stock 1x

U-$^{13}C_6$ glucose 40mM

Essential amino acid mix components:

Ingredient	Stock Concentration g/l (50x)	ASM concentration g/l (0.375x)
L-Arginine HCl	6.32	0.0474
L-Cystine • 2HCl	1.564	0.01173
L-Histidine•HCl•H2O	2.1	0.01575
L-Isoleucine	2.625	0.0196875
L-Leucine	2.62	0.01965
L-Lysine•HCl	3.625	0.0271875
L-Methionine	0.755	0.0056625
L-Phenylalanine	1.65	0.012375
L-Threonine	2.38	0.01785
L-Tryptophan	0.51	0.003825
L-Tyrosine	1.8	0.0135
L-Valine	2.34	0.01755

Non-essential amino acid mix components:

Ingredient	Stock Concentration g/l (100x)	ASM concentration g/l (0.5 x)
L-Alanine (free base)	0.89	0.00445
L-Asparagine•H2O	1.5	0.0075
L-Aspartic Acid	1.33	0.00665
L-Glutamic Acid	1.47	0.00735
Glycine	0.75	0.00375
L-Proline	1.15	0.00575
L-Serine	1.05	0.00525

1.2 M9 minimal media recipes for *Pseudomonas aeruginosa* culture

Na_2HPO_4 $7H_2O$ 64g/L

KH_2PO_4 15g/L

NaCl 2.5g/L

NH_4Cl 5g/L

$MgSO_4$ 1mM

$CaCl_2$ 0.1mM

Succinate 40mM

2. Sample Preparation and Data Acquisition

2.1 Bacterial strains

Pseudomonas aeruginosa FLR19 and *Rothia mucilaginosa* RmFLR01 were isolated from the sputum of an adult CF patient. The genomes from both of these strains are publicly available on the PATRIC database to anyone with a PATRIC account:

https://www.patricbrc.org/workspace/tgallagh@patricbrc.org/Genomes/FLR01
https://www.patricbrc.org/workspace/tgallagh@patricbrc.org/Genomes/Rm

2.2 Glycolysis, TCA cycle metabolites and amino acids

50 uL of bacterial cells or supernatant were used for extraction. Polar metabolites were extracted with 1mL acetonitrile, isopropanol and water (3:3:2) and dried down in the speed vacuum concentration system, followed by methoximation and tert.butyldimethylsilylation. Agilent

5977A GC-quadrupole mass spectrometer was used for data acquisition in electron ionization mode.

2.3 Pentose phosphate pathway metabolites

50uL bacterial cells or media were used for extraction. Metabolites were extracted with 1mL acetonitrile, isopropanol and water (3:3:2) and dried down in the speed vacuum concentration system, followed by methoximation and trimethylsilylation. Agilent 7200 GC-accurate-mass QTOF was used for data acquisition in methane chemical ionization mode.

2.4 Short Chain Fatty Acids

50uL bacterial cells or media were used for short chain fatty acid analysis. Metabolites were extracted with 700uL of water, hydrochloric acid and methyl tert-butyl ether (5:1:1), followed by dehydration by anhydrous sodium sulfate and tert.butyldimethylsilylation. Agilent 5977A GC-quadrupole mass spectrometer was used for data acquisition in electron ionization mode.

2.5 Long Chain Fatty Acids

50uL bacterial cells or media were used for lipidomics analysis. 225uL methanol, 750uL methyl tert-butyl ether and 188uL water were used as extraction buffers. Samples were dried down in the speed vacuum concentration system and re-suspended with 110uL of methanol and toluene (9:1) with 50ng/mL CUDA (N-cyclohexyl-N'-dodecanoic acid urea). Data was acquired by Agilent 6550 Accurate-Mass QTOF LC/MS with CSH column in negative mode.