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NLDM: A Defined Medium for Cultivation and Exometabolite Profiling of Soil Bacteria

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ABSTRACT

This is the protocol for the preparation of NLDM medium as described in de Raad et al., 2022 - A Defined Medium for Cultivation and Exometabolite Profiling of Soil Bacteria.

The Northen Lab Defined Medium (NLDM) is a defined medium and thus suited for exometabolomic experiments. It is based on the metabolite composition of R2A and soil, metabolite usage by microbes, and biologically relevant elemental stoichiometry of these compounds. NLDM supports the growth of a wide range of phylogenetically diverse soil bacterial isolates and all of its metabolites are trackable through LC-MS/MS analysis. This demonstrates the viability and utility of the constructed NLDM medium for growing and characterizing diverse microbial isolates and communities. We anticipate that the NLDM medium will enable the examination of microbial substrate utilization for a broad range of isolates both directly and through dilution and amendments. We speculate that this media may have additional value in supporting microbial isolations and additional types of microbial characterization.

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KEYWORDS

Defined medium, defined media, exometabolomics, liquid chromatography mass spectrometry, soil bacteria, R2A

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MATERIALS TEXT

GENERAL NOTES

- Store NLDL solutions 1-5 at -80°C
- Store NLDL Salt solutions 1-4 at 4°C
- Both the Vitamin Solution and Trace Mineral Solution are from ATCC ([ATCC® MD-VS™](#) and [ATCC® MD-TMS™](#))

NLDL Solution 1-5

A	B	C	D	E	F	G	H	I	J	K
NLDL Solution 1 - 'Sugars' - 10X										
#	Compound	formula	class	Supplier	Supplier #	MW (avg)	Final_Conc_mmol/L	Conc_Factor	Conc mmol/L	mg for 50 mL stock solution
1	sucrose	C12H22O11	sugar	Sigma	S3929-1KG	342.3	0.875	10	8.75	149.8
2	D-glucose	C6H12O6	sugar	Sigma	G8270-1KG	180.16	0.875	10	8.75	78.8
3	trehalose dihydrate	C12H22O11 x 2H2O	sugar	Santa Cruz Biotechnologies	sc-202368	378.33	0.875	10	8.75	165.5
4	myo-inositol	C6H12O6	sugar	Sigma	I5125-50G	180.16	0.875	10	8.75	78.8
5	xylose	C5H10O5	sugar	Spectrum	XY110	150.13	0.875	10	8.75	65.7
6	D-mannitol	C6H14O6	sugar	Sigma	M4125-500G	182.17	0.875	10	8.75	79.7
7	n-acetyl glucosamine	C8H15NO6	sugar	Sigma	A4106-25G	221.21	0.875	10	8.75	96.8
NLDL Solution 2 - 'Organic_Acids' - 10X										
#	Compound	formula	class	Supplier	Supplier #	MW (avg)	Final_Conc_mmol/L	Conc_Factor	Conc mmol/L	mg for 50 mL stock solution
8	sodium-DL-lactate	C3H5NaO2	organic acid	Sigma	71720-5G	112.06	0.525	10	5.25	29.4
9	DL-Malic acid	C4H6O5	organic acid	Sigma	240176-50G	134.09	0.525	10	5.25	35.2
10	Citric acid	C6H8O7	organic acid	Sigma	C4540-100G	192.12	0.525	10	5.25	50.4
11	Succinic acid	C4H6O4	organic acid	Sigma	S3674-100G	118.09	0.525	10	5.25	31.0
12	sodium pyruvate	C3H3O3Na	organic acid	Sigma	P2256-25G	110.04	0.525	10	5.25	28.9
13	alpha ketoglutaric acid	C5H6O5	organic acid	Sigma	K1750-100G	146.1	0.525	10	5.25	38.4
14	L-Citrulline	C6H13N3O3	organic acid	Sigma	C7629-5G	175.19	0.525	10	5.25	46.0
NLDL Solution 3 - 'Amino_Acids' - 10X										

#	Compound	formula	class	Supplier	Supplier #	MW (avg)	Final_Conc_mmol/L	Conc_Factor	Conc mmol/L	mg for 50 mL stock solution
15	L-Alanine	C3H7NO2	amino acid	Sigma	A7627-100G	89.09	0.175	10	1.75	7.8
16	L-Arginine	C6H14N4O2	amino acid	Sigma	A8094-100G	174.2	0.175	10	1.75	15.2
17	L-Asparagine	C4H8N2O3	amino acid	Sigma	A0884-25G	132.12	0.175	10	1.75	11.6
18	L-Aspartic acid	C4H7NO4	amino acid	Sigma	A9256-100G	133.1	0.175	10	1.75	11.6
19	L-Cysteine	C3H7NO2S	amino acid	Sigma	30089-25G	121.16	0.175	10	1.75	10.6
20	L-Glutamic acid	C5H9NO4	amino acid	Sigma	49449-100G	147.13	0.175	10	1.75	12.9
21	L-Glutamine	C5H10N2O3	amino acid	Sigma	G3126-100G	146.14	0.175	10	1.75	12.8
22	Glycine	C2H5NO2	amino acid	Sigma	410225-50G	75.07	0.175	10	1.75	6.6
23	L-Histidine	C6H9N3O2	amino acid	Sigma	H8000-10G	155.15	0.175	10	1.75	13.6
24	L-Isoleucine	C6H13NO2	amino acid	Sigma	I2752-25G	131.17	0.175	10	1.75	11.5
25	L-Leucine	C6H13NO2	amino acid	Sigma	L8912-25G	131.17	0.175	10	1.75	11.5
26	L-Lysine	C6H14N2O2	amino acid	Sigma	L5501-25G	146.19	0.175	10	1.75	12.8
27	L- Methionine	C5H11NO2S	amino acid	Sigma	M5308-25G	149.21	0.175	10	1.75	13.1
28	L-Phenylalanine	C9H11NO2	amino acid	Sigma	P5482-25G	165.19	0.175	10	1.75	14.5
29	L-Proline	C5H9NO2	amino acid	Sigma	P5607-25G	115.13	0.175	10	1.75	10.1
30	L-Serine	C3H7NO3	amino acid	Sigma	S4311-25G	105.09	0.175	10	1.75	9.2
31	L-Threonine	C4H9NO3	amino acid	Sigma	T8441-25G	119.12	0.175	10	1.75	10.4
32	L-Tryptophan	C11H12N2O2	amino acid	Sigma	T8941-25G	204.23	0.175	10	1.75	17.9
33	L-Tyrosine	C9H11NO3	amino acid	Sigma	T8566-25G	181.19	0.175	10	1.75	15.9
34	L-Valine	C5H11NO2	amino acid	Sigma	94619-25G	117.15	0.175	10	1.75	10.3
NOTE: Dissolve all amino acids in 4 mM HCl. Sonicate for 30 minutes to dissolve all amino acids										
NLDM Solution 4 - 'Nucleobases/Nucleosides' - 50X										
#	Compound	formula	class	Supplier	Supplier #	MW (avg)	Final_Conc_mmol/L	Conc_Factor	Conc mmol/L	mg for 50 mL stock solution
35	5-methyluridine	C10H14N2O6	nucleoside	Sigma	535893-25G	258.23	0.0175	50	0.875	11.3
36	Adenine	C5H5N5	nucleobase	Sigma	A8626-5G	135.13	0.0175	50	0.875	5.9
37	Adenosine	C10H13N5O4	nucleoside	Sigma	A4036-5G	267.24	0.0175	50	0.875	11.7
38	Cytidine	C9H13N3O5	nucleoside	Sigma	C4654-5G	243.22	0.0175	50	0.875	10.6
39	Cytosine	C4H5N3O	nucleobase	Sigma	C3506-1G	111.1	0.0175	50	0.875	4.9
40	Guanine	C5H5N5O	nucleobase	Sigma	G11950-100G	151.13	0.0175	50	0.875	6.6

41	Guanosine	C10H13N5O5	nucleoside	Sigma	G6264-5G	283.24	0.0175	50	0.875	12.4
42	Hypoxanthine	C5H4N4O	nucleobase	Sigma	H9636-5G	136.11	0.0175	50	0.875	6.0
43	Inosine	C10H12N4O5	nucleoside	Sigma	I4125-100G	268.23	0.0175	50	0.875	11.7
44	Thymine	C5H6N2O2	nucleobase	Sigma	T0895-5G	126.11	0.0175	50	0.875	5.5
45	Uracil	C4H4N2O2	nucleobase	Sigma	U1128-25G	112.09	0.0175	50	0.875	4.9
46	Uridine	C9H12N2O6	nucleoside	Sigma	U3003-5G	244.2	0.0175	50	0.875	10.7
47	Xanthine	C5H4N4O2	nucleobase	Sigma	X0626-5G	152.11	0.0175	50	0.875	6.7
48	Xanthosine dihydrate	C10H12N4O6	nucleobase	Sigma	X0750-5G	320.26	0.0175	50	0.875	14.0
NOTE: Dissolve all nucleobases and nucleosides in 0.08M NaOH										
NLDM Solution 5 - 'Other_Cpds' - 50X										
#	Compound	formula	class	Supplier	Supplier #	MW (avg)	Final_Conc_mmol/L	Conc_Factor	Conc mmol/L	mg for 50 mL stock solution
49	gamma-Aminobutyric acid	C4H9NO2	other	Sigma	A2129-25G	103.12	0.0175	50	0.875	4.5
50	Ectoine	C6H10N2O2	other	Sigma	81619-1G-F	142.16	0.0175	50	0.875	6.2
51	Betaine	C5H11NO2	amino acid deriv	Sigma	61962-50G	117.15	0.0175	50	0.875	5.1
52	N-acetyl-L-Glutamic acid	C7H11NO5	amino acid deriv	Sigma	855642-25G	189.17	0.0175	50	0.875	8.3
53	Nicotinamide	C6H6N2O	vitamin	Sigma	72340-100G	122.12	0.0175	50	0.875	5.3
54	Ornithine HCl	C5H12N2O2	amino acid deriv	Sigma	O2375-5G	168.62	0.0175	50	0.875	7.4
55	Shikimic acid	C7H10O5	other	Sigma	S5375-1G	174.15	0.0175	50	0.875	7.6
56	spermidine	C7H19N3	other	Sigma	S0266-1G	145.25	0.0175	50	0.875	6.4
57	taurine	C2H7NO3S	other	Sigma	T0625-10G	125.15	0.0175	50	0.875	5.5
58	Trigonelline HCl	C7H7NO2 x HCl	other	Sigma	T5509-5G	173.6	0.0175	50	0.875	7.6
59	L-Carnitine HCl	C7H16NO3 x HCl	other	Sigma	C0283-1G	197.66	0.0175	50	0.875	8.6
60	Choline Cl	C5H14NO	other	Sigma	C7017-5G	139.62	0.0175	50	0.875	6.1
61	N-acetyl-L-Glutamine	C7H12N2O4	amino acid deriv	Sigma	A9125-25G	188.18	0.0175	50	0.875	8.2
62	n-acetyl-L-lysine	C8H16N2O3	amino acid deriv	Sigma	A4021-1G	188.22	0.0175	50	0.875	8.2
63	n-acetylmuramic acid	C11H19NO8	other	Sigma	A3007-500MG	293.27	0.0175	50	0.875	12.8
64	sn-glycero-3-phosphocholine	C8H20NO6P	other	Cayman	20736	257.22	0.0175	50	0.875	11.3

NLDM Salt Solution 1-4

A	B	C	D	E	F	G	H	I	J	K
NLDM Salt solution 1 - 'MgSO4' - 50X										
#	Compound	formula	class	Supplier	Supplier #	MW (avg)	Final_Conc_mmol/L	Conc_Factor	Conc mmol/L	mg/50ml
S1	Magnesium sulfate heptahydrate	MgSO4 * 7H2O	Salt	Sigma	M2773-500G	246.47	0.812	50	40.6	500.3
NLDM Salt solution 2 - 'NH4Cl' - 50X										
#	Compound	formula	class	Supplier	Supplier #	MW (avg)	Final_Conc_mmol/L	Conc_Factor	Conc mmol/L	mg/50ml
S2	Ammonium chloride	NH4Cl	Salt	Sigma	A9434-500G	53.49	1	50	50	133.7
NLDM Salt solution 3 - 'CaCl2' - 50X										
#	Compound	formula	class	Supplier	Supplier #	MW (avg)	Final_Conc_mmol/L	Conc_Factor	Conc mmol/L	mg/50ml
S3	Calcium chloride dihydrate	CaCl2 * 2 H2O	Salt	Sigma	C7902-500G	147.01	0.68	50	34	249.9
NLDM Salt solution 4 - 'PO4' - 50X										
#	Compound	formula	class	Supplier	Supplier #	MW (avg)	Final_Conc_mmol/L	Conc_Factor	Conc mmol/L	mg/50ml
S4	Sodium phosphate monobasic	NaH2PO4	Salt	Sigma	S0751-500G	119.98	1.665	50	83.25	499.4
S5	Potassium phosphate dibasic	K2HPO4	Salt	Sigma	P3786-500G	174.18	3.33	50	166.5	1450.0

Vitamin Solution - 100X

- Vitamin Supplement (ATCC® MD-VS™) based on Wolfe's Vitamin Solution
- Order at [ATCC](#)

Trace Mineral Solution - 100X

- Trace Mineral Supplement (ATCC® MD-TMS™) based on Wolfe's mineral solution
- Order at [ATCC](#)

NLDM Components

1 NLDM stock solutions

- See Materials section on how to prepare stock solutions

A
Medium components
NLDM Solution 1 - 'Sugars' - 10X
NLDM Solution 2 - 'Organic_Acids' - 10X
NLDM Solution 3 - 'Amino_Acids' - 10X
NLDM Solution 4 - 'Nucleobases/Nucleosides' - 50X
NLDM Solution 5 - 'Other_Cpds' - 50X
NLDM Salt solution 1 - 'MgSO4' - 50X
NLDM Salt solution 2 - 'NH4Cl' - 50X
NLDM Salt solution 3 - 'CaCl2' - 50X
NLDM Salt solution 4 - 'PO4' - 50X
Vitamin solution - 100X
Trace Mineral solution - 100X

NLDM PREPARATION

- For the preparation of 500 mL NLDM medium mix the following stock solutions:

A	B
	Volume (mL)
NLDM Solution 1 - 'Sugars' - 10X	50
NLDM Solution 2 - 'Organic_Acids' - 10X	50
NLDM Solution 3 - 'Amino_Acids' - 10X	50
NLDM Solution 4 - 'Nucleobases/Nucleosides' - 50X	10
NLDM Solution 5 - 'Other_Cpds' - 50X	10
NLDM Salt solution 1 - 'MgSO4' - 50X	10
NLDM Salt solution 2 - 'NH4Cl' - 50X	10
NLDM Salt solution 3 - 'CaCl2' - 50X	10
NLDM Salt solution 4 - 'PO4' - 50X	10
Vitamin solution - 100X	5
Trace Mineral solution - 100X	5
Milli-Q Water	280
TOTAL	500

NLDM pH

- Adjust the pH of the NLDM medium 7.0 - 7.2 if needed

STERILIZATION

- Filter sterilize the NLDM medium using a 0.22 um filter

STORAGE

- Store the NLDM medium at 4°C in the dark