Electronic Supplementary Material (ESI) for Chemical Science.

In silico MS/MS prediction for peptidoglycan profiling uncovers novel anti-inflammatory peptidoglycan fragments of the gut microbiota

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Materials and Methods

Bacterial culturing conditions

For culturing model bacteria, a single colony of the respective bacteria was used to inoculate an overnight culture of 20 mL with appropriate media (LB: *E. coli*; TSB: *E. faecium*, *E. faecalis*, *S. aureus*; MRS: *L. plantarum*). For culturing gut bacteria, starter cultures were grown from a single colony with 5 mL of appropriate culture media (DSMZ Bifidobacterium medium: *B. adolescentis*, *B. bifidum*, *B. infantis*; modified PYG: *F. nucleatum*; BHI supplemented with 0.1% mucin: *A. muciniphila*) under anaerobic conditions. The starter cultures were used to inoculate overnight cultures (50 mL) once the cultures were sufficiently dense. All organisms were grown at 37 °C. Only model bacteria were shaken at 200 rpm. Cells were harvested with centrifugation at 4,000 rpm, for 5 min.

Isolation of soluble PGNs from bacterial sacculi

Peptidoglycan was extracted from overnight bacterial culture following Kühner *et al*'s methods with slight modifications.[1] Briefly, the bacterial cell pellets were resuspended in 1 mL PBS and transferred to an Eppendorf tube. The supernatant was discarded after centrifugation at 10,000 rpm, for 5 min (subsequent centrifugation steps have the same settings). The cell pellet was resuspended in solution A (0.5% SDS, 0.1M Tris/HCl pH 6.8) and boiled for >20 min. The suspension was then centrifuged, and the supernatant was discarded. Excess SDS was removed by washing with 1 mL ddH_2O for 3-5 times. The washed sacculi pellet was resuspended in ddH_2O and sonicated for 30 minutes prior to trypsin digestion (50 μg/mL trypsin solution, 500 μL) for 60 minutes at 37 °C, 200 rpm. Trypsin was deactivated by boiling the suspension for 3 min on a heating block. The suspension was pelleted, the supernatant discarded and washed with 1 mL ddH_2O twice. For Gram-positive species, the pellet was further treated with 1 M HCl (500 μL) for 4 hrs at 37 °C, 200 rpm. Remaining acid was removed by washing with ddH_2O until pH ~6, as indicated by pH paper. Finally, the pellet was resuspended in 500 μL digestion buffer (12.5 mM NaH_2PO_4, pH 5.5) and digested with mutanolysin (5 U/mL, 50 μL) overnight at 37 °C, 200 rpm. Mutanolysin was deactivated by boiling the suspension for 3 min on a heating block, before pelleting. The supernatant was collected and lyophilized. Dried muropeptides were resuspended in 100 μL ddH_2O and kept at -20 °C until further analysis.

LC-MS/MS methods for bacterial PGNs analysis

LC-MS/MS analysis was performed using Vanquish 3000 HPLC coupled to Orbitrap Exploris 120 MS (Thermo Scientific) in the positive mode. Separation was performed with a Poroshell 120 EC-C18 (4.6 x 100 mm, 2.7μm, Agilent) column with solvent A (HPLC/LC grade water with 0.05% formic acid) and solvent B (HPLC/LC acetonitrile with 0.05% formic acid) with a flow rate of 0.5 mL/min and the following conditions: initial 1% B; 1-3% B for 2 min, 3-10% B for 4 min, 10-30% B for 16 min, 30-40% for 2 min, 40-90% B for 0.5 min, 90% B for 2.5 min, 90-1% B for 1 min and finally, 1% B for 2 min. MS/MS spectra were collected with Data Dependent Acquisition. For each scan (*m/z*: 120 – 1600), the top 3 most abundant ions were subjected to MS/MS at 30% HCD. Dynamic exclusion was used to exclude previously scanned ions for 12 seconds.

Generation of *in silico* PGN_MS2 Library

For each bacterial species, two MS2 libraries were created using PGN_MS2. The initial library is used to determine which modifications are present in the PGN composition, and thus, only PGN monomers are created. In the initial library, both *N*-acetylated and *O*-deacetylated modified glycans were included as possible glycans. For the stem peptide, the default sequence Ala_1-γ-Glu/isoGln$_2$-AA_3-Ala_4-Ala_5 was used as a template, where AA_3 is Lys, mDAP, or Lan depending on the species. Other than Ala, fifteen other amino acids were also included at the fourth and fifth positions to screen for non-canonical amino acid (NCDAA) substitution at these positions. For species with bridge peptides, the canonical bridge peptide was included. Shortened bridge peptides were included whenever relevant (e.g., Ala bridges were included with the canonical Ala-Ala bridges in *E. faecalis*). After analyzing the data with the initial library in MS-DIAL, a second, more focused library is made with PGN monomers, dimers, and trimers. Absent glycans and bridge peptides are removed from library generation and absent amino acids are removed during the generation of the PGN polymers. This library is used for the final analysis.

PGN deconvolution with PGN_MS2 reference library in MS-DIAL software

MS-DIAL was used to process LC-MS data, which was able to identify compounds via *m/z*, MS1 isotopic pattern, and MS/MS spectra similarity.[2] PGN_MS2 library was imported into MS-DIAL to provide the

MS/MS reference spectra for matching with experimental data. We consider PGNs identified if their MS-DIAL score for MS/MS spectral matching exceeds 60%. For PGNs with lower identification scores (~60-70%) or unusual structures, we need to verify them manually by checking for signature fragments.

MS-DIAL was also used to calculate the relative abundance of PGNs. For quantification, the EICs of the identified PGNs were automatically integrated. For PGNs that resolve as distinct anomeric peaks or manifest multiple proton adducts, the peak areas of these features were subsequently combined (outside MS-DIAL) and corrected. In addition, peak areas were also corrected for natural isotopic distribution,[3] by dividing the peak area by the theoretical relative intensity of its monoisotopic peak (calculated with RDKit[4]). In addition, peak areas were multiplied by the number of PGN monomeric units present in the PGN polymers (such as dimers and trimers etc.) to yield relative intensities that are proportionate to the number of PGN monomeric units.[5]
The crosslinking index and chain length of peptidoglycan for each bacteria were calculated using Glauner *et al*'s methods.[6] All other statistics (% total composition) were calculated by summing each compound's peak area contribution and dividing this sum by the total peak area. A compound's peak area contribution is calculated as such (using isoGln as an example): (Number of isoGln Present X Compound Peak Area) / No. of PGN Units.

Cloning, overexpression, and purification of putative *Bifidobacterium* LTs

The gene sequences encoding for the respective proteins lacking the transmembrane helix were amplified from genomic DNA with suitable primers (**Table S15**), and inserted into the pET28a(+) vector with XhoI and NheI-HF restriction enzymes (New England Biolabs). The resulting plasmids were transformed into *E. coli* DH5*α cells. The correct insertion was verified by DNA sequencing.*
The recombinant MltG and RpfB proteins were overexpressed and purified according to previously published protocols with modifications.[7,8] Briefly, *E. coli* BL21 (DE3)-RIPL containing the plasmid of interest was grown in 1 L LB supplemented with appropriate antibiotics at 37 °C, 200 rpm. Protein overexpression was induced with the addition of IPTG (see **Table S18** for detailed conditions). Cell pellets were harvested by centrifugation (4,000 *x g*, 30 min), resuspended in 30 mL cold lysis buffer B (**Table S18**) with added 1 x protease inhibitor, and lysed by sonication (30% amplitude, 10s on, 10s off, for 20 min). The cell suspension was clarified by centrifugation (12,000 *x g*, 20 min, 4 °C), and cell lysate was incubated with 1 mL pre-equilibrated TALON® metal affinity resin (Takara Bio) for 2 h, 4 °C. Sample was loaded onto gravity-flow column and resin was washed with 30 mL buffer B (**Table S18**) containing 20 mM imidazole and 30 mL buffer B containing 40 mM imidazole. Bound proteins were eluted with 15 mL of buffer B containing 200 mM imidazole. The eluted fractions containing the desired proteins were combined and concentrated in a 3 kDa MWCO Amicon Ultra Centrifuge Filter Device (Merck, Millipore).

Site-directed mutagenesis of BaMltG_E200A

Site-directed mutagenesis primers (**Table S15**) were designed to substitute the catalytic Glu residue in BaMltG into Ala. The pET28a(+)_BaMltG_E200A plasmid was generated via the Q5 Site-Directed Mutagenesis Kit (New England Biolabs). The mutant BaMltG protein was overexpressed and purified according to the abovementioned protocols.

Biochemical characterization of *Bifidobacterium* MltG with nascent peptidoglycan

Methods from Taguchi *et al.* were used with slight modifications.[7] Briefly, dried *E. faecalis* Lipid II was resuspended in 20 µL reaction buffer (20 mM MES buffer, pH 6.5, 10 mM $CaCl_2$, and 10% DMSO) with 5 µM SgtB and 5 µM MltG at 30 °C overnight. The reaction was quenched by boiling the mixtures for 3 min, followed by the addition of mutanolysin for muropeptide digest. The supernatant was subjected to LC-MS analysis using the abovementioned conditions.

Biochemical characterization of *B. adolescentis* RpfB with sacculi

Methods from Lee *et al.* were adapted with slight modification.[9] Briefly, 50 µL of *E. coli* sacculi suspension (10 mg/mL) was incubated with 5 µM for recombinant RpfB-FL or RpfB-Truncated in a total volume of 200 µL digestion buffer (12.5 mM NaH_2PO_4, pH 5.5) for incubation overnight at 37 °C, 200 rpm. The reaction was quenched by boiling the sample for 3 min. The supernatant was subjected to LC-MS analysis using the abovementioned conditions.

In vitro anti-inflammatory assay with RAW264.7 cells

RAW264.7 cells (American Type Culture Collection; ATCC, Manassas, VA, USA) were cultured in Iscove's modified Dulbecco's medium (IMDM) (Gibco/BRL; Burlington, ON, Canada) supplemented with 2% heat-inactivated fetal bovine serum (FBS) (Gibco/BRL; Burlington, ON, Canada) and 1% Pen Strep antibiotics (life technologies corporation, Grand Island, NY, USA) in 37 °C at 100% humidity in 5% CO_2. For treatment, RAW264.7 cells (1.0 × 10000 cells/well) were plated into 24-well plates and pretreated with ah-PGN of interest (20 μM) for 24h, followed with LPS (20 ng/ml) stimulation for another 4 h. RAW264.7 cells were then collected and total RNA was isolated using the total RNA Miniprep Kit (Monarch, NEB #T2010) according to the manufacturer's instructions to test for cytokine transcriptional levels via quantitative PCR (qPCR). The quantity of total RNA was determined at 260 and 280 nm.
Total RNA was reverse transcribed into complementary DNA (cDNA) using a first-strand cDNA synthesis kit according to the vendor's instructions (Vazyme, Nanjing, China). Target genes were then amplified by the CFX96 Touch Real-Time PCR System (Bio-Rad) with specific oligo dT primers purchased from Integrated DNA Technologies. Reactions were set up in a 10 μL reaction containing 1 μL cDNA template, 5 μL of 2 x SYBR Green qPCR MasterMix (Vazyme), 0.2 μL each of the forward and reverse primers (10 μM), and 3.6 μL ddH2O according to the instructions. The quantitative PCR analysis was performed using the following profile: one cycle at 95 °C for 30 s, followed by 40 cycles at 95 ◦C for 10 s, the primer-specific annealing temperature for 5 s, elongation at 60 °C for 30 s, and a final single cycle at 95 °C for 15 s. At the end of each run, melting curve profiles were achieved by cooling the sample to 60 °C for 60 s and then steadily increasing to 95 °C with continuous measurement of fluorescence to confirm the amplification of specific transcripts. The specificity of designed primers was verified by subjecting the cDNA synthesized to electrophoresis on a 2% agarose gel and visualization by SYBR staining (Thermo Fisher Scientific). Relative mRNA expression was calculated using the $2^{-\Delta\Delta Ct}$ method with reference to the *β*-actin gene expression. The data is presented as the mean ± standard error (SE) of the relative mRNA expression level from three consecutive studies. The student t-test was used to determine the significance of gene expression levels. The primers used are listed in **Table S15**.

In vitro NOD2 activation assay with HEK-Blue™ hNOD2 reporter cells

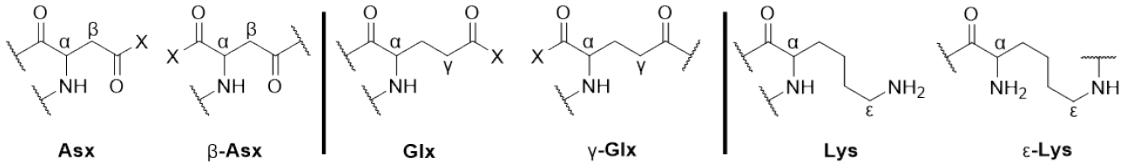
HEK cells expressing the NOD2 receptor and carrying the NF-κB SEAP reporter gene (InvivoGen) were used according to the manufacturer's instructions to assess the NOD2 stimulatory activities of the crude PGNs from various bacteria or the synthetic ah-PGN. Briefly, after attachment of HEK-Blue NOD2 or Null2 cells seeded in 96-well plates, 20 μL of the reconstituted PGNs were added and incubated for 16-18 h at 37 °C (total volume: 200 μL per well). For SEAP detection, 20 μL of supernatant from PGN-treated wells was added to 180 μL of QUANTI-blue substrate (Invivogen) in another 96-well microtiter plate. Supernatants from untreated cells were used as negative controls. The mixture was incubated at 37 °C and SEAP activity was assessed by OD650 nm measurement. All PGNs were reconstituted in endotoxin-free water. Results are shown as means ± standard error (SE). Statistical significance was determined by ANOVA followed by the Duncan method for multiple comparisons.

Supplementary Figures

A

Abbrev.	Meaning	Abbrev.	Meaning
	Non-canonical amino acids		Glycans*
m	Meso-diaminopimelic acid (mDAP)	**(G)**	Glucosamine ($GlcNH_2$)
m(NH2)	Amidated meso-diaminopimelic acid (mDAP(NH2))	**(NAG)**	N-acetylated glucosamine (GlcNAc)
O	Ornithine (Orn)	**(DAG)**	N,O-diacetylated glucosamine (GlcNAcOAc)
q	γ-isoGlutamine (γ-isoGln)	**(MUR)**	Muramic acid ($MurNH_2$)
e	γ-Glutamate (γ-Glu)	**(NAM)**	N-acetylated muramic acid (MurNAc)
d	β-Aspartate (β-Asp)	**(DAM)**	N,O-diacetylated muramic acid (MurNAcOAc)
n	β-isoAsparagine (β-isoAsn)	**an-**	Prefix indicating 1,6 anhydro- modification, e.g. (anNAM)
k	ε-Lysine (ε-Lys)	**[r]**	Postfix indicating reduction, e.g. (NAG)(NAM)[r]

B



C

Notation	Branch peptide	Notation	Polymerisation
	[N-COOH or NH2-Peptide seq.]		Monomer;(Polymer Type)-Monomer Glycosidic polymerisations are indicated with (G-G)** All other crosslink types are formatted as such: (Ns or br-M)
N	Position of amino acid connected to branch peptide	**N**	Position of amino acid in donor stem peptide
COOH or NH2	Indicates connection to branch peptide: NH2 = connected to amino group COOH = connected to carboxyl group	**s/br**	Indicates connection to acyl donor: s = connected to amino group directly br = connected to amino group of branch peptide
Peptide seq.	Peptide sequence indicated from closest to furthest amino acid (from the stem peptide)	**M**	Position of amino acid in acceptor stem peptide

D

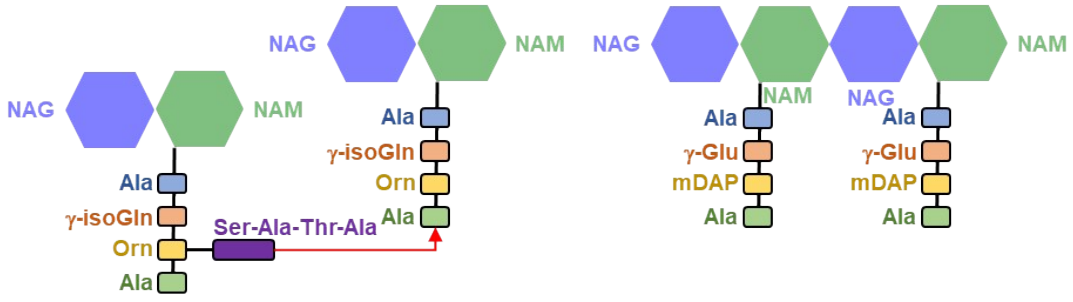
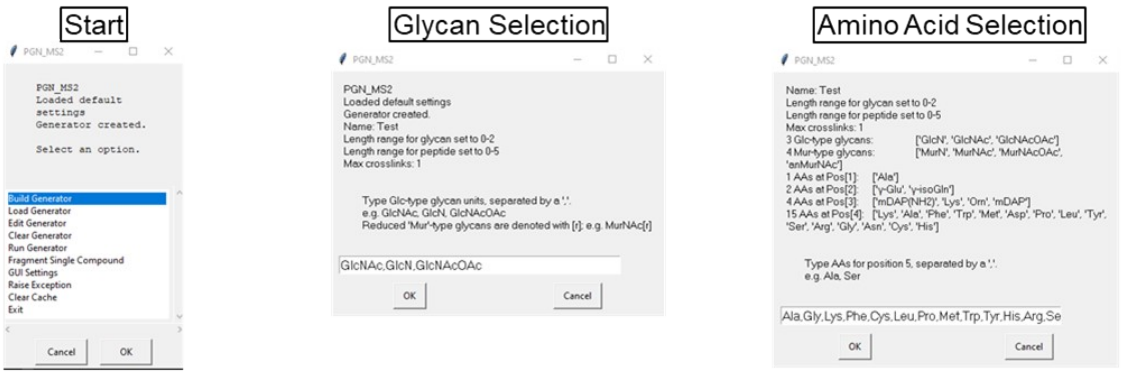


Figure S1. PGN Nomenclature used in PGN_MS2.

(**A**) Abbreviations used for non-canonical amino acids and glycans. (**B**) Chemical structures of amino acids Asx, Glx and Lys with their respective isomeric forms. Although Asp & β-Asp share the same chemical structure, they are connected to the stem peptide through different COOH groups (α-COOH vs β-COOH) and are given separate names to emphasize this difference. The same is true for Glu & γ-Glu (α-COOH vs γ-COOH); and Lys & ε-Lys (α-NH_2 vs ε-NH_2). These amino acids are highlighted here as they are commonly found in peptidoglycan: γ-Glu/γ-isoGln (2nd position); β-Asp/β-isoAsn (*E. faecium*)[10]; Lys (3rd position); ε-Lys (Braun's lipoprotein)[11,12]. (**C**) Notations used for descriptions of branch peptides and peptidoglycan polymerizations. (**D**) Example PGNs with their schematic representations and names. *To distinguish the glycans from the amino acids, glycans are encased in brackets. E.g., Glucosamine (abbrev: (G)) can be distinguished from glycine (abbrev: G). **Peptidoglycan polymers connected by glycosidic bonds (i.e., tetra saccharides) use a similar notation as crosslinked polymers, with the polymerization type indicated by (G-G).

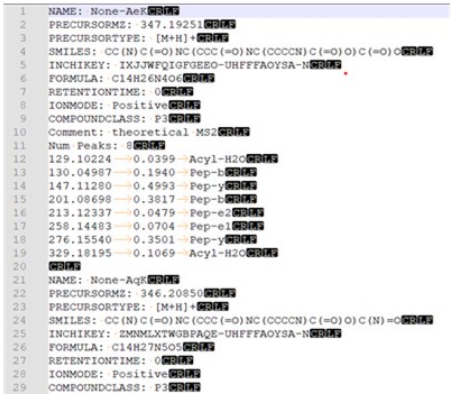
A



B

Subfolder	Filename*	File Description
compounds	[prefix].xlsx	MS1 database in spreadsheet format. Monomers, dimers and trimers are shown on separate sheets.
	[prefix].pickle	MS1 Database in pickle format (in batches of 5,000 compounds)
	[prefix].yaml	User-defined settings saved in yaml format
	[prefix]_graphical_summary.svg	Graphical summary of user-defined settings. See example in Figure 2.
msp	[prefix].msp	MS2 database. Different adduct forms are given as separate entries. Fragment ions with same *m/z* (but different structures) are combined and only the top 50 most intense ions are saved. See example in (C).
	[prefix]_[number].pickle	MS2 Database in pickle format. Saved in batches of 5,000 compounds, which is indicated by [number].
	[prefix].txt	Text file referencing the MS1 pickle file used to generate the MS2 database.
peaklists	[prefix]_spectradata.xlsx	MS2 database in spreadsheet format. Each batch has its own sheet. Each compound is presented as its own table containing the top 200 most intense ions. See example in (D).
	[prefix]_iondata.xlsx	All ions and their respective structures are tabulated in this file.

C



D

	A	B	C	D	E	F	G	H	I	J
1	Parent Name	Parent Synonym	Parent Mass	Parent Formula	Paren t	Parent SMILES				
2	None-AeK	None-Ala.isoGlu.Lys	346.18523	C14H26N4O6	IXJJW FQIG FGEE	CC(N)C (=O)NC (CCC(=				
3	mz	intensity	frag_type	idx	gen	p_idx	formula	SMILES		
4	0	1	Parent	0	0		C14H26N4O6	CC(N)C(=O)NC(CCC(=O)NC(CCCCN)C(=O)O)C(=O)O		
5	129.10224	0.0399	Acyl-H2O	8	2	2	C6H13N2O+	NCCCCC(N)C#[O+]		
6	130.04987	0.194	Pep-b	5	2	1	C5H8NO3+	NC(CCC#[O+])C(=O)O		
7	147.1128	0.4993	Pep-y	2	1	0	C6H15N2O2+	NCCCCC([NH3+])C(=O)O		
8	201.08698	0.3817	Pep-b	3	1	0	C8H13N2O4+	CC(N)C(=O)NC(CCC#[O+])C(=O)O		
9	213.12337	0.0479	Pep-e2	7	2	1	C10H17N2O3+	C#CCC(=[OH+])NC(CCCCN)C(=O)O		
10	258.14483	0.0391	Pep-e1	6	2	1	C11H20N3O4+	NCCCCC(NC(=O)CCC(N)C#[O+])C(=O)O		
11	258.14483	0.0313	Acyl-H2O	9	2	1	C11H20N3O4+	NCCCCC(C#[O+])NC(=O)CCC(N)C(=O)O		
12	276.1554	0.3501	Pep-y	1	1	0	C11H22N3O5+	NCCCCC(NC(=O)CCC([NH3+])C(=O)O)C(=O)O		
13	329.18195	0.1069	Acyl-H2O	4	1	0	C14H25N4O5+	CC(N)C(=O)NC(C#[O+])CCC(=O)NC(CCCCN)C(=O)O		

Figure S2. Screenshots of the graphical user interface for PGN_MS2 and generated outputs.

(**A**) The graphical user interface of PGN_MS2 guides the user through the creation of user-defined settings for compound database creation. Screenshots of the start, glycan selection and amino acid selection menu are shown. A detailed, step-by-step guide is available in the SI. (**B**) Outputs of PGN_MS2 and their descriptions. All outputs are saved in a parent folder and prefixed with a timestamp and a user-defined name: e.g., 202304041419_Akm. (**C**) Screenshot of the .msp format MS2 library. .msp format files can be inspected with any text editor. To reduce file size, the fragment structures are omitted. (**D**) A companion spreadsheet is created for each library. Herein, the user can find the precursor, fragment type, chemical structure, and formula for each fragment and for each compound.

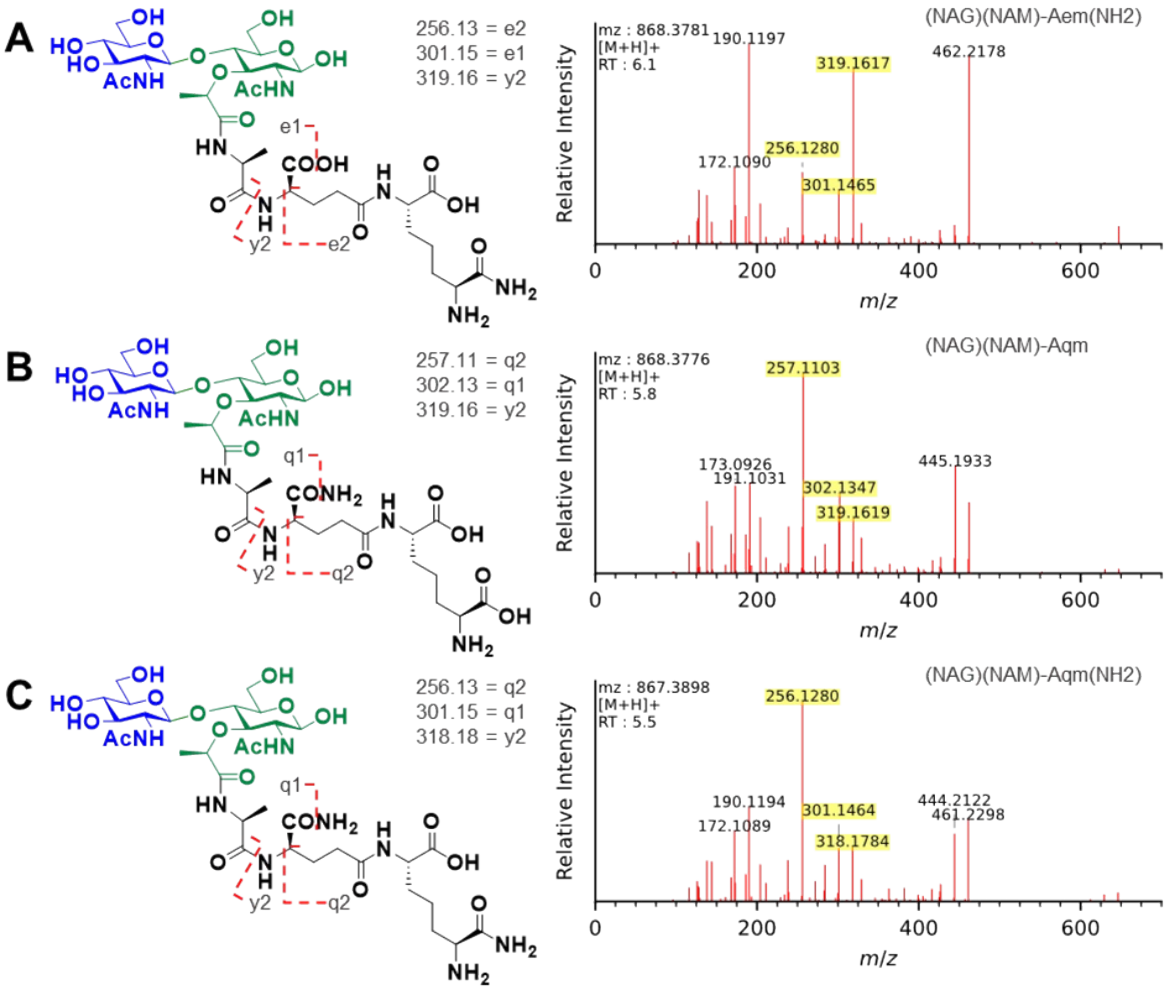
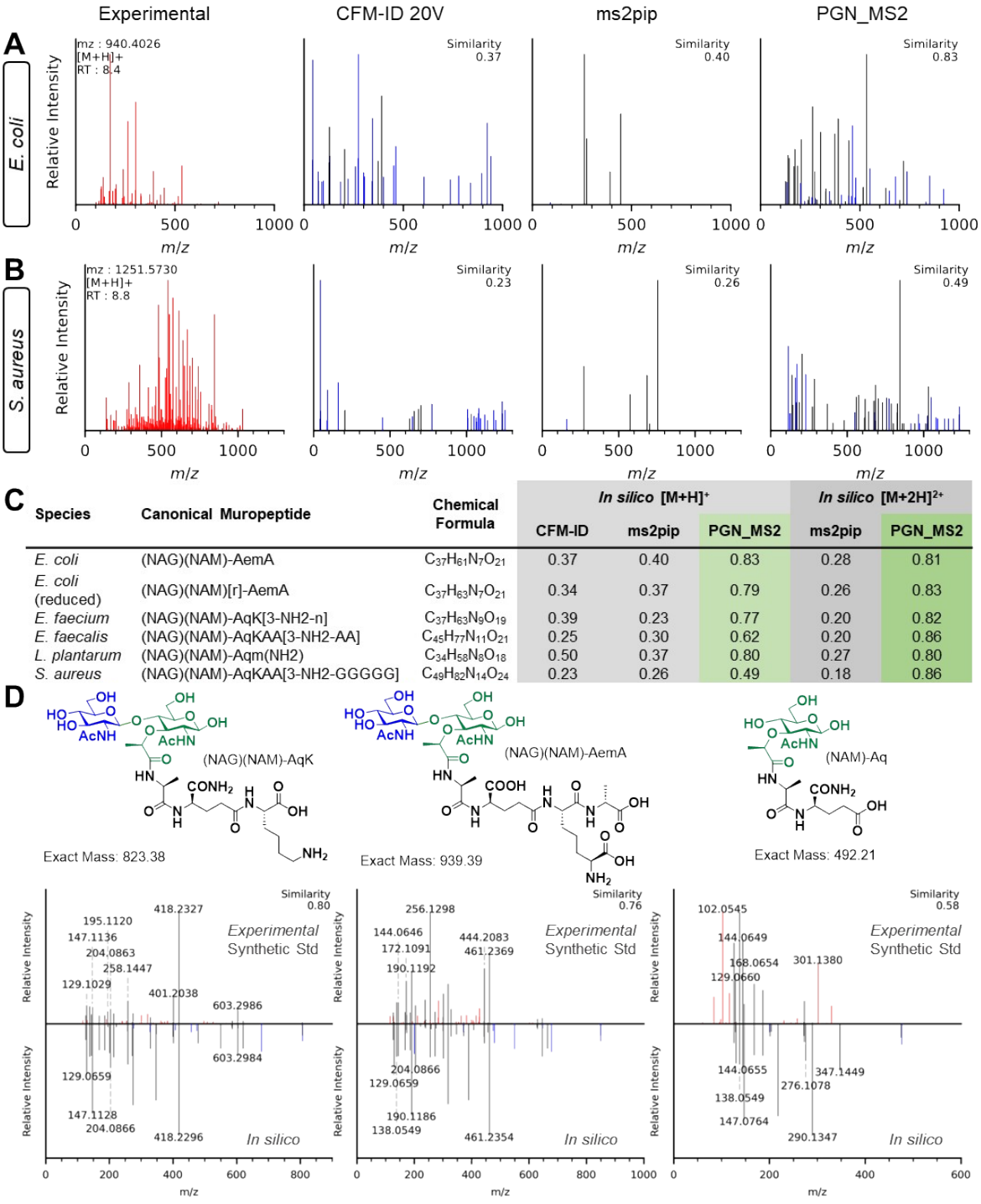


Figure S3. MS/MS fragmentations (e1/e2/q1/q2) pertain amidation status of γ-Glu and γ-isoGln in PGN stem peptides.

(**A-C**) Chemical structures and corresponding MS/MS spectra of three PGNs in *L. plantarum*. The structures of the respective y2 fragment and its derivatives, q1/e1 and q2/e2 are indicated, with corresponding peaks highlighted in yellow in the MS/MS spectra. The q1 and q2 fragments form intense peaks in the γ-isoGln-containing PGNs, B and C, with q2 being the more intense peak. A and B represent PGN isomers (*m/z* = 868.38) that can be distinguished by q1/q2 fragments.



Species	Canonical Muropeptide	Chemical Formula	*In silico* [M+H]⁺			*In silico* [M+2H]²⁺	
			CFM-ID	ms2pip	PGN_MS2	ms2pip	PGN_MS2
E. coli	(NAG)(NAM)-AemA	$C_{37}H_{61}N_7O_{21}$	0.37	0.40	0.83	0.28	0.81
E. coli (reduced)	(NAG)(NAM)[r]-AemA	$C_{37}H_{63}N_7O_{21}$	0.34	0.37	0.79	0.26	0.83
E. faecium	(NAG)(NAM)-AqK[3-NH2-n]	$C_{37}H_{63}N_9O_{19}$	0.39	0.23	0.77	0.20	0.82
E. faecalis	(NAG)(NAM)-AqKAA[3-NH2-AA]	$C_{45}H_{77}N_{11}O_{21}$	0.25	0.30	0.62	0.20	0.86
L. plantarum	(NAG)(NAM)-Aqm(NH2)	$C_{34}H_{58}N_8O_{18}$	0.50	0.37	0.80	0.27	0.80
S. aureus	(NAG)(NAM)-AqKAA[3-NH2-GGGGG]	$C_{49}H_{82}N_{14}O_{24}$	0.23	0.26	0.49	0.18	0.86

Figure S4. PGN_MS2 outperforms other existing *in silico* fragmentation tools in metabolomics and proteomics for PGN identification.

The performance of PGN_MS2 (highlighted in green) was compared against other *in silico* tools that could generate *in silico* spectra; CFM-ID (20V),[13] and ms2pip.[14] CFM-ID spectra were generated using the 'Spectral Prediction' function and inputting each muropeptide's SMILES. ms2pip spectra were generated from each muropeptide's peptide sequence, with its glycans, branch peptides, mDAP and mDAP(NH2) (as modifications of K), added as post-translational modifications. (**A**) and (**B**) show how the three *in silico* spectra (in blue) compare to the experimental $[M+H]^+$ spectra for the most abundant muropeptides in *E. coli* and *S. aureus* respectively. The similarity score between the experimental spectrum and each *in silico* spectrum is shown in the top right corner of each *in silico* spectrum. Matching peaks (within +/- 50 mDa) are plotted in black instead of red/blue. The similarity scores of these comparisons are tabulated in (**C**) for *E. coli*, *S. aureus* and four other samples (spectra not

shown). The *in silico* spectra generated by CFM-ID and ms2pip are less similar to the experimental spectra compared to those generated by PGN_MS2 (scores between 0.49-0.83). (**D**) Experimental spectra of synthetic muropeptides (top spectra) compared with PGN_MS2 *in silico* spectra (bottom spectra). Both Lys-type and mDAP-type PGN match well with their *in silico* counterparts. The match is poorer for MDP (far right) as we are unable to determine the structure of the 301.1380 fragment and hence unable to simulate it with our current fragmentation rules.

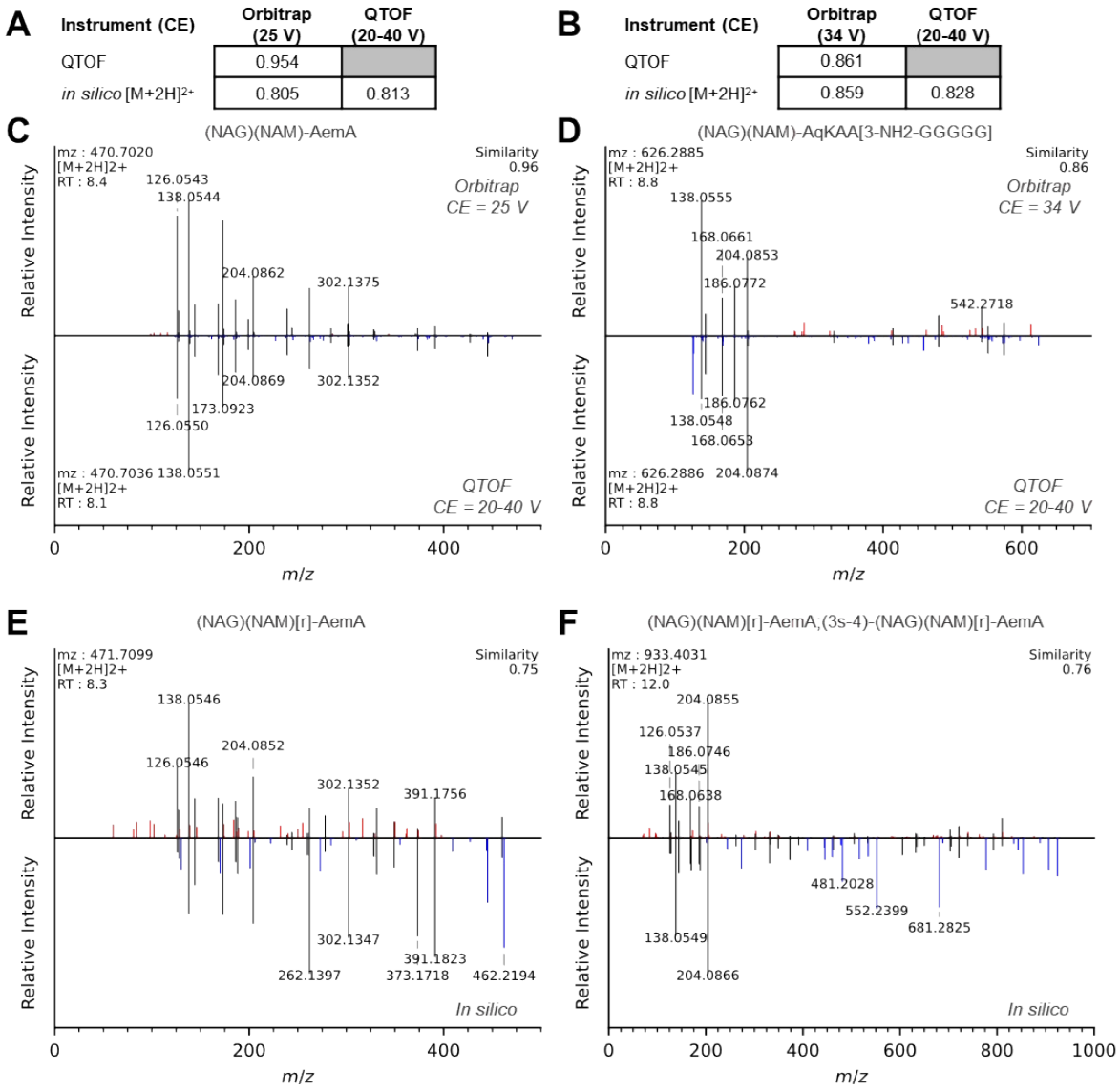


Figure S5. Predicted *in silico* MS/MS patterns by PGN_MS2 show good matches to experimental spectra.

Similarity scores for MS/MS spectra of canonical PGNs in *E. coli* (**A**) and *S. aureus* (**B**) in Q-TOF and Orbitrap mass spectrometers, and *in silico* spectra. Comparison of MS/MS spectra from Orbitrap (top) and Q-TOF mass spectrometers for *E. coli* and *S. aureus* (**C** and **D** respectively). Two examples of how PGN_MS2 generated *in silico* spectra match well with *P. aeruginosa* MS/MS spectra deposited by Anderson *et al*. for PGN monomers (**E**) and dimers (**F**).[15]

A *E. coli*

Compound	Formula	Chemical Similarity*	*In silico* [M+H]⁺ Matched Peaks	*In silico* [M+H]⁺ Spectral Similarity	*In silico* [M+2H]²⁺ Matched Peaks	*In silico* [M+2H]²⁺ Spectral Similarity
(DAG)(MUR)-AemA	$C_{37}H_{61}N_7O_{21}$	0.733	14	0.756	13	0.781
(G)(DAM)-AemA		0.727	19	0.795	15	0.737
(NAG)(NAM)-AeKD		0.746	18	0.625	13	0.700
(NAG)(NAM)-emAA		0.912	19	0.672	13	0.719
NAG)(NAM)-AemA		1.000	25	0.827	18	0.805

B *S. aureus*

Compound	Formula	Chemical Similarity*	*In silico* [M+H]⁺ Matched Peaks	*In silico* [M+H]⁺ Spectral Similarity	*In silico* [M+2H]²⁺ Matched Peaks	*In silico* [M+2H]²⁺ Spectral Similarity
(DAG)(MUR)-AqKAA[3-NH2-GGGGG]	$C_{49}H_{82}N_{14}O_{24}$	0.772	15	0.384	9	0.704
(G)(DAM)-AqKAA[3-NH2-GGGGG]		0.762	23	0.471	11	0.688
(NAG)(NAM)-AqKAAGG[3-NH2-GGG]		0.886	25	0.415	9	0.809
(NAG)(NAM)-qKAAA[2-NH2-GGGGG]		0.947	27	0.382	11	0.831
(NAG)(NAM)-AqKAA[3-NH2-GGGGG]		1.000	31	0.488	14	0.859

Figure S6. PGN_MS2 enables accurate identification of PGNs among closely related isomers via spectral matching.

Four isomers of *E. coli* (NAG)(NAM)-AemA, and four isomers of *S. aureus* (NAG)(NAM)-AqKAA[3-NH2-GGGGG] were generated, respectively. The experimental MS/MS spectra of (NAG)(NAM)-AemA and NAG)(NAM)-AqKAA[3-NH2-GGGGG] were compared with the *in silico* predicted spectra ($[M+H]^+$ and $[M+2H]^{2+}$) of the relevant isomers. Their similarity scores and the number of matched peaks are tabulated in (**A**) and (**B**) respectively for *E. coli* and *S. aureus*. The correct PGNs were identified, which show the highest spectral similarity score (highlighted in green) by a small margin. Matching experimental spectra against those generated *in silico* spectra allows for the differentiation of isomers. *Tanimoto chemical similarity was calculated with RDKit.

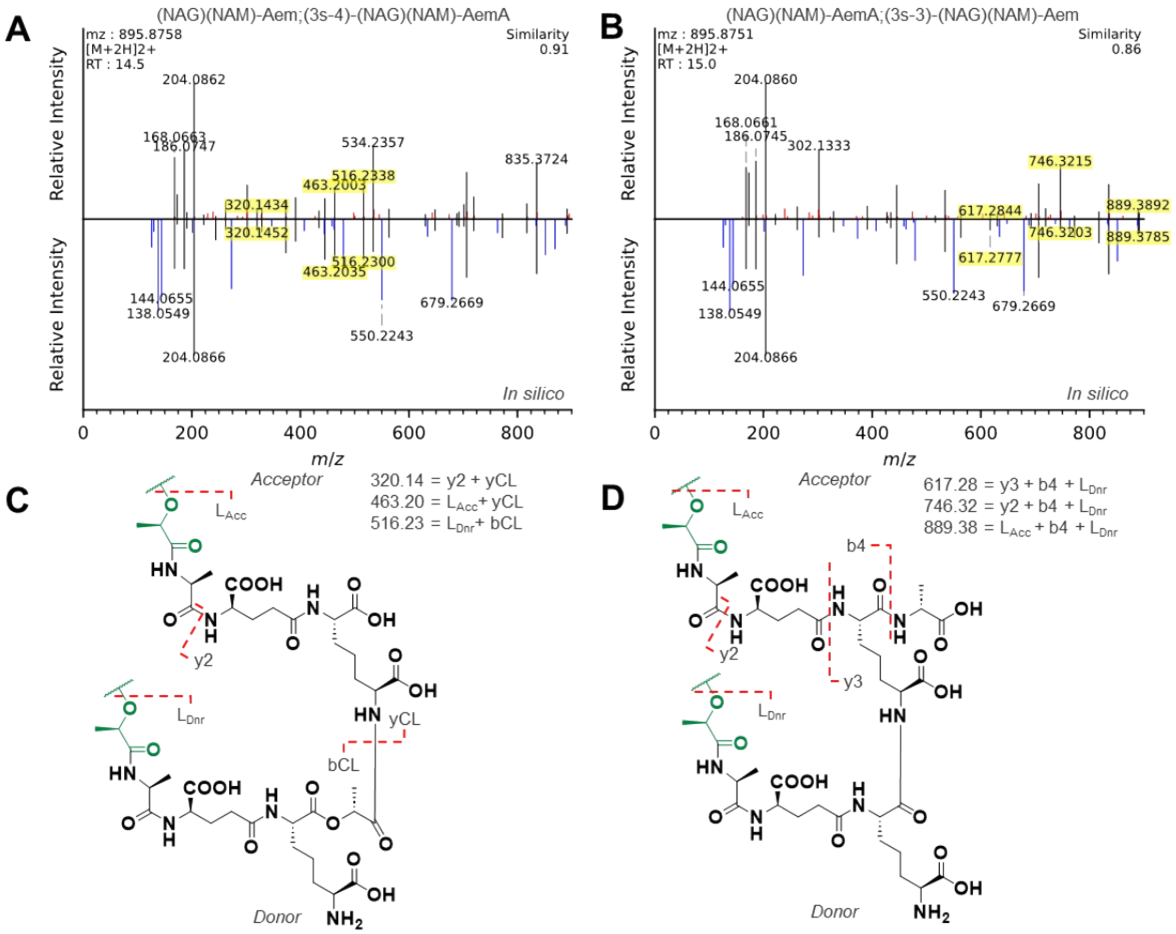


Figure S7. PGN_MS2 enables the differentiation of 3-4 and 3-3 crosslinked PGN isomers.

Analysis of MS/MS spectra allows differentiation between isomers and pinpoint the exact mode of PGN crosslinking in *E. coli*. (**A-B**) MS/MS spectra for 3-4 crosslinked (NAG)(NAM)-Aem;(3s-4)-(NAG)(NAM)-AemA; and 3-3 crosslinked (NAG)(NAM)-AemA;(3s-3)-(NAG)(NAM)-Aem, respectively. Peaks essential for differentiating the 3-4 and 3-3 crosslinked PGN isomers are highlighted in yellow with their structures shown in (**C-D**). For clarification, the crosslinked bond is elongated and the glycan moieties are not shown.

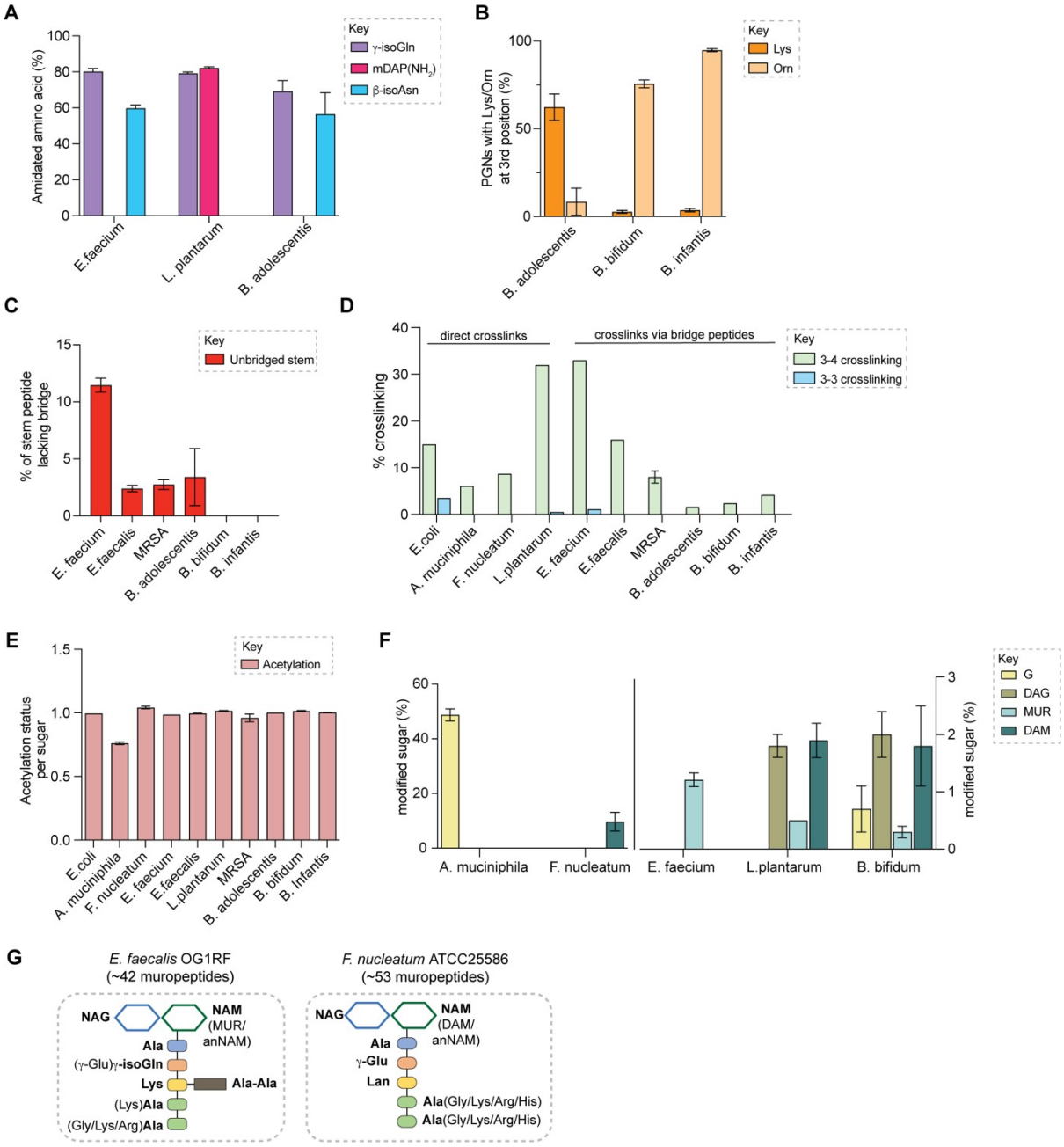
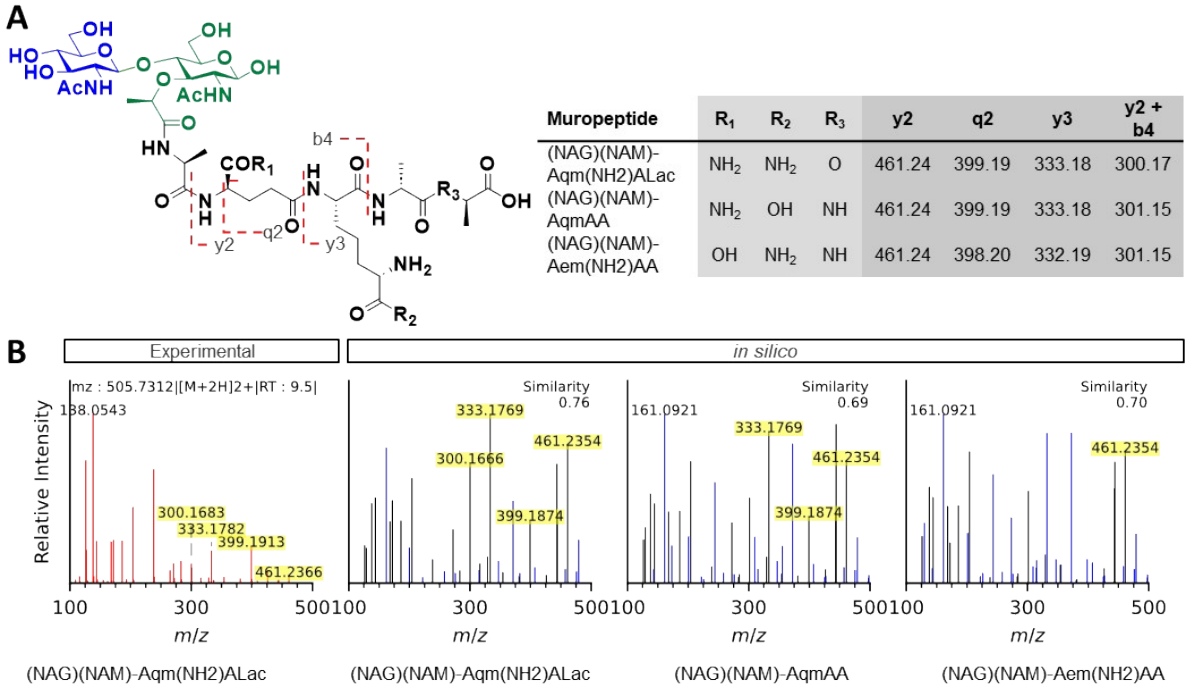


Figure S8. Summary of PGN compositions and features in model and gut bacteria.

(**A**) Distribution of amidation status on stem peptides in PGNs from species with two possible amidated residues. (**B**) *Bifidobacterium* spp. manifest distinct preference for Lys or Orn at the third position in stem peptides. (**C**) The proportion of stem peptides without bridges in PGNs across relevant species. (**D**) The proportion of crosslinked PGNs across bacteria. (**E**) Average acetylation rate (per glycan) in PGN across bacteria. (**F**) Frequency of glycans with altered acetylation states across bacteria. (**G**) PGN compositions in *E. faecalis* and *F. nucleatum*.



Muropeptide	R_1	R_2	R_3	y2	q2	y3	y2 + b4
(NAG)(NAM)-Aqm(NH2)ALac	NH_2	NH_2	O	461.24	399.19	333.18	300.17
(NAG)(NAM)-AqmAA	NH_2	OH	NH	461.24	399.19	333.18	301.15
(NAG)(NAM)-Aem(NH2)AA	OH	NH_2	NH	461.24	398.20	332.19	301.15

Figure S9. PGN_MS2 enables the identification of amidation positions of stem peptides in PGNs.

Analysis of MS/MS spectra allows differentiation between isomers and pinpoint the exact sites of amidation of stem peptides in PGNs of *L. plantarum*. (**A**) Chemical structures of three PGN isomers with identical molecular formula $C_{40}H_{67}N_9O_{21}$, and tabulation of key MS/MS fragments. (**B**) Experimental (left, in red) MS/MS spectra of (NAG)(NAM)-Aqm(NH2)ALac and *in silico* spectra for each isomer (in blue). The *in silico* spectrum corresponding to the correct PGN affords the highest similarity score (0.79) to the experimental, which manifests all four key MS/MS fragment peaks (highlighted in yellow).

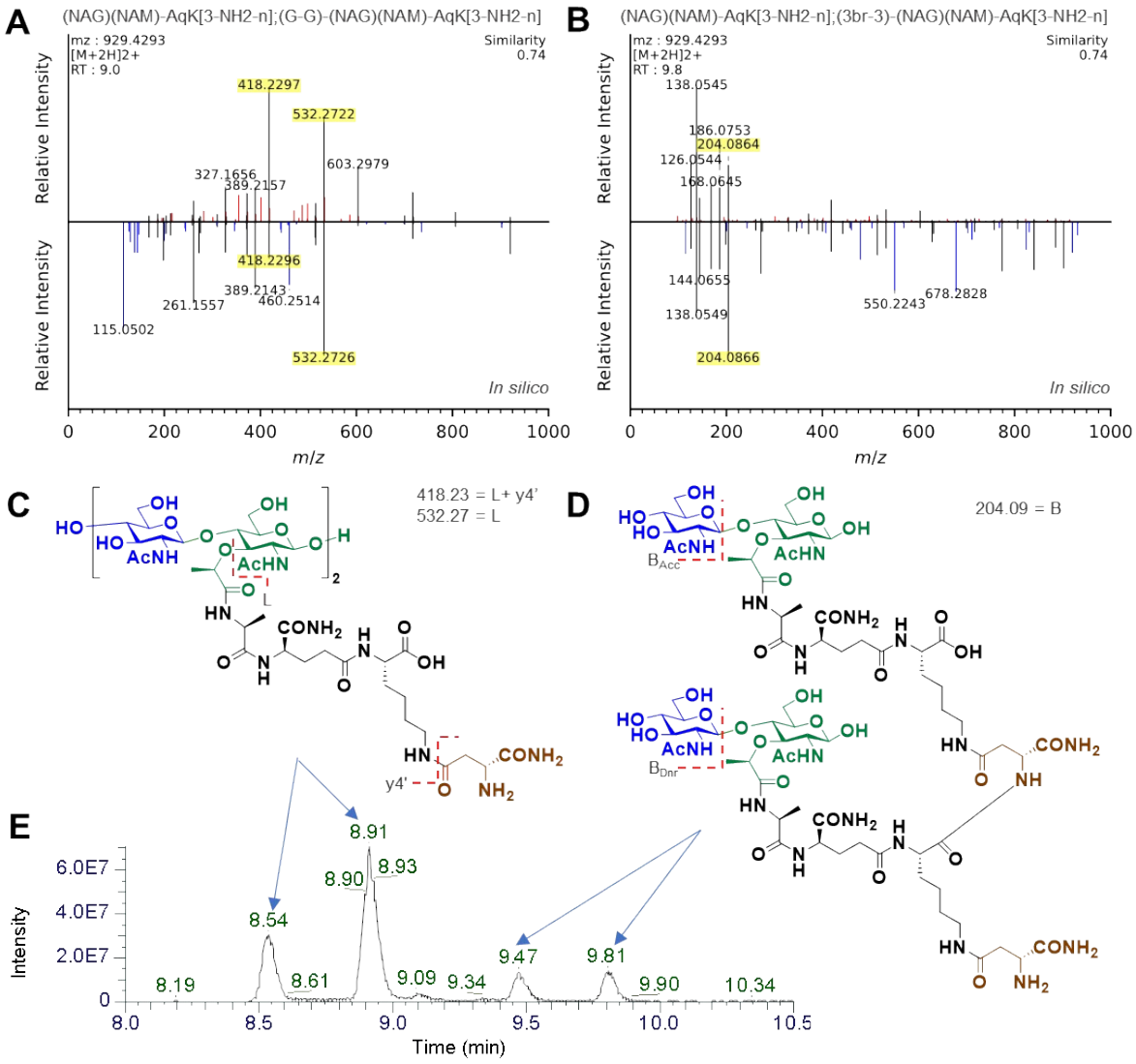


Figure S10. PGN_MS2 enables the differentiation of tetra-saccharide and crosslinked PGN isomers.

Analysis of MS/MS allows differentiation between tetra-saccharide and crosslinked isomers in *E. faecium*. (**A-B**) MS/MS spectra for tetra-saccharide (NAG)(NAM)-AqK[3-NH2-n];(G-G)-(NAG)(NAM)-AqK[3-NH2-n] and 3s-3 crosslinked (NAG)(NAM)-AqK[3-NH2-n];(3br-3)-(NAG)(NAM)-AqK[3-NH2-n], respectively. Peaks essential for differentiating the two PGN isomers are highlighted in yellow, with their structures shown in (**C-D**) for tetra-saccharide and 3-3 crosslinked isomers respectively. (**E**) Extracted ion chromatograms (EICs) of both PGNs isomers at *m/z* = 929.4260. The two isomers can also be differentiated by retention time. The crosslinked dimer (**D**) elutes later compared to the tetra-saccharide (**C**) in reverse-phase LC. Two peaks are observed for each isomer due to the mutarotation of the reducing end muramic acid.[16]

.

A

Species	Anhydro-muropeptide (most abundant)	Chemical Formula	RT (min)
B. adolescentis	(NAG)(anNAM)-AqKA[3-NH2-n]	$C_{40}H_{66}N_{10}O_{19}$	10.0
B. bifidum	(NAG)(anNAM)-AqOA[3-NH2-SD]	$C_{42}H_{68}N_{10}O_{22}$	9.6
B. infantis	(NAG)(anNAM)-AqOA[3-NH2-SATA]	$C_{48}H_{80}N_{12}O_{23}$	9.9

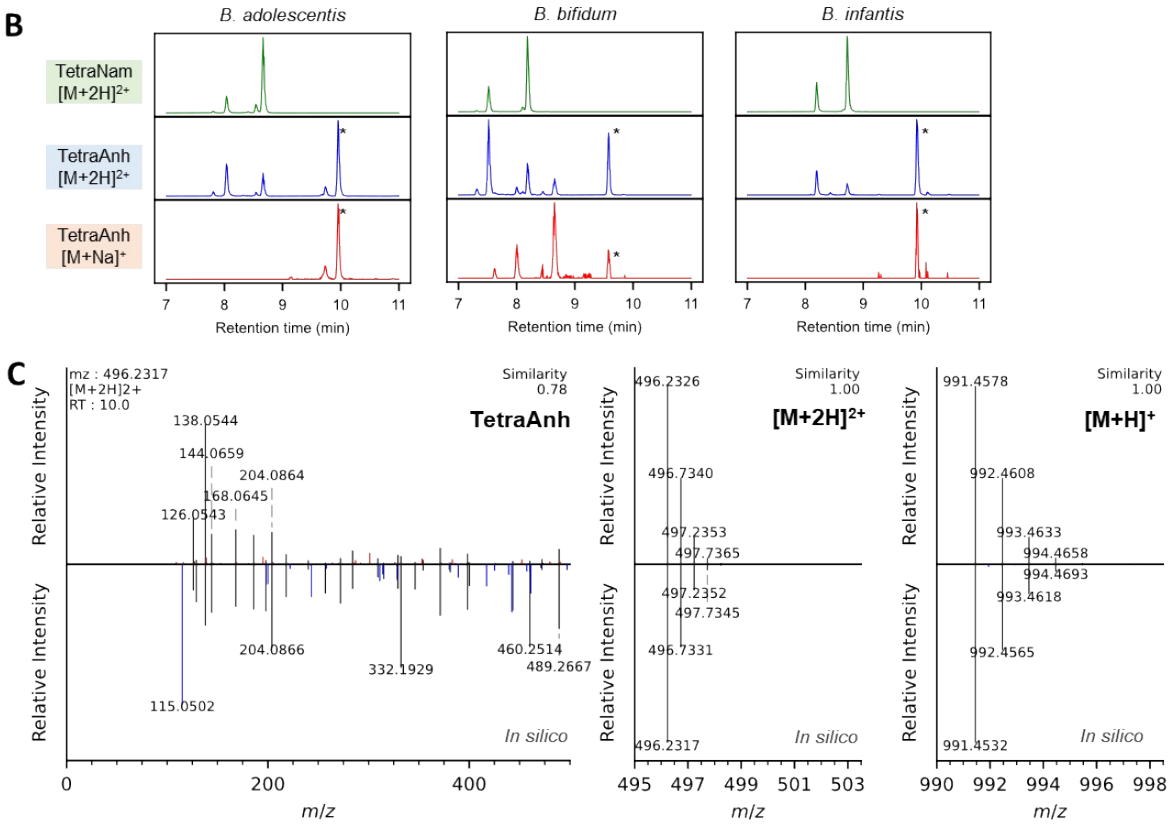


Figure S11. LC-MS/MS spectra of anhydro-PGNs in *Bifidobacterium spp*.

(**A**) Summary of the major anhydro-PGNs identified three *Bifidobacterium* species with their respective sequences, formulae, and RTs. (**B**) Extracted ion chromatograms (EICs) of anhydro-PGNs in three *Bifidobacterium* species. We noted that in source fragmentation (-H_2O) of the (NAM)-containing PGNs also produce ions with the same *m/z* as the target anhydro-PGNs. Nevertheless, the genuine anhydro-PGNs (marked with a *) can be distinguished from in-source fragments due to their later retention times (compare green and blue traces) and coeluting sodium adducts (red trace). (**C**) Isotopic pattern and spectral matching of experimental MS/MS data with the *in silico* predicted spectra confirmed the identity of the anhydro-PGN in *B. adolescentis.*

A

Analogue of…	Domain	*B. adolescentis* ATCC 15703	*B. bifidum* ATCC 15696	*B. infantis* ATCC 15697
E. coli MltA	IPR005300	-	-	-
E. coli MltB	IPR031304	-	K2I6H0*	-
E. coli MltC-F, Slt70 *B. subtilis* CwlQ	IPR008258	-	-	-
E. coli MltG	IPR003770	A1A1A0	A0A286TEX7	B7GS99
E. coli RlpA	IPR034718	-	-	-
M. tuberculosis and *S. coelicolor* RpfB**	IPR023346** & IPR011098	A1A348 & A1A3W2***	A0A286TDF0***	B7GPE2 & B7GUJ6***
B. subtilis SleB / CwlJ	IPR011105	-	-	-

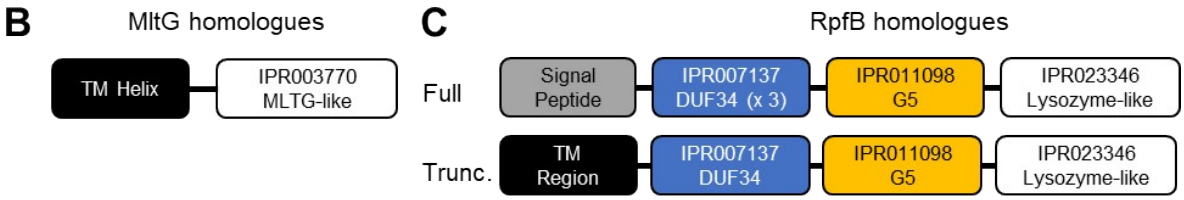


Figure S12. Identification of putative LTs in *Bifidobacterium* by homology searching.

(**A**) We searched for homologous proteins of known LTs in both Gram-negative and Gram-positive bacteria in *Bifidobacterium spp* on UniProt.[17] All three *Bifidobacterium spp.* contain homologs of *E. coli* MltG, *M. tuberculosis* and *S. coelicolor* RpfB. (**B**) The MltG homologs in *Bifidobacterium spp.* contain the same catalytic domain and protein architecture as *E. coli* MltG. (**C**) The RpfB homologs in *Bifidobacterium spp.* show weaker homology** but share the same protein architecture (full). Both *B. adolescentis* and *B. infantis* contain truncated forms of RpfB with two fewer DUF34 domains (truncated homologs indicated with ***). *Unconfirmed protein from shotgun genome sequencing data (as indicated on UniProt). **The catalytic domain of RpfB in *M. tuberculosis* and *S. coelicolor* can be represented with IPR010618 (more specific) or IPR023346 (more general, lysozyme-like domain). No proteins in *Bifidobacterium* show strong homology to RpfB that contain both IPR010618 and G5 domain IPR011098. However, we identified weaker RpfB homologs with both IPR023346 catalytic and G5 domains instead. ***Truncated homologs of RpfB with two absent DUF34 IPR007137 domains. InterPro domain numbers are used in this figure.

Figure S13. ClustalOmega sequence alignment of *Bifidobacterium* MltG and RpfB.

(**A**) Sequence alignment of three *Bifidobacterium* MltGs with three MltG homologs with known activities (*E. coli, B. subtilis* – LT, *S. pneumoniae* – muramidase).[7,18,19] The residue investigated by Taguchi *et al.* is marked with a red arrow,[7] and the catalytic glutamate is indicated with a blue arrow. (**B**) As the RpfB homologs showed weaker sequence conservation, we only aligned the catalytic domains of *B. adolescentis* RpfB homologs with known Rpfs to determine their catalytic sites (blue arrow). Sequences were aligned with ClustalOmega and visualised with MView.[20] Protein sequences are colored by their identities.

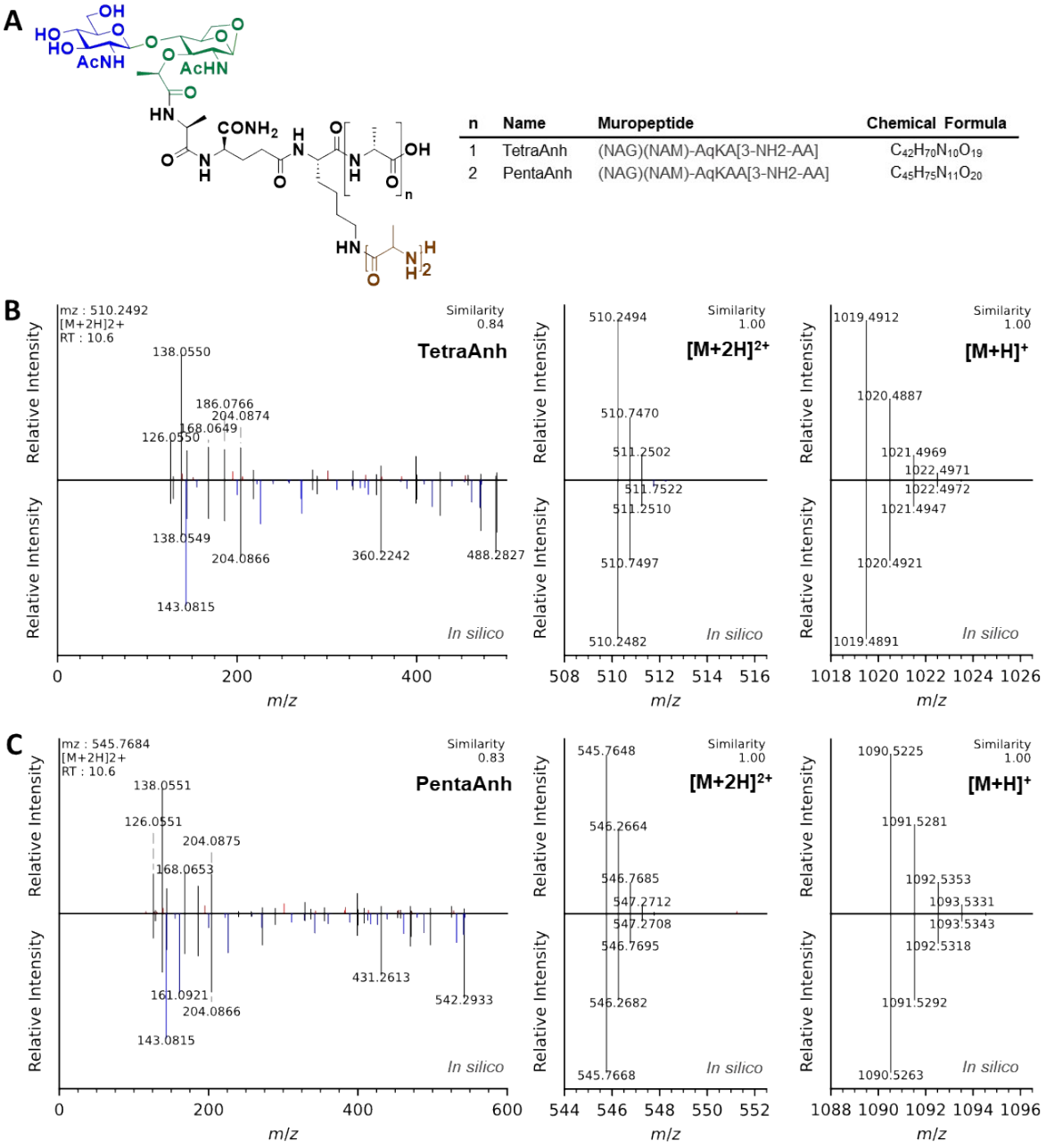


Figure S14. Anhydro-PGNs are generated by recombinant *Bifidobacterium* MltG from nascent peptidoglycan as substrates.

(**A-C**) Chemical structures of anhydro-PGNs (i.e. TetraAnh/PentaAnh) with their corresponding experimental (top) and *in silico* mass spectra. MS/MS spectra are shown on the left and the MS1 isotopic distributions are shown on the right for both adduct forms. Nascent peptidoglycan strands were produced by SgtB polymerization of Lipid II isolated from *E. faecalis*.

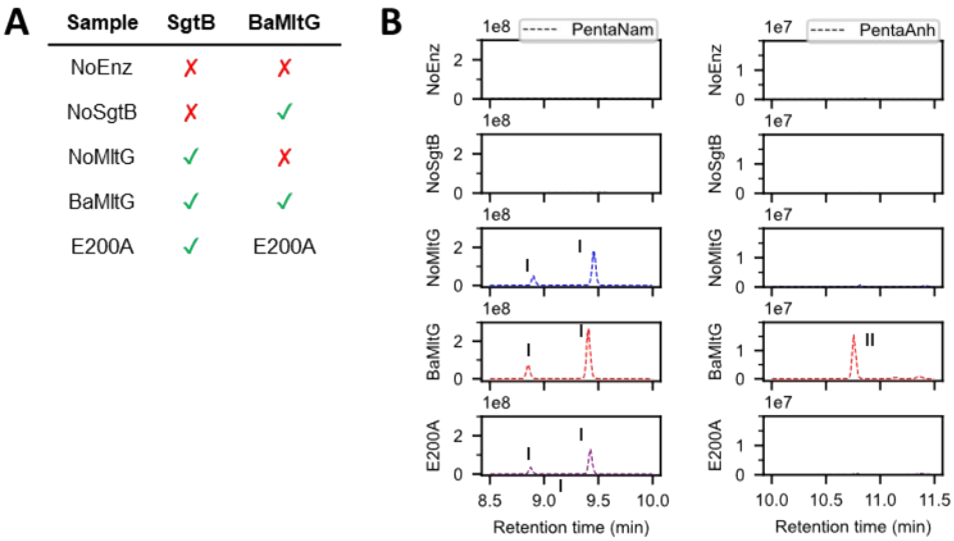


Figure S15. Additional controls for recombinant *Bifidobacterium* MltG biochemical assay

(**A**) Experimental setup for each sample (n=3). (**B**) Representative EICs for products **I** and **II** ((NAG)(NAM)-AqKAA[3-NH2-AA] and (NAG)(anNAM)-AqKAA[3-NH2-AA] respectively, see Figure 6 for structures).

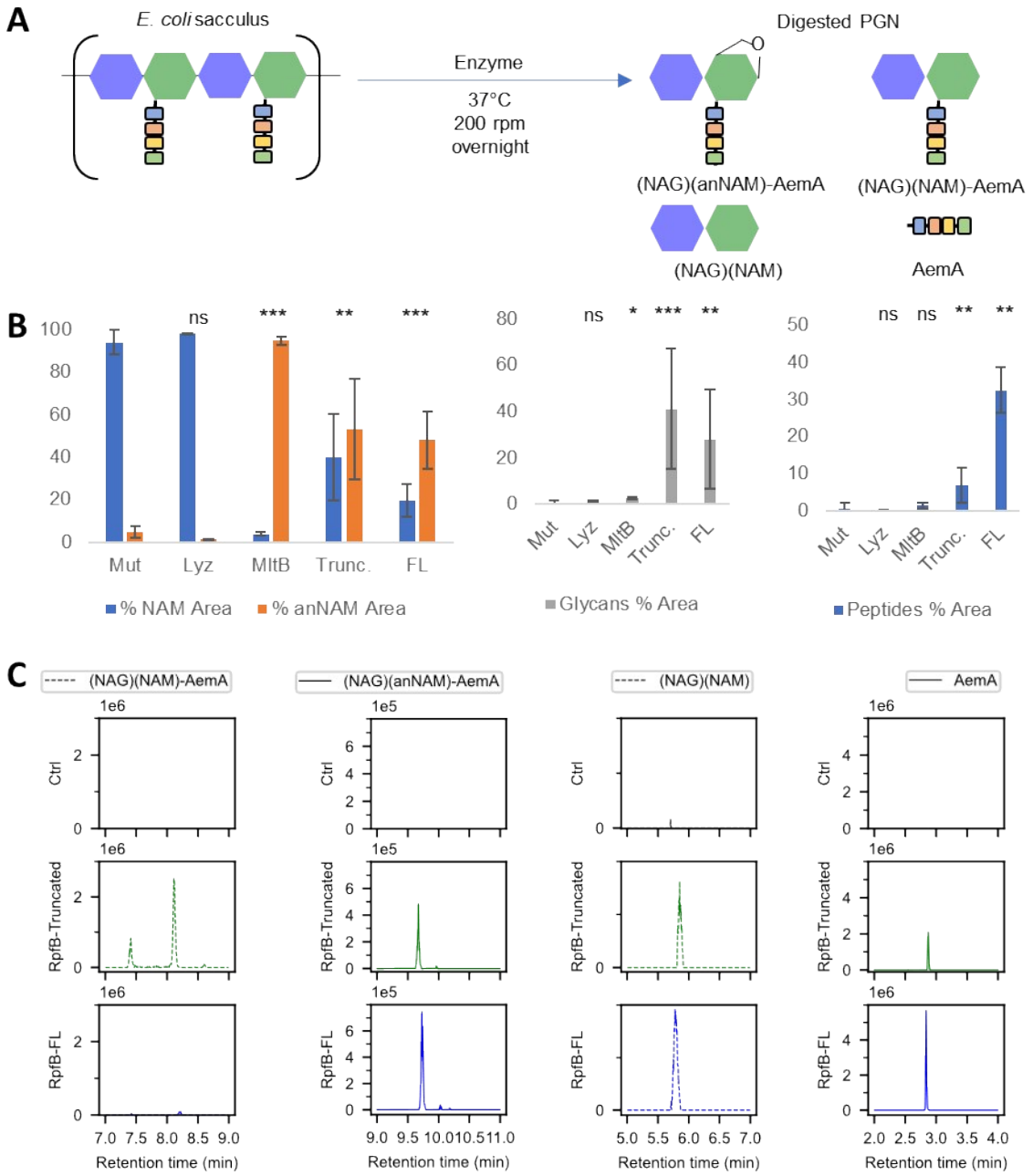


Figure S16. *B. adolescentis* RpfBs exhibit LT activities with sacculi as substrates.

(**A**) Schematics for biochemical reconstitution of the recombinant *B. adolescentis* RpfBs (RpfB-Truncated and RpfB-FL). (**B**) Summary of % compositions of peptides, glycans, and NAM/anNAM-containing PGNs formed by RpfBs. Statistical analysis was performed using a two-tailed student's t-test with mutanolysin digestion as the control. (**C**) Extracted ion chromatograms (EICs) of the amidase and LT products from *B. adolescentis* RpfBs. Both *B. adolescentis* RpfB-Truncated and RpfB-FL exhibit dual LT/amidase activities with sacculi as substrates. All values shown are the average of three replicates except for mutanolysin (six replicates).

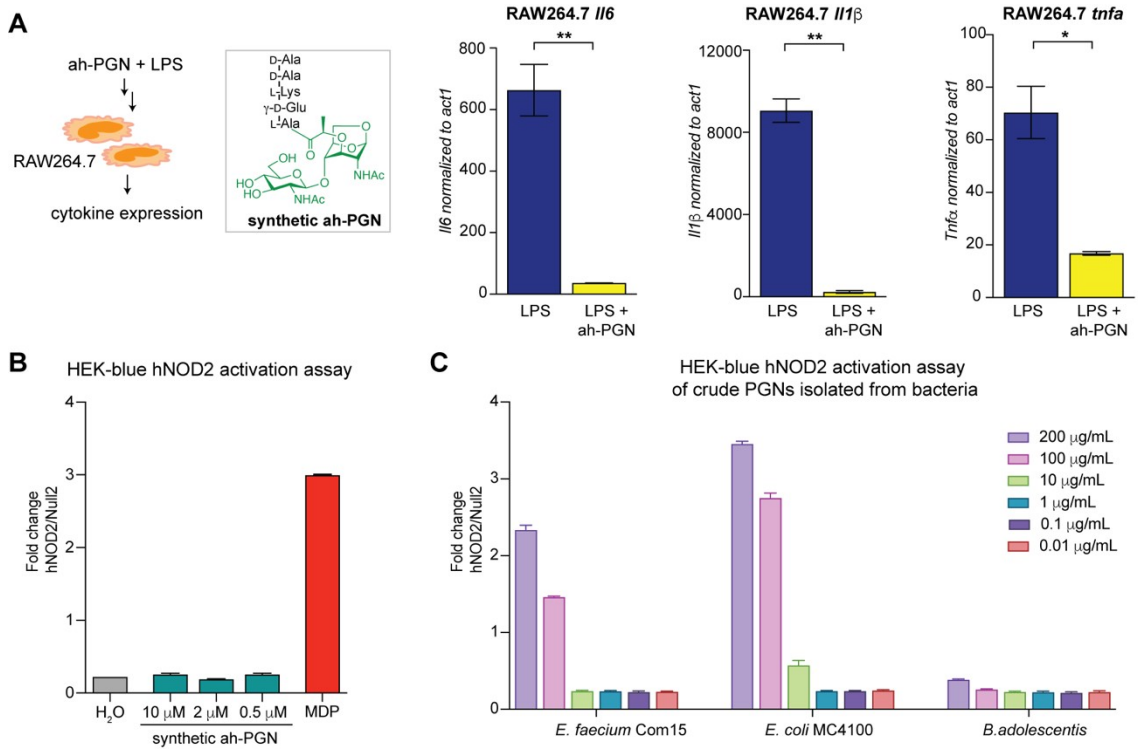


Figure S17. Anti-inflammatory activities of synthetic ah-PGN and crude bacterial PGNs.

(**A**) Schematic of *in vitro* anti-inflammatory assays in RAW264.7 cells (left) and the relative gene expression levels of cytokines by LPS with or without ah-PGN pre-treatment (right). (**B-C**) HEK-blue hNOD2 reporter assay shows that synthetic ah-PGN is not a NOD2 agonist **(B)**. Consistently, crude PGNs of *B. adolescentis* (which contain substantial anhydro-PGNs) do not stimulate NOD2 compared to crude PGNs isolated from *E. faecium*, *E. coli* (**C**).

Supplementary Tables

Table S1

Including excessive numbers of structural modifications in monomeric PGNs would result in a combinatorial explosion, creating an unreasonable amount of possible PGN polymers. This can significantly compromise data analysis time. Therefore, depending on the potential applications, it may be useful to reduce the number of PGN polymers with a more targeted and focused PGN database. For instance, the canonical makeup of *E. faecalis* PGNs contains NAG, NAM, Ala, g-isoGln, Lys, and Ala-Ala (branch peptide), 0 or 2 glycans (glycan length), 4-5 amino acids (stem peptide length), which altogether account for ~70% of its total PGNs (see **Figure 2**); hence for building a focused PGN polymer pool, we excluded minor PGN monomers that differ excessively from the canonical makeup (default: >1 difference). In addition, for 3-4 crosslinked PGNs in *E. faecalis,* we also specified the donor and acceptor stem peptides can only include either the canonical (Ala) or the most common substituted amino acid (Gly) at the terminus By applying such restrictions, the pool of *E. faecalis* PGN dimers generated is significantly reduced from 46,164 to 2,880 The species- and polymerisation-specific criteria are useful for building a focused PGN polymer pool.

Glucosamine Range	Muramic Acid Range	Differences Allowed*	Crosslink Peptide AA4/5	Monomers	G-G Dimers	3br-4 Dimers	Total Dimers	Reduction
GlcNAc, GlcN	MurNAc, anMurNAc, MurN	1	None	2717	17644	28520	46164	N/A
		0	None	2717	128	240	368	99.2%
		1	Ala, Gly	2717	1248	1632	2880	93.8%
		1	Ala, Gly, Ser	2717	1680	2296	3976	91.4%
GlcNAc, GlcN, GlcNAcOAc	MurNAc, anMurNAc, MurN, MurNAcOAc	1	None	4905	23296	32732	56028	N/A
		0	None	4905	128	240	368	99.3%
		1	Ala, Gly	4905	2040	2160	4200	92.5%
		1	Ala, Gly, Ser	4905	2584	2914	5498	90.2%

*The same number of dimers was created when the number of differences allowed was set to 1 or 2.

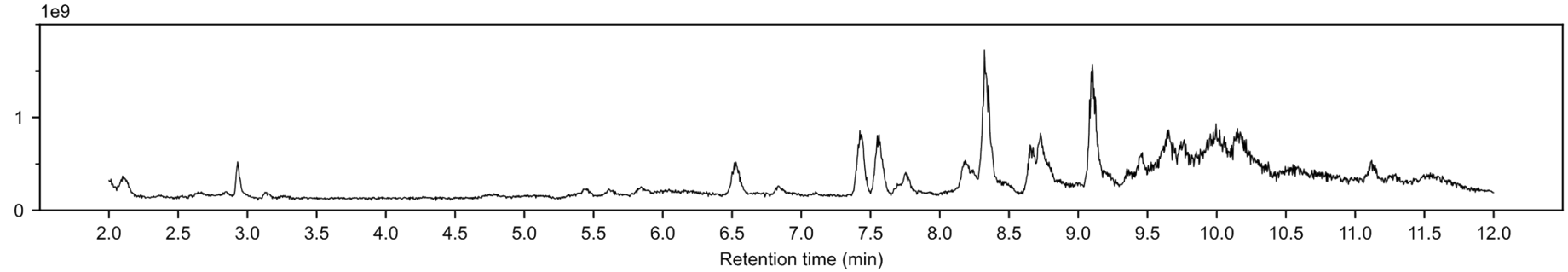
Table S2

Comparison against other tools that can analyze/simulate MS/MS spectra. As there are no tools specialized for peptidoglycan, we looked at tools that could handle ESI-MS/MS spectra for metabolomics (M) or proteomics (P) experiments.

Tool	Usage (M/P)	Limitations	Max. Sim.	Link
CFM-ID[13]	M	Can only simulate [M+H]+.	0.50	https://cfmid.wishartlab.com/
MetFrag[21]	M	Doesn't provide relative intensity estimates.	N/A	https://ipb-halle.github.io/MetFrag/
CSI:FingerID with SIRIUS[22]	M	Can only simulate [M+H]+ and requires days for compounds with >800 Da	N/A	https://bio.informatik.uni-jena.de/software/sirius/
ms2pip[14]	P	NAG-NAM, mDAP and bridge peptides not natively supported	0.40	https://iomics.ugent.be/ms2pip/

Table S3

Representative TIC and identified peptidoglycan in *E. coli* MG1655.



SN	Compound	Formula	Monoisotopic Mass	MS-DIAL score			RT / min	Rel. Abundance	
				[M+H]+	[M+2H]2+	[M+3H]3+		Mean	S.D
1	(G)(NAM)-None	C17H30N2O12	454.17987	86.3			3.2	0.05%	0.00%
2	(NAG)(anNAM)-None	C19H30N2O12	478.17987	75.4			8.5	1.47%	0.36%
3	(NAG)(NAM)-None	C19H32N2O13	496.19044	76.8			5.0|6.1	0.18%	0.01%
4	(NAG)(anNAM)-Ae	C27H42N4O16	678.25958	63.5			11.0	0.19%	0.02%
5	(NAG)(NAM)-Ae	C27H44N4O17	696.27015	66.7			8.4|9.1	1.59%	0.05%
6	(NAG)(anNAM)-Aem	C34H54N6O19	850.34437	74.8	71.8		9.1	0.84%	0.19%
7	(NAG)(NAM)-Aem	C34H56N6O20	868.35494	74.4	74.7		6.5|7.5	8.68%	0.29%
8	(NAG)(anNAM)-AemG	C36H57N7O20	907.36584	70.8	63.9		9.4	0.11%	0.01%
9	(NAG)(anNAM)-AemA	C37H59N7O20	921.38149	MS1	70		10.0	2.80%	0.32%
10	(NAG)(NAM)-AemG	C36H59N7O21	925.3764	72.6	70.7		6.8|7.8	2.55%	0.07%
11	(NAG)(NAM)-AemA	C37H61N7O21	939.39205	71.6	71.1		7.6|8.4	19.33%	0.08%
12	(NAG)(NAM)-None; (G-G)-(NAG)(anNAM)-None	C38H60N4O24	956.35975		67.3		5.2	0.04%	0.02%
13	(NAG)(NAM)-AemC	C37H61N7O21S	971.36412	68.5	MS1		8.2|8.9	0.06%	0.00%
14	(NAG)(NAM)-AemN	C38H62N8O22	982.39787	MS1			6.2|7.2	0.05%	0.00%
15	(NAG)(anNAM)-AemH	C40H61N9O20	987.40329		76.3		8.6|8.7	0.04%	0.00%

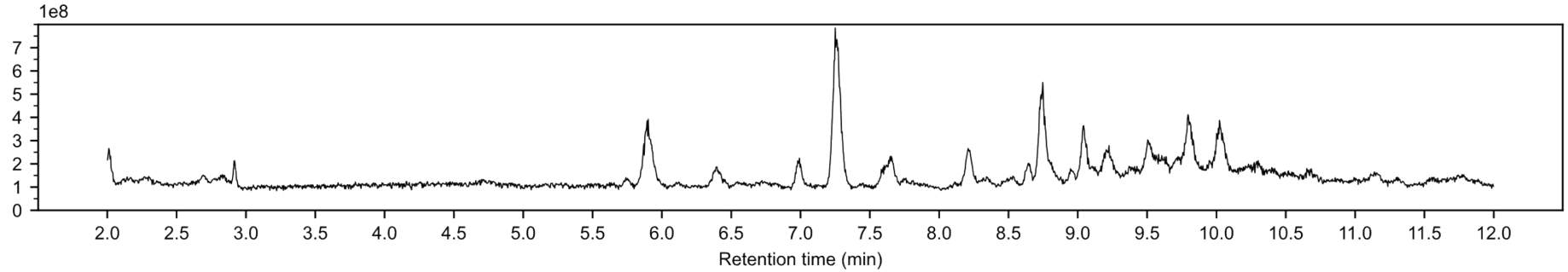
SN	Compound	Formula	Monoisotopic Mass	MS-DIAL score			RT / min	Rel. Abundance	
16	(NAG)(NAM)-AemAG	C39H64N8O22	996.41352	64.2	69.9		7.3|8.1	0.04%	0.00%
17	(NAG)(NAM)-AemK	C40H68N8O21	996.4499		75.5		5.6|6.7	0.12%	0.01%
18	(NAG)(anNAM)-AemF	C43H63N7O20	997.41279	63.4			15.4	0.01%	0.00%
19	(NAG)(NAM)-AemM	C39H65N7O21S	999.39542	65.6	MS1		10.6|11.4	0.06%	0.00%
20*	(NAG)(NAM)-AemH	C40H63N9O21	1005.41385	MS1	72.9		5.9|6.2|6.9|7.2	0.55%	0.01%
21	(NAG)(NAM)-AemF	C43H65N7O21	1015.42335	64.8	68		13.5|14.3	0.49%	0.02%
22	(NAG)(NAM)-AemR	C40H68N10O21	1024.45605		62.9		6.3|7.3	0.08%	0.00%
23	(NAG)(NAM)-AemY	C43H65N7O22	1031.41827	66.5	MS1		11.2|11.6	0.05%	0.00%
24	(NAG)(NAM)-AemAK	C43H73N9O22	1067.48701		72.8		6.2|7.1	0.02%	0.00%
25	(NAG)(NAM)-AemAH	C43H68N10O22	1076.45096		72		6.4|7.3	0.04%	0.00%
26	(NAG)(NAM)-AemA; (3s-4)-None-mA	C47H78N10O25	1182.51396		77		7.9|8.6	0.21%	0.04%
27	None-Aem; (3s-3)-(NAG)(NAM)-Aem	C49H80N10O27	1240.51944		71.2		7.3|7.9	0.41%	0.07%
28	(NAG)(NAM)-Aem; (3s-3)-None-Aem	C49H80N10O27	1240.51944		71.1		7.6|8.2	0.16%	0.02%
29	(G)(NAM)-None; (G-G)-(NAG)(NAM)-Aem	C51H84N8O31	1304.52425		73.6		6.4|7.1	0.83%	0.03%
30	(NAG)(NAM)-Aem; (3s-4)-None-AemA	C52H85N11O28	1311.55655		66.8		8.4	0.12%	0.01%
31	None-Aem; (3s-4)-(NAG)(NAM)-AemA	C52H85N11O28	1311.55655		76.4		7.7|8.2	0.14%	0.00%
32	(NAG)(NAM)-None; (G-G)-(NAG)(NAM)-Aem	C53H86N8O32	1346.53481		75		7.6|8.3	0.27%	0.02%
33	(G)(NAM)-AemA; (G-G)-(NAG)(NAM)-None	C54H89N9O32	1375.56136		73.5		7.9|8.2	0.61%	0.00%
34	(NAG)(NAM)-None; (G-G)-(NAG)(NAM)-AemA	C56H91N9O33	1417.57193		73.2		8.3|8.9	0.28%	0.04%
35	(NAG)(anNAM)-Aem; (3s-3)-(NAG)(anNAM)-Aem	C68H106N12O37	1682.67818		63.7		11.8	0.03%	0.00%
36	(NAG)(NAM)-Aem; (3s-3)-(NAG)(anNAM)-Aem	C68H108N12O38	1700.68875		71.2		10.3|10.7	0.38%	0.01%
37	(NAG)(NAM)-Aem; (G-G)-(NAG)(NAM)-Aem	C68H110N12O39	1718.69931		72	76	7.7|8.2	5.14%	0.26%
38	(NAG)(NAM)-Aem; (3s-3)-(NAG)(NAM)-Aem	C68H110N12O39	1718.69931		73.8		9.3|9.6	0.54%	0.07%
39	(NAG)(anNAM)-AemA; (3s-3)-(NAG)(anNAM)-Aem	C71H111N13O38	1753.7153		68.8		11.4	0.08%	0.01%
40	(NAG)(anNAM)-Aem; (3s-4)-(NAG)(anNAM)-AemA	C71H111N13O38	1753.7153		70.8		12.0	0.04%	0.00%

SN	Compound	Formula	Monoisotopic Mass	MS-DIAL score		RT / min	Rel. Abundance	
42	(NAG)(anNAM)-Aem; (3s-4)-(NAG)(NAM)-AemA	C71H113N13O39	1771.72586	73.2		10.5|10.9	0.46%	0.02%
41	(NAG)(NAM)-AemG; (3s-3)-(NAG)(NAM)-Aem	C70H113N13O40	1775.72078	70.5		9.1|9.5	0.19%	0.03%
43	(NAG)(NAM)-AemG; (G-G)-(NAG)(NAM)-Aem	C70H113N13O40	1775.72078	75.6	MS1	7.9||8.4	1.40%	0.06%
44	(NAG)(NAM)-AemA; (G-G)-(NAG)(NAM)-Aem	C71H115N13O40	1789.73643	77.5	76.6	8.3|8.7	8.78%	0.79%
45	(NAG)(NAM)-Aem; (3s-4)-(NAG)(NAM)-AemA	C71H115N13O40	1789.73643	76.5		9.5|9.8	1.33%	0.20%
46	(NAG)(NAM)-AemA; (3s-3)-(NAG)(NAM)-Aem	C71H115N13O40	1789.73643	74	MS1	9.6|9.9	1.72%	0.26%
47	(NAG)(anNAM)-AemA; (3s-4)-(NAG)(anNAM)-AemA	C74H116N14O39	1824.75241	69.7		11.3	0.61%	0.07%
48	(NAG)(NAM)-AemG; (3s-4)-(NAG)(anNAM)-AemA	C73H116N14O40	1828.74732	71.4		10.8	0.12%	0.01%
49	(NAG)(NAM)-AemG; (G-G)-(NAG)(NAM)-AemG	C72H116N14O41	1832.74224	75.4	73.8	8.0|8.4	0.44%	0.01%
50	(NAG)(anNAM)-AemA; (3s-4)-(NAG)(NAM)-AemA	C74H118N14O40	1842.76297	71.4	MS1	10.9|11.2|11.3	1.67%	0.16%
51	(NAG)(NAM)-AemG; (3s-4)-(NAG)(NAM)-AemA	C73H118N14O41	1846.75789	68.9	MS1	9.3|9.4|9.7	1.36%	0.14%
52	(NAG)(NAM)-AemG; (G-G)-(NAG)(NAM)-AemA	C73H118N14O41	1846.75789	74.8	72.5	8.4|8.8	3.21%	0.04%
53	(NAG)(NAM)-AemA; (3s-4)-(NAG)(NAM)-AemA	C74H120N14O41	1860.77354	72.5	75.4	9.8|10.1	8.61%	1.60%
54	(NAG)(NAM)-AemA; (G-G)-(NAG)(NAM)-AemA	C74H120N14O41	1860.77354	74.6	75.1	8.8|9.1	20.81%	1.23%
55	(NAG)(NAM)-AemA; (3s-4)-(NAG)(NAM)-AemA; (3s-4)-(NAG)(NAM)-AemA	C111H179N21O61	2782.15503	MS1	MS1	10.6|10.9	0.62%	0.14%

*Two stereoisomers of AemH suspected

Table S4

Representative TIC and identified peptidoglycan in *E. coli* MG1655 (reduced with $NaBH_4$)



SN	Compound	Formula	Monoisotopic Mass	MS-DIAL score			RT / min	Rel. Abundance	
				[M+H]+	[M+2H]2+	[M+3H]3+		Mean	S.D
1	(G)(NAM)[r]-None	C17H32N2O12	456.19552	71.6			2.9	0.04%	0.01%
2	(NAG)(anNAM)-None	C19H30N2O12	478.17987	75.4			8.5	0.87%	0.16%
3	(NAG)(anNAM)-Ae	C27H42N4O16	678.25958	60.5			11.1	0.11%	0.01%
4	(NAG)(NAM)[r]-Ae	C27H46N4O17	698.2858	68.1			7.6	1.10%	0.06%
5	(NAG)(anNAM)-Aem	C34H54N6O19	850.34437	74.5	70.7		9.1	0.49%	0.05%
6	(NAG)(NAM)-Aem	C34H56N6O20	868.35494	73.5	MS1		7.5	0.03%	0.03%
7	(NAG)(NAM)[r]-Aem	C34H58N6O20	870.37059	75.1	76		5.9	8.86%	0.14%
8	(NAG)(anNAM)-AemG	C36H57N7O20	907.36584	71.9	MS1		9.4	0.07%	0.01%
9	(NAG)(anNAM)-AemA	C37H59N7O20	921.38149	68.7	70.1		10.1	0.31%	0.07%
10	(NAG)(NAM)[r]-AemG	C36H61N7O21	927.39205	MS1	64.7		6.4	2.63%	0.09%
11	(NAG)(NAM)-AemA	C37H61N7O21	939.39205	70.2			7.6|8.4	0.06%	0.05%
12	(NAG)(NAM)[r]-AemA	C37H63N7O21	941.4077	73.1	74.5		7.3	21.82%	1.06%
13	(NAG)(NAM)[r]-AemC	C37H63N7O21S	973.37977	MS1	67.1		7.8	0.06%	0.00%
14*	(NAG)(NAM)[r]-AemH	C40H65N9O21	1007.4295		73.1		5.5|5.8	0.37%	0.01%
15	(NAG)(NAM)[r]-AemF	C43H67N7O21	1017.439		67.4		12.1	0.06%	0.03%
16	(NAG)(NAM)[r]-AemR	C40H70N10O21	1026.4717		MS1		6.0	0.05%	0.00%

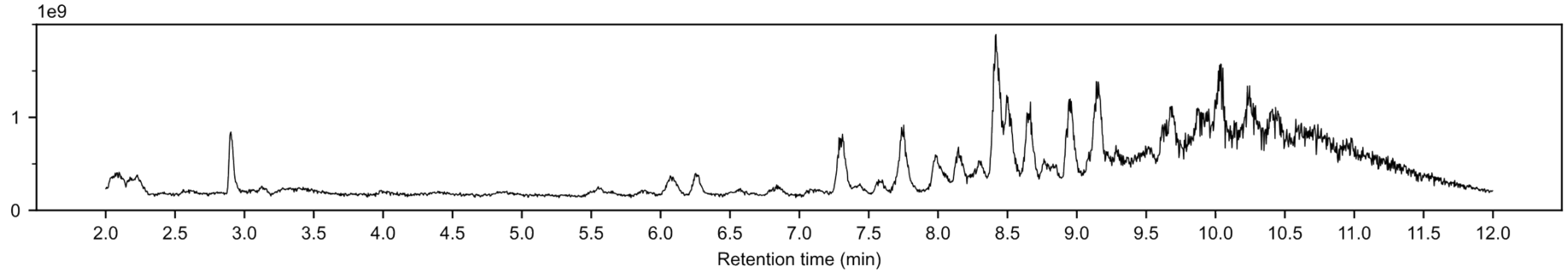
SN	Compound	Formula	Monoisotopic Mass	MS-DIAL score			RT / min	Rel. Abundance	
				[M+H]+	[M+2H]2+	[M+3H]3+		Mean	S.D
17	None-mA; (3s-4)-(NAG)(NAM)[r]-AemA	C47H80N10O25	1184.52961		73.3		7.6	0.14%	0.01%
18	None-Aem; (3s-3)-(NAG)(NAM)[r]-Aem	C49H82N10O27	1242.53509		MS1		8.8	5.16%	0.21%
19	(NAG)(NAM)-None; (G-G)-(NAG)(NAM)[r]-Aem	C53H88N8O32	1348.55046		69.7		7.5	0.15%	0.02%
20	(NAG)(NAM)-None; (G-G)-(NAG)(NAM)[r]-AemA	C56H93N9O33	1419.58758		64.9		8.3	0.21%	0.01%
21	(NAG)(anNAM)-Aem; (3s-3)-(NAG)(NAM)[r]-Aem	C68H110N12O38	1702.7044		67.5		10.2	0.19%	0.00%
22	(NAG)(NAM)[r]-Aem; (3s-3)-(NAG)(NAM)[r]-Aem	C68H114N12O39	1722.73061		73.2	MS1	8.4	0.49%	0.06%
23	(NAG)(anNAM)-AemA; (3s-3)-(NAG)(anNAM)-Aem	C71H111N13O38	1753.7153		63.2		11.4	0.05%	0.00%
24	(NAG)(anNAM)-Aem; (3s-4)-(NAG)(anNAM)-AemA	C71H111N13O38	1753.7153		MS1		12.0	0.03%	0.00%
25	(NAG)(NAM)[r]-Aem; (3s-4)-(NAG)(anNAM)-AemA	C71H115N13O39	1773.74151		66		9.4	0.99%	0.11%
26	(NAG)(anNAM)-Aem; (3s-4)-(NAG)(NAM)[r]-AemA	C71H115N13O39	1773.74151		65.8		9.6	1.18%	0.20%
27	(NAG)(anNAM)-AemA; (3s-3)-(NAG)(NAM)[r]-Aem	C71H115N13O39	1773.74151		65.9		9.8	2.19%	0.15%
28	(NAG)(NAM)-AemA; (G-G)-(NAG)(NAM)[r]-Aem	C71H117N13O40	1791.75208		76.9	71.4	8.2	7.77%	0.53%
29	(NAG)(NAM)[r]-Aem; (3s-4)-(NAG)(NAM)[r]-AemA	C71H119N13O40	1793.76773		69.7	69.5	8.7|8.8	2.63%	0.36%
30	(NAG)(anNAM)-AemA; (3s-4)-(NAG)(anNAM)-AemA	C74H116N14O39	1824.75241		MS1		12.4	0.03%	0.00%
31	(NAG)(NAM)[r]-AemG; (3s-4)-(NAG)(anNAM)-AemA	C73H118N14O40	1830.76297		MS1		9.2	0.55%	0.08%
32	(NAG)(anNAM)-AemG; (3s-4)-(NAG)(NAM)[r]-AemA	C73H118N14O40	1830.76297		67.3		9.4	0.55%	0.04%
33	(NAG)(anNAM)-AemA; (3s-4)-(NAG)(NAM)[r]-AemA	C74H120N14O40	1844.77862		63.7		10.0	8.99%	0.43%
34	(NAG)(NAM)[r]-AemA; (3s-4)-(NAG)(anNAM)-AemA	C74H120N14O40	1844.77862		64.5		9.8	6.47%	1.16%
35	(NAG)(NAM)-AemA; (G-G)-(NAG)(NAM)[r]-AemG	C73H120N14O41	1848.77354		72.1		8.4	1.54%	0.09%
36	(NAG)(NAM)-AemA; (G-G)-(NAG)(NAM)[r]-AemA	C74H122N14O41	1862.78919		77.8	74.2	8.8	17.36%	1.88%
37	(NAG)(NAM)[r]-AemA; (3s-4)-(NAG)(NAM)[r]-AemA	C74H124N14O41	1864.80484		65		9.1	5.26%	0.69%

SN	Compound	Formula	Monoisotopic Mass	MS-DIAL score			RT / min	Rel. Abundance	
				[M+H]+	[M+2H]2+	[M+3H]3+		Mean	S.D
38	(NAG)(NAM)[r]-AemA; (3s-4)-(NAG)(NAM)[r]-AemA; (3s-4)-(NAG)(anNAM)-AemA	C111H181N21O60	2768.17576			66.4	10.4|10.9	1.13%	0.37%

*Two stereoisomers of AemH suspected

Table S5

Representative TIC and identified peptidoglycan in *E faecium* COM15.



SN	Compound	Formula	Monoisotopic Mass	MS-DIAL score			RT / min	Rel. Abundance	
				[M+H]+	[M+2H]2+	[M+3H]3+		Mean	S.D
1	None-AqKA	C17H32N6O6	416.23833	68			2.7	0.01%	0.00%
2	(NAG)(anMUR)-None	C17H28N2O11	436.16931	74.4			5.8	0.01%	0.00%
3	(NAG)(MUR)-None	C17H30N2O12	454.17987	80.1			2.3|3.3	0.02%	0.00%
4	None-AqK[3-NH2-n]	C18H33N7O7	459.24415	67.9			2.9	0.10%	0.01%
5	None-AqK[3-NH2-d]	C18H32N6O8	460.22816	69.5			3.2	0.05%	0.00%
6	(NAG)(NAM)-None	C19H32N2O13	496.19044	80.7			4.9|6.1	2.27%	0.26%
7	None-AqKA[3-NH2-n]	C21H38N8O8	530.28126	MS1			3.6	0.03%	0.00%
8	None-AqKA[3-NH2-d]	C21H37N7O9	531.26528	69	63.9		5.5|5.6	0.09%	0.00%
9	(NAG)(NAM)-A	C22H37N3O14	567.22755	65.5			8.2|9.1	0.63%	0.03%
10	(NAG)(anMUR)-Aq	C25H41N5O14	635.265	63.5			7.3	0.04%	0.01%
11	(NAG)(MUR)-Aq	C25H43N5O15	653.27557	62.6			6.2|6.4	0.02%	0.00%
12	(NAG)(NAM)-Aq	C27H45N5O16	695.28613	MS1			7.6|8.5	1.36%	0.24%
13	(NAG)(NAM)-Ae	C27H44N4O17	696.27015	65.8			9.1	0.11%	0.02%
14	(NAG)(anMUR)-AqK	C31H53N7O15	763.35996	MS1	73.1		5.9	0.10%	0.01%
15	(NAG)(MUR)-AqK	C31H55N7O16	781.37053		71.2		4.9|5.1	0.05%	0.00%
16	(NAG)(NAM)-AqK	C33H57N7O17	823.38109	74.6	73.3		6.3|7.3	4.14%	0.31%

SN	Compound	Formula	Monoisotopic Mass	MS-DIAL score			RT / min	Rel. Abundance	
				[M+H]+	[M+2H]2+	[M+3H]3+		Mean	S.D
17	(NAG)(NAM)-AeK	C33H56N6O18	824.36511	77.8	72.8		6.8|7.7	0.35%	0.03%
18	(NAG)(MUR)-AqKA	C34H60N8O17	852.40764		67.3		6.0|7.2	0.03%	0.00%
19	(NAG)(anMUR)-AqK[3-NH2-n]	C35H59N9O17	877.40289	64	66.5		7.4	0.24%	0.02%
20	(NAG)(anMUR)-AqK[3-NH2-d]	C35H58N8O18	878.38691	69.7	70		7.9	0.12%	0.02%
21	(NAG)(NAM)-AqKG	C35H60N8O18	880.40256	69.7	66.4		6.5|7.6	0.09%	0.01%
22	(NAG)(NAM)-AqKA	C36H62N8O18	894.41821	69.8	67.4		7.3|8.2	2.63%	0.19%
23	(NAG)(NAM)-AeKA	C36H61N7O19	895.40222	76.2	71.3		7.8|8.5	0.26%	0.00%
24	(NAG)(MUR)-AqK[3-NH2-n]	C35H61N9O18	895.41346	MS1	68.9		6.9|7.0	0.15%	0.03%
25	(NAG)(MUR)-AqK[3-NH2-d]	C35H60N8O19	896.39747		72.3		7.4|7.5	0.06%	0.00%
26	(NAG)(NAM)-AqKS	C36H62N8O19	910.41312	62.7	MS1		6.8|7.7	0.04%	0.00%
27	(NAG)(anNAM)-AqK[3-NH2-n]	C37H61N9O18	919.41346	68.1	70.2		9.8	0.13%	0.02%
28	(NAG)(anNAM)-AqK[3-NH2-d]	C37H60N8O19	920.39747	69.4	MS1		10.1	0.06%	0.01%
29	(NAG)(NAM)-None; (G-G)-(NAG)(MUR)-None	C36H60N4O24	932.35975		69.3		7.3|8.2	0.24%	0.03%
30	(NAG)(NAM)-AqK[3-NH2-n]	C37H63N9O19	937.42402	70	71.8		7.7|8.4	13.22%	0.99%
31	(NAG)(NAM)-AqK[3-NH2-d]	C37H62N8O20	938.40804	72	75.4		8.0|8.7	5.61%	0.33%
32	(NAG)(NAM)-AeK[3-NH2-n]	C37H62N8O20	938.40804	69.4			8.8	0.41%	0.06%
33	(NAG)(NAM)-AeK[3-NH2-d]	C37H61N7O21	939.39205	73.8	78.6		8.4|9.0	0.33%	0.03%
34	(NAG)(anMUR)-AqKA[3-NH2-n]	C38H64N10O18	948.44001	MS1	73.5		8.0	0.10%	0.01%
35	(NAG)(anMUR)-AqKA[3-NH2-d]	C38H63N9O19	949.42402	MS1	70.7		8.5	0.15%	0.04%
36	(NAG)(NAM)-AqKAG	C38H65N9O19	951.43967	67.9	66.3		7.0|7.9	0.04%	0.00%
37	(NAG)(NAM)-AqKGA	C38H65N9O19	951.43967	64.9			7.5|8.2	0.02%	0.00%
38	(NAG)(NAM)-AqKAA	C39H67N9O19	965.45532	70.8	68		7.7|8.5	0.75%	0.03%
39	(NAG)(NAM)-AeKAA	C39H66N8O20	966.43934	69	67.1		8.1|8.8	0.07%	0.00%
40	(NAG)(MUR)-AqKA[3-NH2-n]	C38H66N10O19	966.45057		69.6		7.4	0.05%	0.00%
41	(NAG)(MUR)-AqKA[3-NH2-d]	C38H65N9O20	967.43459		73.9		8.0	0.08%	0.01%
42	(NAG)(NAM)-None; (G-G)-(NAG)(NAM)-None	C38H62N4O25	974.37031	66.2	80.6		7.2|8.0	0.90%	0.27%
43	(NAG)(NAM)-AqKG[3-NH2-n]	C39H66N10O20	994.44548	65.7	67.5		7.6|8.4	0.08%	0.00%

SN	Compound	Formula	Monoisotopic Mass	MS-DIAL score			RT / min	Rel. Abundance	
				[M+H]+	[M+2H]2+	[M+3H]3+		Mean	S.D
44	(NAG)(NAM)-AqKG[3-NH2-d]	C39H65N9O21	995.4295	67.4	74.2		7.8|8.6	0.10%	0.01%
45	(NAG)(NAM)-AqKA[3-NH2-n]	C40H68N10O20	1008.4611	70.6	69.4		8.3|8.9	6.00%	0.45%
46	(NAG)(NAM)-AqKA[3-NH2-d]	C40H67N9O21	1009.4452	70	75.1		8.5	1.79%	0.27%
47	(NAG)(NAM)-AeKA[3-NH2-n]	C40H67N9O21	1009.4452	70.7	72.2		9.1	6.35%	0.70%
48	(NAG)(NAM)-AeKA[3-NH2-d]	C40H66N8O22	1010.4292	72.8	71.8		8.8|9.4	0.42%	0.04%
49	(NAG)(NAM)-AqKS[3-NH2-n]	C40H68N10O21	1024.4561	MS1	MS1		7.2|8.0	0.37%	0.02%
50	(NAG)(NAM)-AqKS[3-NH2-d]	C40H67N9O22	1025.4401	MS1	MS1		7.4|8.2	0.51%	0.04%
51	(NAG)(NAM)-AqKAG[3-NH2-n]	C42H71N11O21	1065.4826	66.6	68.4		7.9|8.6	0.11%	0.01%
52	(NAG)(NAM)-AqKAG[3-NH2-d]	C42H70N10O22	1066.4666	64.8	70.6		8.1|8.8	0.13%	0.02%
53	(NAG)(NAM)-AqKAA[3-NH2-n]	C43H73N11O21	1079.4983	69.6	72.4		8.5|9.1	1.80%	0.05%
54	(NAG)(NAM)-AqKAA[3-NH2-d]	C43H72N10O22	1080.4823	66.3	76		8.7|9.3	1.60%	0.22%
55	(NAG)(NAM)-AeKAA[3-NH2-d]	C43H71N9O23	1081.4663	68.5	73.1		9.0|9.6	0.11%	0.02%
56	(NAG)(MUR)-None; (G-G)-(NAG)(anNAM)-AqK	C50H83N9O27	1241.5398	66.6	71.9		9.2	0.11%	0.02%
57	None-K[3-NH2-n]; (3br-4)-(NAG)(NAM)-AqKA[3-NH2-n]	C50H86N14O23	1250.599		68.1		7.3	0.13%	0.02%
58	(NAG)(NAM)-AqK[3-NH2-n]; (3br-4)-None-KA[3-NH2-n]	C50H86N14O23	1250.599	65.1	66.3		7.7|8.3	4.08%	0.52%
59	(NAG)(NAM)-AqK[3-NH2-n]; (3br-4)-None-KA[3-NH2-d]	C50H85N13O24	1251.583		68.9		8.4|8.9	1.24%	0.21%
60	(NAG)(MUR)-None; (G-G)-(NAG)(NAM)-AqK	C50H85N9O28	1259.5504		73.8		6.2|7.0	0.23%	0.04%
61	(NAG)(NAM)-AqK; (G-G)-(NAG)(NAM)-None	C52H87N9O29	1301.561		70.7		7.4|8.1	0.49%	0.06%
62	None-KA[3-NH2-n]; (3br-4)-(NAG)(NAM)-AqKA[3-NH2-n]	C53H91N15O24	1321.6361		68		8.1|8.6	0.80%	0.07%
63	(NAG)(NAM)-AqKA[3-NH2-n]; (3br-4)-None-KA[3-NH2-d]	C53H90N14O25	1322.6202		65.4		8.9|9.3	0.56%	0.05%
64	(NAG)(NAM)-AqKA[3-NH2-d]; (3br-4)-None-KA[3-NH2-d]	C53H89N13O26	1323.6042		70.4		9.6	0.09%	0.01%
65	(NAG)(NAM)-AqKA; (G-G)-(NAG)(MUR)-None	C53H90N10O29	1330.5875		72		7.0|7.6	0.04%	0.01%
66	None-AqK[3-NH2-n]; (3br-4)-(NAG)(NAM)-AqKA	C54H93N15O24	1335.6518		65.1		7.5|8.0	0.26%	0.02%
67	(NAG)(NAM)-AqK[3-NH2-n]; (3br-4)-None-AqKA	C54H93N15O24	1335.6518		68.4		7.7|8.2	0.16%	0.02%

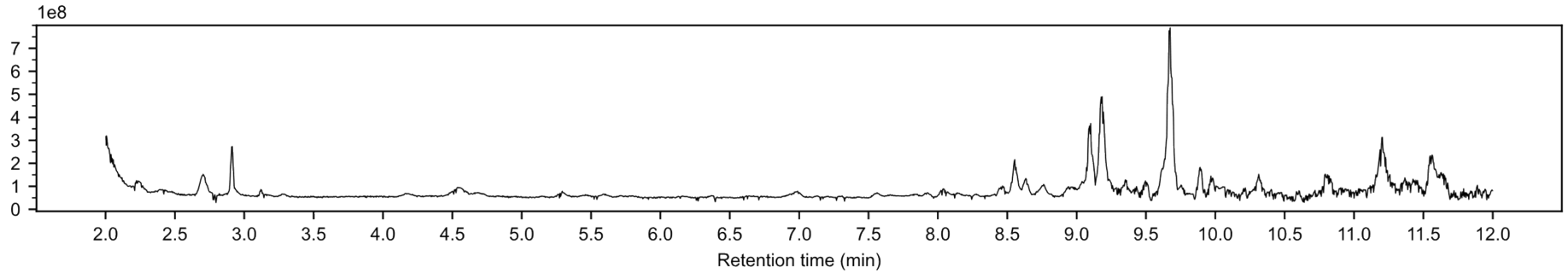
SN	Compound	Formula	Monoisotopic Mass	MS-DIAL score			RT / min	Rel. Abundance	
				[M+H]+	[M+2H]2+	[M+3H]3+		Mean	S.D
68	(NAG)(MUR)-None; (G-G)-(NAG)(anNAM)-AqK[3-NH2-n]	C54H89N11O29	1355.5828	66.6	70.2		9.3|9.8	0.30%	0.03%
69	(NAG)(NAM)-AqKA; (G-G)-(NAG)(NAM)-None	C55H92N10O30	1372.5981		71.1		8.1|8.7	0.11%	0.02%
70	(NAG)(NAM)-AqK[3-NH2-n]; (G-G)-(NAG)(MUR)-None	C54H91N11O30	1373.5933		72.4		7.4|7.9	0.49%	0.14%
71	None-AqK[3-NH2-n]; (3br-3)-(NAG)(NAM)-AqK[3-NH2-n]	C55H94N16O25	1378.6576		67.5		7.8|8.3	0.22%	0.01%
72	(NAG)(NAM)-AqKAA[3-NH2-n]; (3br-4)-None-KA[3-NH2-n]	C56H96N16O25	1392.6733		61.2		8.7	0.07%	0.01%
73	None-AqKA[3-NH2-n]; (3br-4)-(NAG)(NAM)-AqKA	C57H98N16O25	1406.6889		60.9		8.0|8.5	0.09%	0.00%
74	(NAG)(NAM)-AqK[3-NH2-n]; (G-G)-(NAG)(NAM)-None	C56H93N11O31	1415.6039	63.9	69.8		8.3|8.8	0.68%	0.10%
75	(NAG)(NAM)-AqKA[3-NH2-d]; (G-G)-(NAG)(MUR)-None	C57H95N11O32	1445.6145		69.5		8.1|8.6	0.11%	0.01%
76	None-AqK[3-NH2-n]; (3br-4)-(NAG)(NAM)-AqKA[3-NH2-n]	C58H99N17O26	1449.6947		69.3		8.1|8.5	2.01%	0.19%
77	(NAG)(NAM)-AqK[3-NH2-n]; (3br-4)-None-AqKA[3-NH2-d]	C58H98N16O27	1450.6787		64.2		9.1	0.22%	0.01%
78	(NAG)(NAM)-AqKA[3-NH2-n]; (G-G)-(NAG)(NAM)-None	C59H98N12O32	1486.641		71.6		8.8|9.3	0.17%	0.02%
79	(NAG)(NAM)-AqKA[3-NH2-d]; (G-G)-(NAG)(NAM)-None	C59H97N11O33	1487.625	MS1	72.4		9.0|9.5	0.32%	0.08%
80	(NAG)(NAM)-AqKA[3-NH2-n]; (3br-4)-None-AqKA[3-NH2-n]	C61H104N18O27	1520.7318		67.2		8.6|9.0	0.54%	0.09%
81	None-AqKA[3-NH2-d]; (3br-4)-(NAG)(NAM)-AqKA[3-NH2-n]	C61H103N17O28	1521.7159		66.1		9.3	0.20%	0.01%
82	None-AqKA[3-NH2-n]; (3br-4)-(NAG)(NAM)-AqKA[3-NH2-d]	C61H103N17O28	1521.7159		68.4		9.4	0.17%	0.01%
83	(NAG)(NAM)-AqK; (G-G)-(NAG)(NAM)-AqK	C66H112N14O33	1628.7516		72	MS1	7.4|7.8|7.9	0.14%	0.00%
84	(NAG)(NAM)-AqK; (G-G)-(NAG)(NAM)-AqKA	C69H117N15O34	1699.7887		67.7		7.8|8.3	0.09%	0.00%
85	(NAG)(NAM)-AqK[3-NH2-n]; (G-G)-(NAG)(NAM)-AqK	C70H118N16O35	1742.7946		72.4		8.0|8.4	0.53%	0.01%
86	(NAG)(NAM)-AqK[3-NH2-n]; (3br-3)-(NAG)(NAM)-AqK	C70H118N16O35	1742.7946		67		9.0|9.4	0.09%	0.01%
87	(NAG)(NAM)-AqK; (G-G)-(NAG)(NAM)-AqKA[3-NH2-n]	C73H123N17O36	1813.8317		69.7		8.3|8.7	0.28%	0.01%
88	(NAG)(NAM)-AqK[3-NH2-n]; (3br-4)-(NAG)(NAM)-AqKA	C73H123N17O36	1813.8317		68.4	66.7	9.4|9.7	1.18%	0.11%
89	(NAG)(NAM)-AqKA[3-NH2-d]; (G-G)-(NAG)(NAM)-AqK	C73H122N16O37	1814.8157		74.6	68.9	9.0	0.51%	0.07%

SN	Compound	Formula	Monoisotopic Mass	MS-DIAL score			RT / min	Rel. Abundance	
				[M+H]+	[M+2H]2+	[M+3H]3+		Mean	S.D
90	(NAG)(NAM)-AqKA[3-NH2-d]; (3br-3)-(NAG)(NAM)-AqK	C73H122N16O37	1814.8157		60.3		9.6|9.9	0.47%	0.00%
91	(NAG)(NAM)-AqK[3-NH2-n]; (G-G)-(NAG)(NAM)-AqK[3-NH2-n]	C74H124N18O37	1856.8375		68.9	68.3	8.6|8.9|9.0	1.83%	0.16%
92	(NAG)(NAM)-AqK[3-NH2-n]; (3br-3)-(NAG)(NAM)-AqK[3-NH2-n]	C74H124N18O37	1856.8375		62.2		9.5|9.8	0.30%	0.04%
93	(NAG)(NAM)-AqKA[3-NH2-n]; (G-G)-(NAG)(NAM)-AqKA	C76H128N18O37	1884.8688		72.6		9.1|9.3	0.15%	0.05%
94	(NAG)(NAM)-AqKA[3-NH2-n]; (3br-4)-(NAG)(NAM)-AqKA	C76H128N18O37	1884.8688		68.5	MS1	9.7|10.0	0.43%	0.05%
95	(NAG)(NAM)-AqKA[3-NH2-d]; (3br-4)-(NAG)(NAM)-AqKA	C76H127N17O38	1885.8528		68.9	MS1	10.2	0.20%	0.02%
96	(NAG)(NAM)-AqKA[3-NH2-n]; (G-G)-(NAG)(NAM)-AqK[3-NH2-n]	C77H129N19O38	1927.8746		66.4	71.9	8.8|9.2|9.3	1.32%	0.08%
97	(NAG)(NAM)-AqK[3-NH2-n]; (3br-4)-(NAG)(NAM)-AqKA[3-NH2-n]	C77H129N19O38	1927.8746		67.6	72	9.7|10.0	7.55%	0.47%
98	(NAG)(NAM)-AqKA[3-NH2-d]; (G-G)-(NAG)(NAM)-AqK[3-NH2-n]	C77H128N18O39	1928.8586		74.2	73.7	9.5	1.90%	0.14%
99	(NAG)(NAM)-AqK[3-NH2-n]; (3br-4)-(NAG)(NAM)-AqKA[3-NH2-d]	C77H128N18O39	1928.8586		67.8	69.1	9.9|10.2	5.86%	0.27%
100	(NAG)(NAM)-AqKA[3-NH2-n]; (3br-4)-(NAG)(NAM)-AqKA[3-NH2-n]	C80H134N20O39	1998.9117		67.9	68.5	10.0|10.4	2.50%	0.22%
101	(NAG)(NAM)-AqKA[3-NH2-n]; (G-G)-(NAG)(NAM)-AqKA[3-NH2-n]	C80H134N20O39	1998.9117		72	70.3	9.1|9.2|9.5	0.26%	0.01%
102	(NAG)(NAM)-AqKA[3-NH2-n]; (3br-4)-(NAG)(NAM)-AqKA[3-NH2-d]	C80H133N19O40	1999.8957			69.3	10.2	0.21%	0.03%
103	(NAG)(NAM)-AqKA[3-NH2-d]; (3br-4)-(NAG)(NAM)-AqKA[3-NH2-n]	C80H133N19O40	1999.8957		65.7		10.2	1.11%	0.11%
104	(NAG)(NAM)-AeKA[3-NH2-n]; (3br-4)-(NAG)(NAM)-AqKA[3-NH2-n]	C80H133N19O40	1999.8957		66.1	69.5	10.6	1.74%	0.12%
105	(NAG)(NAM)-AqKA[3-NH2-n]; (G-G)-(NAG)(NAM)-AqKA[3-NH2-d]	C80H133N19O40	1999.8957		75.2	76.5	9.8	0.73%	0.07%
106	(NAG)(NAM)-AqKA[3-NH2-d]; (3br-4)-(NAG)(NAM)-AqKA[3-NH2-d]	C80H132N18O41	2000.8797		65.7		10.8	0.52%	0.08%
107	(NAG)(NAM)-AqKAA[3-NH2-n]; (3br-4)-(NAG)(NAM)-AqKA[3-NH2-n]	C83H139N21O40	2069.9488		68.3		10.1|10.4	0.40%	0.01%
108	(NAG)(NAM)-AqKAA[3-NH2-n]; (G-G)-(NAG)(NAM)-AqKA[3-NH2-n]	C83H139N21O40	2069.9488		71.9		9.5	0.07%	0.00%
109	(NAG)(NAM)-AqKAA[3-NH2-d]; (3br-4)-(NAG)(NAM)-AqKA[3-NH2-n]	C83H138N20O41	2070.9328		66.5	66.4	10.3|10.6	0.48%	0.06%
110	(NAG)(NAM)-AqKAA[3-NH2-n]; (G-G)-(NAG)(NAM)-AqKA[3-NH2-d]	C83H138N20O41	2070.9328		71.1		9.8	0.14%	0.02%
111	(NAG)(NAM)-AqKAA[3-NH2-d]; (3br-4)-(NAG)(NAM)-AqKA[3-NH2-d]	C83H137N19O42	2071.9169		62.7		10.8	0.07%	0.01%

SN	Compound	Formula	Monoisotopic Mass	MS-DIAL score			RT / min	Rel. Abundance	
				[M+H]+	[M+2H]2+	[M+3H]3+		Mean	S.D
112	(NAG)(NAM)-AqKA[3-NH2-n]; (3br-4)-(NAG)(NAM)-AqKA[3-NH2-n]; (3br-4)-(NAG)(NAM)-AqKA[3-NH2-n]	C120H200N30O58	2989.3623		MS1	MS1	10.8|11.1	0.48%	0.07%
113	(NAG)(NAM)-AqKAA[3-NH2-n]; (3br-4)-(NAG)(NAM)-AqKA[3-NH2-n]; (3br-4)-(NAG)(NAM)-AqKA[3-NH2-n]	C123H205N31O59	3060.3994			MS1	10.8|11.1	0.05%	0.00%

Table S6

Representative TIC and identified peptidoglycan in *E. faecalis* OG1RF.

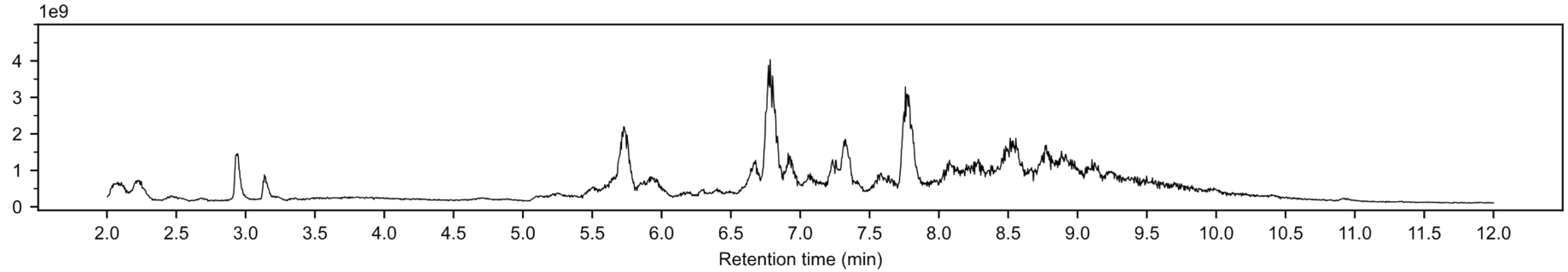


SN	Compound	Formula	Monoisotopic Mass	MS-DIAL score			RT / min	Rel. Abundance	
				[M+H]+	[M+2H]2+	[M+3H]3+		Mean	S.D
1	None-K[3-NH2-AA]	C12H24N4O4	288.17976	71.1			2.3	0.03%	0.01%
2	(NAG)(NAM)-None	C19H32N2O13	496.19044	80.5			6.2|7.0	0.23%	0.01%
3	(NAG)(NAM)-A	C22H37N3O14	567.22755	71.5			8.2|9.2	1.19%	0.07%
4	None-AqKAA[3-NH2-AA]	C26H47N9O9	629.34967		MS1		6.4	0.32%	0.18%
5	(NAG)(NAM)-Aq	C27H45N5O16	695.28613	60.3			7.6|8.5	0.67%	0.14%
6	(NAG)(NAM)-Ae	C27H44N4O17	696.27015	66.6			9.1	0.07%	0.01%
7	(NAG)(NAM)-AqK	C33H57N7O17	823.38109	73.4	71.8		6.3|7.3	0.17%	0.03%
8	(NAG)(NAM)-AqKA	C36H62N8O18	894.41821	70	71		8.1|8.8	1.73%	0.18%
9	(NAG)(NAM)-AeK[3-NH2-A]	C36H61N7O19	895.40222	77.3	76.9		8.5|9.2	0.52%	0.09%
10	(NAG)(MUR)-AqK[3-NH2-AA]	C37H65N9O18	923.44476		71.1		7.7|8.3	0.09%	0.00%
11	(NAG)(MUR)-AeK[3-NH2-AA]	C37H64N8O19	924.42877		73		8.2|8.5	0.08%	0.02%
12	(NAG)(NAM)-AqKAA	C39H67N9O19	965.45532	67.1	67.1		7.7|8.4	0.48%	0.12%
13	(NAG)(NAM)-AqK[3-NH2-AA]	C39H67N9O19	965.45532	73.8	76.7		8.6|9.2	14.27%	0.86%
14	(NAG)(NAM)-AeKAA	C39H66N8O20	966.43934		72.7		8.1	0.02%	0.02%
15	(NAG)(NAM)-AeK[3-NH2-AA]	C39H66N8O20	966.43934	75.2	78.1		9.0|9.5	3.03%	0.41%
16	(NAG)(NAM)-AqKAA[3-NH2-A]	C42H72N10O20	1036.49243	67.7	76.2		8.7|9.4	2.66%	0.15%
17	(NAG)(NAM)-AqKA[3-NH2-AA]	C42H72N10O20	1036.49243	MS1	72.4		9.1|9.6	5.10%	0.33%

SN	Compound	Formula	Monoisotopic Mass	MS-DIAL score			RT / min	Rel. Abundance	
				[M+H]+	[M+2H]2+	[M+3H]3+		Mean	S.D
18	(NAG)(NAM)-AeKAA[3-NH2-A]	C42H71N9O21	1037.47645		71.5		9.7	0.19%	0.01%
19	(NAG)(NAM)-AeKA[3-NH2-AA]	C42H71N9O21	1037.47645	71.3	77		9.9	0.48%	0.04%
20	(NAG)(MUR)-AqKAA[3-NH2-AA]	C43H75N11O20	1065.51898		63.5		8.3|8.8	0.16%	0.01%
21	(NAG)(MUR)-AeKAA[3-NH2-AA]	C43H74N10O21	1066.503		68.8		8.7|9.0	0.05%	0.00%
22	(NAG)(anNAM)-AqKAA[3-NH2-AA]	C45H75N11O20	1089.51898	MS1	73.2		11.1	0.15%	0.05%
23	(NAG)(NAM)-AqKAG[3-NH2-AA]	C44H75N11O21	1093.5139	MS1	73.9		8.6|9.2	0.52%	0.05%
24	(NAG)(NAM)-AqKAK[3-NH2-A]	C45H79N11O20	1093.55028		74.8		7.7|8.3	0.30%	0.01%
25	(NAG)(NAM)-AqKAA[3-NH2-AA]	C45H77N11O21	1107.52955	65.1	74.2		9.1|9.7	35.00%	1.16%
26	(NAG)(NAM)-AeKAA[3-NH2-AA]	C45H76N10O22	1108.51356	68	75.7		9.4|10.0	4.22%	0.30%
27	(NAG)(NAM)-AqKAK[3-NH2-AA]	C48H84N12O21	1164.5874	69.2	73.8		8.1|8.7	4.08%	0.22%
28	(NAG)(NAM)-AeKAK[3-NH2-AA]	C48H83N11O22	1165.57141		72.3		8.4|9.0	1.52%	0.38%
29	(NAG)(NAM)-AqKAH[3-NH2-AA]	C48H79N13O21	1173.55135		70.9		7.7|8.3	0.07%	0.01%
30	(NAG)(NAM)-AqK[3-NH2-AA]; (3br-4)-None-AqKA[3-NH2-AA]	C62H107N17O26	1505.75732		68.1		9.7	0.35%	0.02%
31	(NAG)(NAM)-AqKAA[3-NH2-AA]; (3br-4)-None-AqKA[3-NH2-AA]	C68H117N19O28	1647.83154		71.5		10.0	0.15%	0.01%
32	(NAG)(NAM)-AqK[3-NH2-AA]; (G-G)-(NAG)(NAM)-AqK[3-NH2-AA]	C78H132N18O37	1912.90008		76.7	74	9.4|9.8	1.80%	0.18%
33	(NAG)(NAM)-AqK[3-NH2-AA]; (3br-4)-(NAG)(NAM)-AqKA[3-NH2-AA]	C81H137N19O38	1983.93719		68	68.1	10.8|11.2	6.34%	0.48%
34	(NAG)(NAM)-AeKAA[3-NH2-AA]; (3br-4)-(NAG)(NAM)-AeKA	C81H135N17O40	1985.90522		MS1		11.8	0.16%	0.05%
35	(NAG)(NAM)-AqKA[3-NH2-AA]; (3br-4)-(NAG)(NAM)-AqKA[3-NH2-AA]	C84H142N20O39	2054.9743		MS1		11.2|11.6	0.57%	0.05%
36	(NAG)(NAM)-AqKAA[3-NH2-AA]; (G-G)-(NAG)(NAM)-AqK[3-NH2-AA]	C84H142N20O39	2054.9743		75.8		9.7|10.1	0.80%	0.08%
37	(NAG)(NAM)-AqKAA[3-NH2-AA]; (G-G)-(NAG)(NAM)-AqKA[3-NH2-AA]	C87H147N21O40	2126.01142		62.3	73.3	10.0|10.3	1.03%	0.28%
38	(NAG)(NAM)-AqKAA[3-NH2-AA]; (3br-4)-(NAG)(NAM)-AqKA[3-NH2-AA]	C87H147N21O40	2126.01142		66.3	60.2	11.2|11.6	7.87%	1.01%
39	(NAG)(NAM)-AeKAA[3-NH2-AA]; (3br-4)-(NAG)(NAM)-AqKA[3-NH2-AA]	C87H146N20O41	2126.99543		62.8		11.8	0.57%	0.14%
40	(NAG)(NAM)-AqKAA[3-NH2-AA]; (G-G)-(NAG)(NAM)-AqKAA[3-NH2-AA]	C90H152N22O41	2197.04853		66.2	71.8	10.0|10.3	2.43%	0.08%
41	(NAG)(NAM)-AqKAA[3-NH2-AA]; (G-G)-(NAG)(NAM)-AeKAA[3-NH2-AA]	C90H151N21O42	2198.03255			70.2	10.6	0.51%	0.08%

Table S7

Representative TIC and identified peptidoglycan in *L. plantarum* BAA-793.



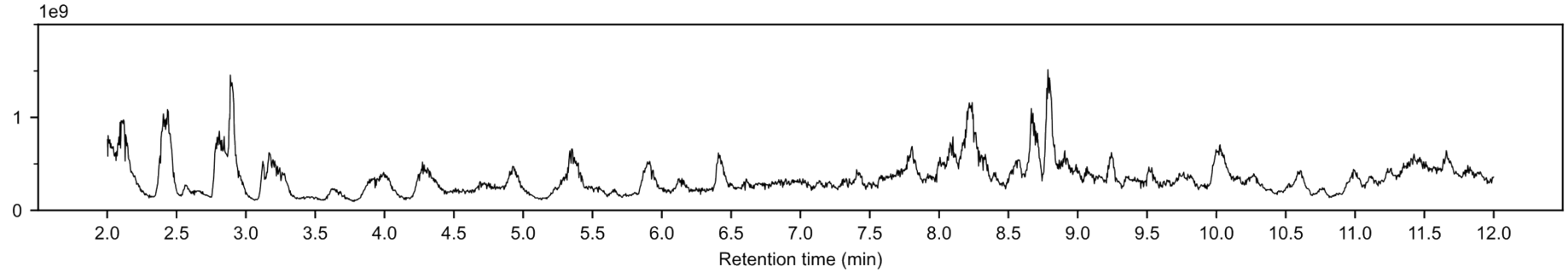
SN	Compound	Formula	Monoisotopic Mass	MS-DIAL score			RT / min	Rel. Abundance	
				[M+H]+	[M+2H]2+	[M+3H]3+		Mean	S.D
1	(NAG)(MUR)-None	C17H30N2O12	454.17987	77.8			2.4	0.01%	0.00%
2	None-Aqm(NH2)A	C18H33N7O7	459.24415	60.1			2.2	0.01%	0.00%
3	(NAG)(NAM)-None	C19H32N2O13	496.19044	80.6			5.1|6.2	0.81%	0.08%
4	(DAG)(NAM)-None	C21H34N2O14	538.201	69.9			10.0	0.02%	0.00%
5	(NAG)(anNAM)-A	C22H35N3O13	549.21699	75.8			11.1	0.01%	0.00%
6	(NAG)(NAM)-A	C22H37N3O14	567.22755	72.3			8.1|9.1	0.78%	0.03%
7	(DAG)(NAM)-A	C24H39N3O15	609.23812	65			10.5|11.9	0.02%	0.00%
8	(NAG)(MUR)-Aq	C25H43N5O15	653.27557	64			6.2|6.4	0.04%	0.00%
9	(NAM)-Aqm(NH2)	C26H45N7O13	663.30753	70.4			3.2	0.13%	0.01%
10	(NAM)-Aem(NH2)	C26H44N6O14	664.29155	77.2			5.0	0.01%	0.00%
11	(NAG)(anNAM)-Aq	C27H43N5O15	677.27557	61			10.4	0.07%	0.01%
12	(NAG)(anNAM)-Ae	C27H42N4O16	678.25958	64.5			11.0	0.01%	0.00%
13	(NAG)(NAM)-Aq	C27H45N5O16	695.28613	64.6			7.6|8.5	2.93%	0.26%
14	(NAG)(NAM)-Ae	C27H44N4O17	696.27015	70.7			9.1	0.21%	0.01%
15	(NAM)-Aqm(NH2)A	C29H50N8O14	734.34465	63.4			6.0	0.03%	0.00%
16	(NAG)(DAM)-Aq	C29H47N5O17	737.2967	MS1			9.3	0.10%	0.01%

SN	Compound	Formula	Monoisotopic Mass	MS-DIAL score			RT / min	Rel. Abundance	
				[M+H]+	[M+2H]2+	[M+3H]3+		Mean	S.D
17	(DAG)(NAM)-Aq	C29H47N5O17	737.2967	MS1			9.7|10.9	0.35%	0.03%
18	(DAG)(NAM)-Ae	C29H46N4O18	738.28071	61.4			10.5|11.6	0.02%	0.00%
19	(NAG)(DAM)-Ae	C29H46N4O18	738.28071	MS1			9.8|10.3	0.03%	0.00%
20	(NAG)(MUR)-Aqm(NH2)	C32H56N8O17	824.37634	69.9	73.4		3.2	0.27%	0.01%
21	(NAG)(anNAM)-Aqm(NH2)	C34H56N8O17	848.37634	73.5	78.3		8.6	0.30%	0.00%
22	(NAG)(anNAM)-Aem(NH2)	C34H55N7O18	849.36036	79.3			8.8	0.02%	0.00%
23	(NAG)(NAM)-Aqm(NH2)	C34H58N8O18	866.38691	74.1	78.7		5.7|6.8	21.93%	0.84%
24	(NAG)(NAM)-Aem(NH2)	C34H57N7O19	867.37092	76.3	81.5		6.3|7.2	1.84%	0.06%
25	(NAG)(MUR)-Aqm(NH2)A	C35H61N9O18	895.41346	MS1	66.6		5.5	0.14%	0.01%
26	(NAG)(DAM)-Aqm(NH2)	C36H60N8O19	908.39747	68.8	70.6		7.7|8.1	0.91%	0.17%
27	(DAG)(NAM)-Aqm(NH2)	C36H60N8O19	908.39747	70.3	73.9		7.8|9.0	0.91%	0.09%
28	(DAG)(NAM)-Aem(NH2)	C36H59N7O20	909.38149	74.5	74.3		8.3|9.4	0.10%	0.01%
29	(NAG)(anNAM)-Aqm(NH2)A	C37H61N9O18	919.41346	67.6	68.5		9.5	0.19%	0.00%
30	(NAG)(NAM)-Aqm(NH2)G	C36H61N9O19	923.40837	68.6	70.8		6.2|7.2	0.13%	0.01%
31	(NAG)(NAM)-Aqm(NH2)A	C37H63N9O19	937.42402	69.2	71.5		6.9|7.8	12.67%	0.41%
32	(NAG)(NAM)-Aem(NH2)A	C37H62N8O20	938.40804	74	75.2		7.4|8.1	0.97%	0.09%
33	(NAG)(NAM)-AqmA	C37H62N8O20	938.40804	66.6	70.7		8.0|8.4	0.71%	0.06%
34	(NAG)(NAM)-AemA	C37H61N7O21	939.39205	MS1			7.7	0.01%	0.00%
35	(DAG)(DAM)-Aqm(NH2)	C38H62N8O20	950.40804	62.9	71.3		10.0	0.02%	0.00%
36	(NAG)(DAM)-Aqm(NH2)A	C39H65N9O20	979.43459	65.4	69.8		8.6|9.0	0.35%	0.06%
37	(DAG)(NAM)-Aqm(NH2)A	C39H65N9O20	979.43459	66.8	65.6		9.7	0.11%	0.02%
38	(DAG)(NAM)-AqmA	C39H64N8O21	980.4186	62.6			9.9	0.01%	0.00%
39	(NAG)(NAM)-Aqm(NH2)AA	C40H68N10O20	1008.46113	66	65.3		8.2	0.07%	0.01%
40	(NAG)(NAM)-Aqm(NH2)ALac	C40H67N9O21	1009.44515	65.5	69.6		8.8|9.5	0.71%	0.06%
41	(NAG)(NAM)-Aem(NH2)ALac	C40H66N8O22	1010.42917	64	66.7		9.8	0.04%	0.01%
42	(DAG)(NAM)-Aqm(NH2)ALac	C42H69N9O22	1051.45571	MS1			8.3	0.08%	0.00%
43	(NAG)(NAM)-Aqm(NH2); (3s-4)-None-m(NH2)A	C44H76N12O21	1108.5248	71.4	73.2		5.7|6.7	15.51%	0.75%

SN	Compound	Formula	Monoisotopic Mass	MS-DIAL score			RT / min	Rel. Abundance	
				[M+H]+	[M+2H]2+	[M+3H]3+		Mean	S.D
44	None-m(NH2)A; (3s-4)-(NAG)(NAM)-Aqm(NH2)A	C47H81N13O22	1179.56191		70.7		5.8|6.7	0.49%	0.01%
45	(NAG)(NAM)-Aqm(NH2)A; (3s-4)-None-m(NH2)A	C47H81N13O22	1179.56191	68.3	68.9		6.4|7.3	6.72%	0.46%
46	(NAG)(NAM)-Aem(NH2)A; (3s-4)-None-m(NH2)A	C47H80N12O23	1180.54593		69.6		7.7	0.66%	0.04%
47	(NAG)(NAM)-Aqm(NH2); (3s-3)-None-Aqm(NH2)	C49H84N14O23	1236.58337		67.4		6.0|6.9	0.39%	0.02%
48	None-Aqm(NH2)A; (3s-4)-(NAG)(NAM)-Aqm(NH2)A	C55H94N16O25	1378.6576		66.8		6.7|7.3	0.16%	0.02%
49	(NAG)(NAM)-Aqm(NH2)A; (3s-4)-None-Aqm(NH2)A	C55H94N16O25	1378.6576		69.7		6.9|7.5	0.16%	0.02%
50	(NAG)(NAM)-Aqm(NH2); (G-G)-(NAG)(NAM)-Aqm(NH2)	C68H114N16O35	1714.76325		75.3	75.8	6.8|7.3	9.95%	0.94%
51	(NAG)(NAM)-Aqm(NH2); (3s-3)-(NAG)(NAM)-Aqm(NH2)	C68H114N16O35	1714.76325		69.8		8.2|8.7	0.16%	0.00%
52	(NAG)(MUR)-Aqm(NH2)A; (G-G)-(NAG)(NAM)-Aqm(NH2)	C69H117N17O35	1743.7898		63.9		8.0	0.05%	0.00%
53	(NAG)(NAM)-Aqm(NH2)A; (G-G)-(NAG)(NAM)-Aqm(NH2)	C71H119N17O36	1785.80036		76.2	75.5	7.3|7.8	5.07%	0.19%
54	(NAG)(NAM)-Aqm(NH2); (3s-4)-(NAG)(NAM)-Aqm(NH2)A	C71H119N17O36	1785.80036		70.6	68.2	8.5|8.9	5.21%	0.27%
55	(NAG)(NAM)-Aqm(NH2); (3s-4)-(NAG)(NAM)-Aem(NH2)A	C71H118N16O37	1786.78438		69.6		9.1	0.65%	0.04%
56	(NAG)(DAM)-Aqm(NH2)A; (G-G)-(NAG)(NAM)-Aqm(NH2)	C73H121N17O37	1827.81093		73.5	75.8	7.9|8.2	0.74%	0.10%
57	(NAG)(NAM)-Aqm(NH2); (3s-4)-(DAG)(NAM)-Aqm(NH2)A	C73H121N17O37	1827.81093		64.5		9.4|9.8|10.1	0.30%	0.09%
58	(NAG)(NAM)-Aqm(NH2)A; (G-G)-(NAG)(NAM)-Aqm(NH2)A	C74H124N18O37	1856.83748		70	72.7	7.8|8.2	2.85%	0.10%
59	(NAG)(NAM)-Aqm(NH2)A; (3s-4)-(NAG)(NAM)-Aqm(NH2)A	C74H124N18O37	1856.83748		63.1	69.8	8.8|9.2	1.90%	0.11%
60	(NAG)(NAM)-Aqm(NH2)A; (3s-4)-(NAG)(NAM)-Aem(NH2)A	C74H123N17O38	1857.82149		60.1		9.4	0.23%	0.01%
61	(NAG)(NAM)-AqmA; (3s-4)-(NAG)(NAM)-Aqm(NH2)A	C74H123N17O38	1857.82149		62.5		9.6|9.8	0.13%	0.01%
62	(NAG)(DAM)-Aqm(NH2)A; (G-G)-(NAG)(NAM)-Aqm(NH2)A	C76H126N18O38	1898.84804		70.5		8.3|8.6	0.28%	0.03%
63	(DAG)(NAM)-Aqm(NH2)A; (3s-4)-(NAG)(NAM)-Aqm(NH2)A	C76H126N18O38	1898.84804		63.9		9.7|10.4	0.08%	0.01%
64	(NAG)(NAM)-Aqm(NH2)ALac; (G-G)-(NAG)(NAM)-Aqm(NH2)ALac	C80H132N18O41	2000.87974		64.6	65.1	9.9|10.2	0.23%	0.03%

Table S8

Representative TIC and identified peptidoglycan in *S. aureus* MW2 and RN450.



SN	Compound	Formula	Monoisotopic Mass	MS-DIAL score			RT / min	MW2 Rel. Abundance		RN450 Rel. Abundance	
				[M+H]+	[M+2H]2+	[M+3H]3+		Mean	S.D	Mean	S.D.
1	(NAM)-None	C11H19NO8	293.11107	88.5			3.4|5.6	0.25%	0.23%	0.01%	0.19%
2	(anNAM)-A	C14H22N2O8	346.13762	MS1			10.5	0.01%	0.01%	0.01%	0.00%
3	(NAM)-A	C14H24N2O9	364.14818	66.9			5.8|7.4	0.10%	0.07%	0.02%	0.05%
4	(NAG)(MUR)-None	C17H30N2O12	454.17987	81.6			2.2	0.07%	0.09%	0.01%	0.04%
5	(anNAM)-Ae	C19H29N3O11	475.18021	62.2			10.5	0.04%	0.06%	0.02%	0.02%
6	(NAG)(anNAM)-None	C19H30N2O12	478.17987	79.5			8.2	0.33%	0.27%	0.09%	0.12%
7	(NAM)-Aq	C19H32N4O11	492.20676	MS1			6.0|7.1	0.06%	0.03%	0.00%	0.02%
8	(NAG)(NAM)-None	C19H32N2O13	496.19044	84.5			4.6|5.9	9.87%	10.44%	0.70%	3.92%
9	None-AqKA[3-NH2-GG]	C21H38N8O8	530.28126		MS1		3.4	0.03%	0.06%	0.01%	0.05%
10	(NAG)(DAM)-None	C21H34N2O14	538.201	74.4			6.9|7.6	0.06%	0.03%	0.02%	0.02%
11	None-AqKAA[3-NH2-G]	C22H40N8O8	544.29691	62.9	MS1		3.3	0.18%	0.16%	0.13%	0.14%
12	(NAG)(anNAM)-A	C22H35N3O13	549.21699	67.4			10.7	0.06%	0.05%	0.01%	0.01%
13	None-AqKAA[3-NH2-A]	C23H42N8O8	558.31256		MS1		5.4	0.05%	0.01%	0.02%	0.00%
14	(NAG)(NAM)-A	C22H37N3O14	567.22755	66.7			8.0|8.9	2.28%	1.86%	0.33%	0.42%
15	None-AqKA[3-NH2-GGG]	C23H41N9O9	587.30272		61.9		3.8	0.05%	0.07%	0.03%	0.06%
16	None-AqKAA[3-NH2-GG]	C24H43N9O9	601.31837		62		4.4	0.13%	0.22%	0.06%	0.20%

SN	Compound	Formula	Monoisotopic Mass	MS-DIAL score			RT / min	MW2 Rel. Abundance		RN450 Rel. Abundance	
				[M+H]+	[M+2H]2+	[M+3H]3+		Mean	S.D	Mean	S.D.
17	(NAG)(MUR)-Aq	C25H43N5O15	653.27557	64.9			6.0	0.01%	0.01%	0.00%	0.00%
18	None-AqKAA[3-NH2-GGG]	C26H46N10O10	658.33984		61.3		4.8	0.10%	0.22%	0.04%	0.21%
19	None-AeKAA[3-NH2-GGG]	C26H45N9O11	659.32385		MS1		5.8	0.01%	0.02%	0.01%	0.01%
20	(NAG)(anNAM)-Aq	C27H43N5O15	677.27557	MS1			10.0	0.04%	0.04%	0.01%	0.00%
21	(NAG)(anNAM)-Ae	C27H42N4O16	678.25958	61.8			10.6	0.02%	0.01%	0.00%	0.01%
22	(NAG)(NAM)-Aq	C27H45N5O16	695.28613	63.7			7.4|8.2	1.42%	1.31%	0.09%	0.26%
23	(NAG)(NAM)-Ae	C27H44N4O17	696.27015	68.8			8.8	0.11%	0.13%	0.02%	0.02%
24	None-AqKAGG[3-NH2-GGG]	C27H47N11O11	701.34565		MS1		3.2	0.03%	0.04%	0.02%	0.03%
25	None-AqKA[3-NH2-GGGGG]	C27H47N11O11	701.34565	63	67		4.9|5.1	1.11%	0.78%	0.99%	0.71%
26	None-AeKA[3-NH2-GGGGG]	C27H46N10O12	702.32967		MS1		5.5	0.00%	0.03%	0.00%	0.02%
27	None-AeKAGG[3-NH2-GGG]	C27H46N10O12	702.32967		63.1		5.7	0.07%	0.06%	0.04%	0.05%
28	None-AqKAA[3-NH2-GGGG]	C28H49N11O11	715.3613		61.9		5.1	0.06%	0.16%	0.02%	0.14%
29	None-AqKAG[3-NH2-GGGGG]	C29H50N12O12	758.36712		73.9		3.4	0.08%	0.34%	0.05%	0.32%
30	None-AqKAA[3-NH2-GGGGG]	C30H52N12O12	772.38277	61.6	74		5.4	0.95%	1.68%	0.62%	2.23%
31	None-AeKAA[3-NH2-GGGGG]	C30H51N11O13	773.36678		67.9		6.0	0.10%	0.16%	0.05%	0.18%
32	None-AqKAA[3-NH2-AGGGG]	C31H54N12O12	786.39842		74.1		6.4	0.12%	0.09%	0.06%	0.11%
33	None-AqKAA[3-NH2-GGSGG]	C31H54N12O13	802.39333		63.1		5.4	0.07%	0.06%	0.04%	0.09%
34	None-AqKAGG[3-NH2-GGGGG]	C31H53N13O13	815.38858		72.3		4.3	0.13%	0.26%	0.06%	0.28%
35	(NAG)(NAM)-AqK	C33H57N7O17	823.38109	76.4	73.9		6.1|7.1	0.10%	0.06%	0.01%	0.00%
36	None-AqKAGGG[3-NH2-GGGGG]	C33H56N14O14	872.41004		69.7		4.7	0.08%	0.18%	0.04%	0.18%
37	(NAG)(NAM)-AqK[3-NH2-G]	C35H60N8O18	880.40256	61.7	71.9		8.1	0.04%	0.04%	0.01%	0.01%
38	(NAG)(NAM)-AqKA	C36H62N8O18	894.41821	71.5	68.8		7.1|7.9	0.33%	0.36%	0.04%	0.03%
39	None-AqKAGGGG[3-NH2-GGGGG]	C35H59N15O15	929.43151		76.2		4.8	0.11%	0.19%	0.05%	0.19%
40	(NAG)(anNAM)-AqKA[3-NH2-G]	C38H63N9O18	933.42911	69.1	72.5		9.9	0.05%	0.05%	0.01%	0.01%
41	(NAG)(anNAM)-AqKAA	C39H65N9O18	947.44476	66.8	60.3		9.6	0.05%	0.02%	0.01%	0.00%
42	(NAG)(NAM)-AqKAG	C38H65N9O19	951.43967	72	69.3		6.8|7.6	0.14%	0.10%	0.03%	0.03%
43	(NAG)(NAM)-AqKA[3-NH2-G]	C38H65N9O19	951.43967	69.2	74.3		7.9|8.6	1.94%	1.99%	0.03%	0.10%
44	(NAG)(NAM)-AeKA[3-NH2-G]	C38H64N8O20	952.42369	66.7	69.2		8.3|8.9	0.16%	0.21%	0.02%	0.02%

SN	Compound	Formula	Monoisotopic Mass	MS-DIAL score			RT / min	MW2 Rel. Abundance		RN450 Rel. Abundance	
				[M+H]+	[M+2H]2+	[M+3H]3+		Mean	S.D	Mean	S.D.
45	(NAG)(NAM)-AqKAA	C39H67N9O19	965.45532	72.3	69.8		7.5|8.2	1.65%	1.21%	0.28%	0.29%
46	(NAG)(NAM)-AqKA[3-NH2-A]	C39H67N9O19	965.45532	72.5	74.1		8.9	0.10%	0.04%	0.01%	0.00%
47	(NAG)(NAM)-AeKAA	C39H66N8O20	966.43934	75.3	74.5		7.9|8.5	0.14%	0.13%	0.03%	0.04%
48	(NAM)-AqKA[3-NH2-GGGGG]	C38H64N12O18	976.44615	60.4	67.5		7.8|8.3	0.12%	0.09%	0.01%	0.08%
49	(NAG)(anNAM)-AqKAA[3-NH2-G]	C41H68N10O19	1004.46622	65.9	71.9		10.1	0.13%	0.11%	0.03%	0.03%
50	(NAG)(NAM)-AqKAGG	C40H68N10O20	1008.46113	68.9	74.5		7.6	0.18%	0.16%	0.04%	0.04%
51	(NAG)(NAM)-AqKA[3-NH2-GG]	C40H68N10O20	1008.46113	68.1	74		8.1|8.7	1.21%	1.38%	0.07%	0.06%
52	(NAG)(NAM)-AqKAG[3-NH2-G]	C40H68N10O20	1008.46113	65.7	73.7		8.2	0.43%	0.41%	0.05%	0.09%
53	(NAG)(NAM)-AeKA[3-NH2-GG]	C40H67N9O21	1009.44515	72.9	75.7		8.4|9.0	0.09%	0.13%	0.01%	0.01%
54	(NAG)(anNAM)-AqKAA[3-NH2-A]	C42H70N10O19	1018.48187	65.6			10.3	0.01%	0.00%	0.00%	0.00%
55	(NAG)(NAM)-AqKAA[3-NH2-G]	C41H70N10O20	1022.47678	67	75.4		8.1|8.7	4.45%	4.81%	0.48%	0.85%
56	(NAG)(NAM)-AeKAA[3-NH2-G]	C41H69N9O21	1023.4608	68.8	72.9		8.5|9.0	0.40%	0.55%	0.09%	0.11%
57	(NAG)(NAM)-AqKAA[3-NH2-A]	C42H72N10O20	1036.49243	70.3	77.2		8.5|9.0	0.75%	0.17%	0.10%	0.03%
58	(NAG)(NAM)-AeKAA[3-NH2-A]	C42H71N9O21	1037.47645	69.5			8.8|9.4	0.02%	0.01%	0.00%	0.00%
59	(NAM)-AqKAA[3-NH2-GGGGG]	C41H69N13O19	1047.48327	MS1	65.5		7.9|8.4	0.23%	0.22%	0.02%	0.20%
60	(NAG)(anNAM)-AqKAA[3-NH2-GG]	C43H71N11O20	1061.48768	61.8	72		10.1	0.07%	0.07%	0.01%	0.01%
61	(NAG)(NAM)-AqKAGGG	C42H71N11O21	1065.4826	71.6	72.4		7.6	0.14%	0.14%	0.03%	0.03%
62	(NAG)(NAM)-AqKAG[3-NH2-GG]	C42H71N11O21	1065.4826	67.8	72.8		7.7|8.4	0.16%	0.16%	0.04%	0.05%
63	(NAG)(NAM)-AqKA[3-NH2-GGG]	C42H71N11O21	1065.4826	66.4	71.7		8.1|8.7	1.12%	1.34%	0.05%	0.06%
64	(NAG)(NAM)-AqKAGG[3-NH2-G]	C42H71N11O21	1065.4826		73.8		8.2	0.20%	0.20%	0.03%	0.05%
65	(NAG)(NAM)-AeKA[3-NH2-GGG]	C42H70N10O22	1066.46661	70	71.7		8.9	0.08%	0.11%	0.01%	0.01%
66	(NAG)(NAM)-AqKAA[3-NH2-GG]	C43H73N11O21	1079.49825	68.2	73		8.2|8.8	2.27%	2.73%	0.29%	0.45%
67	(NAG)(NAM)-AeKAA[3-NH2-GG]	C43H72N10O22	1080.48226	70.6	73.3		8.6|9.1	0.25%	0.36%	0.05%	0.08%
68	(NAG)(NAM)-AqKAA[3-NH2-AG]	C44H75N11O21	1093.5139	69.1	73.7		8.7|9.3	0.34%	0.08%	0.05%	0.02%
69	(NAG)(NAM)-AqK[3-NH2-GGGGG]	C43H72N12O22	1108.48841	66	71.6		7.6|8.3	0.26%	0.28%	0.02%	0.05%
70	(NAG)(anNAM)-AqKAA[3-NH2-GGG]	C45H74N12O21	1118.50915	MS1	68.1		10.1	0.07%	0.05%	0.01%	0.01%
71	(NAG)(NAM)-AqKAGGG[3-NH2-G]	C44H74N12O22	1122.50406	69.1	70.8		7.5|8.2	0.54%	0.54%	0.09%	0.10%
72	(NAG)(NAM)-AqKAGG[3-NH2-GG]	C44H74N12O22	1122.50406	66.7	73.7		7.7|8.4	0.57%	0.62%	0.12%	0.19%

SN	Compound	Formula	Monoisotopic Mass	MS-DIAL score			RT / min	MW2 Rel. Abundance		RN450 Rel. Abundance	
				[M+H]+	[M+2H]2+	[M+3H]3+		Mean	S.D	Mean	S.D.
73	(NAG)(NAM)-AqKA[3-NH2-GGGG]	C44H74N12O22	1122.50406	63.9	67.8		8.1|8.7	0.96%	1.16%	0.06%	0.05%
74	(NAG)(NAM)-AeKA[3-NH2-GGGG]	C44H73N11O23	1123.48808	63.9	65.7		8.9	0.06%	0.10%	0.01%	0.01%
75	(NAG)(NAM)-AqKAA[3-NH2-GGG]	C45H76N12O22	1136.51971	66.4	65.2		8.2|8.8	2.08%	2.60%	0.24%	0.40%
76	(NAG)(NAM)-AeKAA[3-NH2-GGG]	C45H75N11O23	1137.50373	66	65.6		8.6|9.1	0.25%	0.37%	0.04%	0.08%
77	(NAG)(MUR)-AqKA[3-NH2-GGGGG]	C44H75N13O22	1137.51496		67.4		7.3	0.04%	0.06%	0.00%	0.01%
78	(NAG)(NAM)-AqKAA[3-NH2-AGG]	C46H78N12O22	1150.53536	61.9	71.2		8.7|9.3	0.36%	0.09%	0.06%	0.03%
79	(NAG)(anNAM)-AqKAGGGG[3-NH2-G]	C46H75N13O22	1161.51496	61.5	72		9.3|9.4	0.09%	0.07%	0.02%	0.01%
80	(NAG)(anNAM)-AqKA[3-NH2-GGGGG]	C46H75N13O22	1161.51496	MS1	64.5		9.5|9.9	0.36%	0.28%	0.03%	0.02%
81	(NAG)(anNAM)-AqKAA[3-NH2-GGGG]	C47H77N13O22	1175.53061		65.9		10.0	0.14%	0.05%	0.01%	0.01%
82	(NAG)(NAM)-AqKAGGGG[3-NH2-G]	C46H77N13O23	1179.52553	66.4	73.1		7.5	0.46%	0.28%	0.06%	0.01%
83	(NAG)(NAM)-AqKAGG[3-NH2-GGG]	C46H77N13O23	1179.52553	62.5	69.4		7.7|8.4	0.93%	0.87%	0.20%	0.19%
84	(NAG)(NAM)-AqKA[3-NH2-GGGGG]	C46H77N13O23	1179.52553	62.8	65.4		8.1|8.7	9.45%	7.71%	0.62%	0.40%
85	(NAG)(NAM)-AqKAGGG[3-NH2-GG]	C46H77N13O23	1179.52553		67.4		8.5	3.10%	3.78%	2.77%	2.75%
86	(NAG)(NAM)-AeKA[3-NH2-GGGGG]	C46H76N12O24	1180.50954	67.1	67.5		8.4|8.9	0.85%	1.04%	0.08%	0.12%
87	(NAG)(NAM)-AqKAA[3-NH2-GGGG]	C47H79N13O23	1193.54118	61.7	65.9		8.2|8.8	1.79%	2.07%	0.19%	0.30%
88	(NAG)(NAM)-AqKAGGGG[3-NH2-A]	C47H79N13O23	1193.54118		69.8		8.5	0.09%	0.03%	0.01%	0.01%
89	(NAG)(NAM)-AqKA[3-NH2-AGGGG]	C47H79N13O23	1193.54118	67.8	69.3		9.2	0.23%	0.07%	0.01%	0.01%
90	(NAG)(NAM)-AeKAA[3-NH2-GGGG]	C47H78N12O24	1194.52519	60.8	65.8		9.1	0.14%	0.20%	0.04%	0.04%
91	(NAG)(NAM)-AqKAA[3-NH2-AGGG]	C48H81N13O23	1207.55683	60.9	68.4		8.7|9.3	0.26%	0.08%	0.04%	0.01%
92	(NAG)(MUR)-AqKAA[3-NH2-GGGGG]	C47H80N14O23	1208.55207		66.3		7.5	0.12%	0.18%	0.01%	0.01%
93	(NAG)(NAM)-AqKA[3-NH2-GGSGG]	C47H79N13O24	1209.53609	62.7	66.8		8.1|8.7	0.76%	0.22%	0.11%	0.03%
94	(NAG)(anNAM)-AqKAG[3-NH2-GGGGG]	C48H78N14O23	1218.53642		MS1		9.5	0.44%	0.36%	0.12%	0.08%
95	(NAG)(DAM)-AqKA[3-NH2-GGGGG]	C48H79N13O24	1221.53609		61.5		9.2	0.02%	0.02%	0.01%	0.02%
96	(NAG)(anNAM)-AqKAA[3-NH2-GGGGG]	C49H80N14O23	1232.55207	MS1	MS1		10.0	0.31%	0.23%	0.06%	0.04%
97	(NAG)(anNAM)-AeKAA[3-NH2-GGGGG]	C49H79N13O24	1233.53609	MS1			10.3	0.01%	0.01%	0.00%	0.00%
98	(NAG)(NAM)-AqKAGGGG[3-NH2-GG]	C48H80N14O24	1236.54699	64			7.7	0.01%	0.05%	0.01%	0.04%
99	(NAG)(NAM)-AqKAG[3-NH2-GGGGG]	C48H80N14O24	1236.54699	65.3	60.9		7.8|8.4	2.85%	2.65%	0.60%	0.75%
100	(NAG)(NAM)-AeKAG[3-NH2-GGGGG]	C48H79N13O25	1237.53101	61.6	60.6		8.6	0.18%	0.26%	0.04%	0.05%

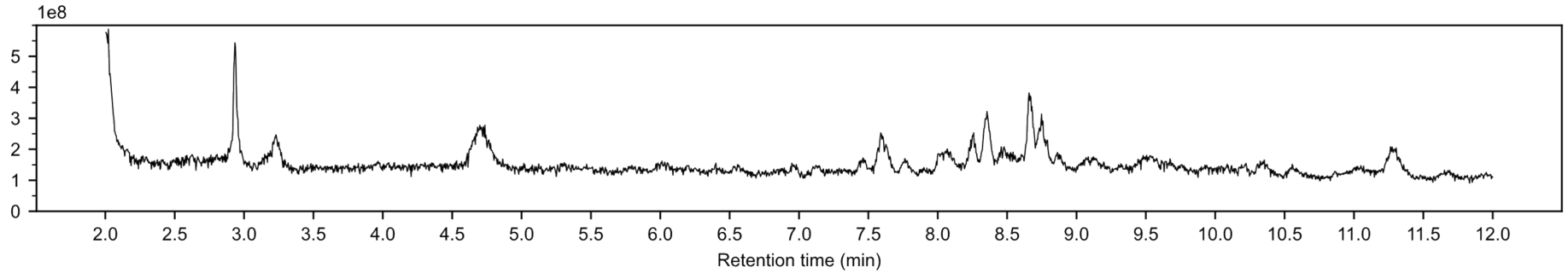
SN	Compound	Formula	Monoisotopic Mass	MS-DIAL score			RT / min	MW2 Rel. Abundance		RN450 Rel. Abundance	
				[M+H]+	[M+2H]2+	[M+3H]3+		Mean	S.D	Mean	S.D.
101	(NAG)(anNAM)-AqKAA[3-NH2-AGGGG]	C50H82N14O23	1246.56772	MS1	63.9		10.5	0.06%	0.01%	0.01%	0.00%
102	(NAG)(NAM)-AqKAA[3-NH2-GGGGG]	C49H82N14O24	1250.56264	60.8	63.2		8.3|8.8	15.97%	16.61%	1.12%	1.23%
103	(NAG)(NAM)-AeKAA[3-NH2-GGGGG]	C49H81N13O25	1251.54666	62.8	65.7		8.6|9.1	1.35%	2.13%	0.08%	0.15%
104	(NAG)(NAM)-AqKAA[3-NH2-AGGGG]	C50H84N14O24	1264.57829	60.9	67.8		8.7|9.3	2.29%	0.39%	0.31%	0.06%
105	(NAG)(NAM)-AqKAA[3-NH2-GGSGG]	C50H84N14O25	1280.5732	MS1	61.6		8.3|8.8	0.79%	0.29%	0.08%	0.10%
106	(NAG)(DAM)-AqKAA[3-NH2-GGGGG]	C51H84N14O25	1292.5732	MS1	61.3		9.4	0.08%	0.06%	0.02%	0.05%
107	(NAG)(NAM)-AqKAGGGG[3-NH2-GGG]	C50H83N15O25	1293.56845		66.8		7.7	0.10%	0.26%	0.02%	0.27%
108	(NAG)(NAM)-AqKAGG[3-NH2-GGGGG]	C50H83N15O25	1293.56845	65.1	64.9		7.8|8.3	1.82%	1.80%	0.31%	0.42%
109	(NAG)(NAM)-AeKAGG[3-NH2-GGGGG]	C50H82N14O26	1294.55247		66.9		8.6	0.10%	0.15%	0.02%	0.03%
110	(NAG)(NAM)-AqKAGG[3-NH2-AGGGG]	C51H85N15O25	1307.5841		65.1		8.8	0.11%	0.10%	0.01%	0.00%
111	(NAG)(NAM)-AqKAGGG[3-NH2-GGGGG]	C52H86N16O26	1350.58992	64.5	61.3		7.8|8.3	1.21%	1.40%	0.11%	0.26%
112	(NAG)(NAM)-AeKAGGG[3-NH2-GGGGG]	C52H85N15O27	1351.57393		61.4		8.6	0.07%	0.09%	0.03%	0.01%
113	(NAG)(NAM)-AqKAGGG[3-NH2-AGGGG]	C53H88N16O26	1364.60557		63.5		8.8	0.09%	0.07%	0.02%	0.02%
114	(NAG)(anNAM)-AqKAGGGG[3-NH2-GGGGG]	C54H87N17O26	1389.60082		MS1		9.4	0.02%	0.02%	0.01%	0.01%
115	(NAG)(NAM)-AqKAGGGG[3-NH2-GGGGG]	C54H89N17O27	1407.61138	66	MS1		7.7|8.3	0.91%	0.91%	0.08%	0.13%
116	(NAG)(NAM)-AeKAGGGG[3-NH2-GGGGG]	C54H88N16O28	1408.5954		61.5		8.0|8.5	0.12%	0.13%	0.02%	0.02%
117	(NAG)(NAM)-AqKAGGGG[3-NH2-AGGGG]	C55H91N17O27	1421.62703		MS1		8.7	0.05%	0.03%	0.00%	0.01%
118	None-AqKA[3-NH2-GGGGG]; (3br-4)-None-AqKA[3-NH2-GGGGG]	C54H92N22O21	1384.68074		MS1	MS1	6.9	0.39%	0.05%	0.19%	0.04%
119	None-AqKAA[3-NH2-GGGGG]; (3br-4)-None-AqKA[3-NH2-GGGGG]	C57H97N23O22	1455.71785		MS1	MS1	6.9	0.42%	0.08%	0.22%	0.08%
120	(NAG)(NAM)-AqKA[3-NH2-GGGGG]; (3br-4)-None-AqKA[3-NH2-G]	C65H110N20O29	1634.77476		63.3		8.1|8.6	0.15%	0.16%	0.01%	0.01%
121	None-AqKAA[3-NH2-GGGGG]; (3br-4)-(NAG)(NAM)-AqKA[3-NH2-G]	C68H115N21O30	1705.81187		67.7		8.2|8.6	0.23%	0.19%	0.01%	0.00%
122	(NAG)(NAM)-AqKA[3-NH2-GGGGG]; (3br-4)-None-AqKA[3-NH2-GGG]	C69H116N22O31	1748.81768		67.9		8.6	0.06%	0.07%	0.01%	0.01%
123	(NAG)(NAM)-AqKA[3-NH2-GGGGG]; (3br-4)-None-AqKA[3-NH2-GGGGG]	C73H122N24O33	1862.86061		66.5	MS1	8.3|8.7	1.06%	1.05%	0.03%	0.12%
124	(NAG)(NAM)-AqKAG[3-NH2-GGGGG]; (3br-4)-None-AqKA[3-NH2-GGGGG]	C75H125N25O34	1919.88208		61.6		8.1|8.4	0.09%	0.09%	0.01%	0.02%
125	(NAG)(NAM)-AqKAA[3-NH2-GGGGG]; (3br-4)-None-AqKA[3-NH2-GGGGG]	C76H127N25O34	1933.89773		66	63.3	8.4|8.7	0.80%	0.89%	0.05%	0.09%
126	None-AqKAA[3-NH2-GGGGG]; (3br-4)-(NAG)(NAM)-AqKA[3-NH2-GGGGG]	C76H127N25O34	1933.89773		62.7	MS1	8.7	0.22%	0.14%	0.01%	0.01%

SN	Compound	Formula	Monoisotopic Mass	MS-DIAL score			RT / min	MW2 Rel. Abundance		RN450 Rel. Abundance	
				[M+H]+	[M+2H]2+	[M+3H]3+		Mean	S.D	Mean	S.D.
127	(NAG)(NAM)-AqKA[3-NH2-GGGGG]; (3br-4)-(NAG)(NAM)-AqKA[3-NH2-G]	C84H140N22O41	2112.95463		64.5		9.5|9.8	0.13%	0.12%	0.03%	0.03%
128	(NAG)(NAM)-AqKA[3-NH2-GGGGG]; (3br-4)-(NAG)(NAM)-AqKA[3-NH2-GG]	C86H143N23O42	2169.9761		60.4		9.6	0.06%	0.05%	0.02%	0.02%
129	(NAG)(NAM)-AqKAA[3-NH2-GGGGG]; (3br-4)-(NAG)(NAM)-AqKA[3-NH2-G]	C87H145N23O42	2183.99175		64.9	MS1	9.6|9.9	0.25%	0.19%	0.08%	0.09%
130	(NAG)(NAM)-AqKAA[3-NH2-GGGGG]; (3br-4)-(NAG)(NAM)-AqKA[3-NH2-GG]	C89H148N24O43	2241.01321		63.8		9.6|9.9	0.16%	0.14%	0.06%	0.07%
131	(NAG)(NAM)-AqKAA[3-NH2-GGGGG]; (3br-4)-(NAG)(NAM)-AqKA[3-NH2-GGG]	C91H151N25O44	2298.03467		62.6	MS1	9.6|9.9	0.20%	0.22%	0.06%	0.11%
132	(NAG)(NAM)-AqKAA[3-NH2-GGGGG]; (3br-4)-(NAG)(NAM)-AqKA[3-NH2-AGG]	C92H153N25O44	2312.05032		MS1	MS1	9.3	0.15%	0.28%	0.02%	0.01%
133	(NAG)(NAM)-AqKA[3-NH2-GGGGG]; (G-G)-(NAG)(NAM)-AqKA[3-NH2-GGGGG]	C92H152N26O45	2341.04049		69.9	67.2	8.9|9.1	0.55%	0.71%	0.06%	0.12%
134	(NAG)(NAM)-AqKA[3-NH2-GGGGG]; (3br-4)-(NAG)(NAM)-AqKA[3-NH2-GGGGG]	C92H152N26O45	2341.04049		62.1	63.5	9.5|9.8	1.06%	0.94%	0.20%	0.32%
135	(NAG)(NAM)-AqKAA[3-NH2-GGGGG]; (3br-4)-(NAG)(NAM)-AqKA[3-NH2-AGGG]	C94H156N26O45	2369.07179		MS1	MS1	8.9|9.3	0.17%	0.37%	0.03%	0.02%
136	(NAG)(NAM)-AqKA[3-NH2-GGSGG]; (G-G)-(NAG)(NAM)-AqKA[3-NH2-GGGGG]	C93H154N26O46	2371.05105		62.2		9.1	0.05%	0.03%	0.01%	0.00%
137	(NAG)(NAM)-AqKAA[3-NH2-GGGGG]; (G-G)-(NAG)(NAM)-AqKA[3-NH2-GGGGG]	C95H157N27O46	2412.0776		71.4	67.9	8.9|9.2	0.65%	0.96%	0.03%	0.14%
138	(NAG)(NAM)-AqKAA[3-NH2-GGGGG]; (3br-4)-(NAG)(NAM)-AqKA[3-NH2-GGGGG]	C95H157N27O46	2412.0776		63.2	MS1	9.6|9.8	1.24%	1.04%	0.37%	0.47%
139	(NAG)(NAM)-AeKAA[3-NH2-GGGGG]; (3br-4)-(NAG)(NAM)-AqKA[3-NH2-GGGGG]	C95H156N26O47	2413.06162		MS1	MS1	10.0	0.21%	0.18%	0.07%	0.09%
140	(NAG)(NAM)-AqKA[3-NH2-AGGGG]; (G-G)-(NAG)(NAM)-AqKAA[3-NH2-GGGGG]	C96H159N27O46	2426.09325		64.5	61.7	9.2	0.09%	0.15%	0.01%	0.01%
141	(NAG)(NAM)-AqKAG[3-NH2-GGGGG]; (G-G)-(NAG)(NAM)-AqKAA[3-NH2-GGGGG]	C97H160N28O47	2469.09906		67.5		9.0	0.05%	0.08%	0.02%	0.01%
142	(NAG)(NAM)-AqKAA[3-NH2-GGGGG]; (G-G)-(NAG)(NAM)-AqKAA[3-NH2-GGGGG]	C98H162N28O47	2483.11471		66.6	61.7	8.9|9.2	1.65%	2.81%	0.26%	0.58%
143	(NAG)(NAM)-AqKAA[3-NH2-GGGGG]; (G-G)-(NAG)(NAM)-AeKAA[3-NH2-GGGGG]	C98H161N27O48	2484.09873		67.1	MS1	9.4	0.20%	0.37%	0.01%	0.04%
144	(NAG)(NAM)-AqKAA[3-NH2-GGGGG]; (G-G)-(NAG)(NAM)-AqKAA[3-NH2-AGGGG]	C99H164N28O47	2497.13036		66.7	61.9	9.2|9.5	0.25%	0.12%	0.01%	0.00%
145	(NAG)(DAM)-AqKAA[3-NH2-GGGGG]; (G-G)-(NAG)(NAM)-AqKAA[3-NH2-GGGGG]	C100H164N28O48	2525.12528		66.5	MS1	9.5|9.6	0.08%	0.18%	0.01%	0.05%
146	None-AqKA[3-NH2-GGGGG]; (3br-4)-None-AqKA[3-NH2-GGGGG]; (3br-4)-None-AqKA[3-NH2-GGGGG]	C81H137N33O31	2068.01582			MS1	7.3	0.04%	0.00%	0.02%	0.00%
147	(NAG)(NAM)-AqKA[3-NH2-GGGGG]; (3br-4)-None-AqKA[3-NH2-GGGGG]; (3br-4)-None-AqKA[3-NH2-GGGGG]	C100H167N35O43	2546.1957			MS1	8.2|8.5	0.19%	0.16%	0.01%	0.01%
148	(NAG)(NAM)-AqKA[3-NH2-GGGGG]; (3br-4)-(NAG)(NAM)-AqKA[3-NH2-GGGGG]; (3br-4)-(NAG)(NAM)-AqKA[3-NH2-GGGGG]	C138H227N39O67	3502.55545			MS1	9.6|10.1|10.3	0.37%	0.31%	0.10%	0.15%

SN	Compound	Formula	Monoisotopic Mass	MS-DIAL score			RT / min	MW2 Rel. Abundance		RN450 Rel. Abundance	
				[M+H]+	[M+2H]2+	[M+3H]3+		Mean	S.D	Mean	S.D.
149	(NAG)(NAM)-AqKAA[3-NH2-GGGGG]; (3br-4)-(NAG)(NAM)-AqKA[3-NH2-GGGGG]; (3br-4)-(NAG)(NAM)-AqKA[3-NH2-GGGGG]	C141H232N40O68	3573.59256			MS1	9.6|10.1|10.3	0.31%	0.24%	0.10%	0.12%

Table S9

Representative TIC and identified peptidoglycan in *A. muciniphila* DSM22959.



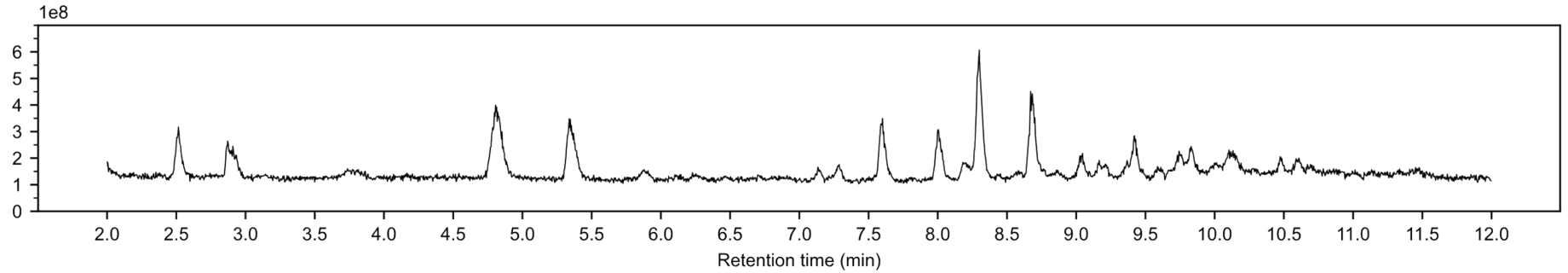
SN	Compound	Formula	Monoisotopic Mass	MS-DIAL score			RT / min	Rel. Abundance	
				[M+H]+	[M+2H]2+	[M+3H]3+		Mean	S.D
1	(G)(anNAM)-None	C17H28N2O11	436.16931	83.3			5.8	0.17%	0.17%
2	(G)(NAM)-None	C17H30N2O12	454.17987	84.4			2.9|3.2	4.20%	4.20%
3	(NAG)(NAM)-None	C19H32N2O13	496.19044	72.1			5.1|6.2	0.96%	0.96%
4	(G)(NAM)-A	C20H35N3O13	525.21699	78.6			6.3|8.0	0.58%	0.58%
5	(NAG)(NAM)-A	C22H37N3O14	567.22755	74.4			8.2|9.1	5.19%	5.19%
6	(G)(NAM)-Ae	C25H42N4O16	654.25958	60.3			7.5|8.6	0.21%	0.21%
7	(NAG)(NAM)-Ae	C27H44N4O17	696.27015	69.4			8.4|9.1	0.69%	0.69%
8	(G)(NAM)-Aem	C32H54N6O19	826.34437	73	80.2		5.8|7.1	3.18%	3.18%
9	(NAG)(NAM)-Aem	C34H56N6O20	868.35494	68.8	78.5		6.5|7.5	2.10%	2.10%
10	(G)(NAM)-AemG	C34H57N7O20	883.36584		67.6		6.1|7.5	0.58%	0.58%
11	(G)(NAM)-AemA	C35H59N7O20	897.38149	71.5	80.7		7.0|8.0	8.87%	8.87%
12	(NAG)(NAM)-AemG	C36H59N7O21	925.3764	70.8	69.9		6.9|7.8	0.62%	0.62%
13	(NAG)(NAM)-AemA	C37H61N7O21	939.39205	75.6	69		7.6|8.4	9.04%	9.04%
14	(G)(NAM)-AemAG	C37H62N8O21	954.40295	MS1	76.6		6.6|7.8	1.00%	1.00%
15	(G)(NAM)-AemAA	C38H64N8O21	968.4186	67.1	71.5		7.5|8.5	2.77%	2.77%
16	(NAG)(NAM)-AemAG	C39H64N8O22	996.41352	66.5	72.7		7.3|8.1	1.57%	1.57%

SN	Compound	Formula	Monoisotopic Mass	MS-DIAL score			RT / min	Rel. Abundance	
				[M+H]+	[M+2H]2+	[M+3H]3+		Mean	S.D
17	(NAG)(NAM)-AemH	C40H63N9O21	1005.41385		68.1		5.9|6.9	0.09%	0.09%
18	(NAG)(NAM)-AemAA	C40H66N8O22	1010.42917	64	69.8		8.1|8.8	4.10%	4.10%
19*	(NAG)(NAM)-AemGK	C42H71N9O22	1053.47136		MS1		5.8|6.8	0.26%	0.26%
20	(G)(NAM)-AemA; (G-G)-(NAG)(NAM)-Aem	C69H113N13O39	1747.72586		80.8	80.9	7.8|8.2	4.10%	4.10%
21	(G)(NAM)-AemA; (3s-4)-(G)(NAM)-AemA	C70H116N14O39	1776.75241		62	71	9.1|9.6	1.92%	1.92%
22	(G)(NAM)-AemA; (G-G)-(G)(NAM)-AemA	C70H116N14O39	1776.75241		68.3	77.9	7.9|8.4	2.77%	2.77%
23	(G)(NAM)-AemA; (G-G)-(NAG)(NAM)-AemA	C72H118N14O40	1818.76297		80	80.2	8.3|8.7	27.48%	27.48%
24	(NAG)(NAM)-AemA; (3s-4)-(G)(NAM)-AemA	C72H118N14O40	1818.76297		68.6	73	9.5|9.9	1.63%	1.63%
25	(G)(NAM)-AemAA; (G-G)-(G)(NAM)-AemA	C73H121N15O40	1847.78952			72	8.6	0.33%	0.33%
26	(NAG)(NAM)-AemA; (G-G)-(NAG)(NAM)-AemA	C74H120N14O41	1860.77354		71.4		8.7|9.1	0.47%	0.47%
27	(NAG)(NAM)-AemA; (3s-4)-(NAG)(NAM)-AemA	C74H120N14O41	1860.77354		65.4		9.8|10.1	0.98%	0.98%
28	(G)(NAM)-AemAG; (G-G)-(NAG)(NAM)-AemA	C74H121N15O41	1875.78444		MS1	60	8.1|8.5	3.27%	3.27%
29	(G)(NAM)-AemAA; (G-G)-(NAG)(NAM)-AemA	C75H123N15O41	1889.80009		74.1	75.7	8.5|8.9	5.51%	5.51%
30	(G)(NAM)-AemAA; (G-G)-(G)(NAM)-AemAA	C76H126N16O41	1918.82664			MS1	8.7	0.21%	0.21%
31	(NAG)(NAM)-AemAA; (G-G)-(NAG)(NAM)-AemA	C77H125N15O42	1931.81065		72.6		9.3	0.16%	0.16%
32	(NAG)(NAM)-AemAA; (3s-4)-(NAG)(NAM)-AemA	C77H125N15O42	1931.81065		MS1		9.9|10.3	0.77%	0.77%
33	(G)(NAM)-AemAA; (G-G)-(NAG)(NAM)-AemAA	C78H128N16O42	1960.8372		67.3	71.6	8.7|9.1	3.28%	3.28%
34	(NAG)(NAM)-AemAA; (G-G)-(NAG)(NAM)-AemAA	C80H130N16O43	2002.84777		70.1	MS1	9.1|9.5	0.93%	0.93%

*Could be AemGK or AemKG

Table S10

Representative TIC and identified peptidoglycan in *F. nucleatum* ATCC25586.



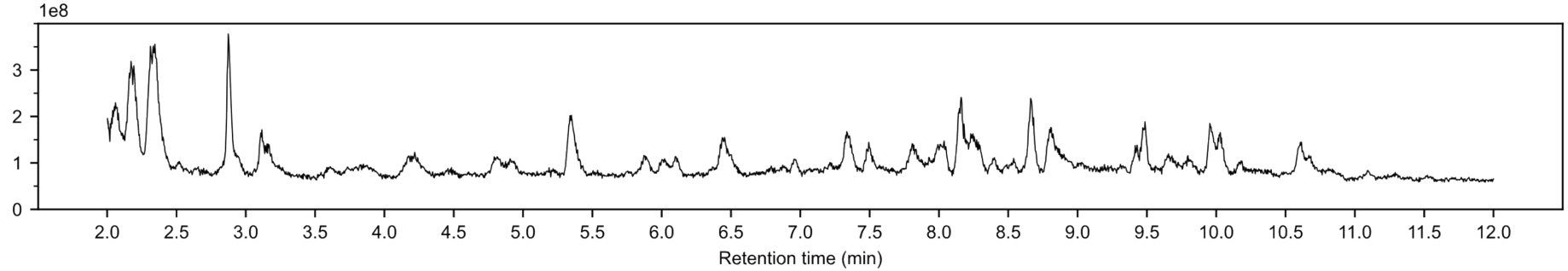
SN	Compound	Formula	Monoisotopic Mass	MS-DIAL score			RT / min	Rel. Abundance	
				[M+H]+	[M+2H]2+	[M+3H]3+		Mean	S.D
1	(NAG)(anNAM)-None	C19H30N2O12	478.17987	64.4			8.2	4.01%	0.51%
2	None-AelA	C17H29N5O9S	479.1686	74.1			2.9	0.19%	0.11%
3	(NAG)(NAM)-None	C19H32N2O13	496.19044	75.1			4.5|5.9	2.62%	0.66%
4	None-AelK	C20H36N6O9S	536.22645	79	61.4		2.2	0.42%	0.73%
5	(NAG)(DAM)-None	C21H34N2O14	538.201	66.3			7.6	0.16%	0.26%
6	(NAG)(NAM)-A	C22H37N3O14	567.22755	64.2			8.0|8.9	0.15%	0.05%
7	(NAG)(NAM)-Ae	C27H44N4O17	696.27015	65			8.2|8.8	0.31%	0.05%
8	(NAG)(anNAM)-Ael	C33H52N6O19S	868.30079	73			8.8	0.10%	0.06%
9	(NAG)(NAM)-Ael	C33H54N6O20S	886.31136	74.8	63.9		6.2|7.2	0.26%	0.10%
10	(NAG)(anNAM)-AelG	C35H55N7O20S	925.32226	70.7			9.1	0.08%	0.02%
11	(NAG)(anNAM)-AelA	C36H57N7O20S	939.33791	72.6			9.8	1.54%	0.70%
12	(NAG)(NAM)-AelG	C35H57N7O21S	943.33282	73.9	MS1		6.8|7.6	0.29%	0.01%
13	(NAG)(NAM)-AelA	C36H59N7O21S	957.34847	73.4	68.3		7.6|8.3	22.77%	2.05%
14	(NAG)(NAM)-None; (G-G)-(NAG)(NAM)-None	C38H62N4O25	974.37031	64.8	82		7.0|7.8	0.60%	0.28%
15	(NAG)(anNAM)-AelAG	C38H60N8O21S	996.35937	67.2			9.4	0.07%	0.01%
16	(NAG)(DAM)-None; (G-G)-(NAG)(anNAM)-None	C40H62N4O25	998.37031		77.6		10.7	0.53%	0.15%
17	(NAG)(DAM)-AelA	C38H61N7O22S	999.35904	71.9	71.1		8.4|9.0	0.95%	1.37%

SN	Compound	Formula	Monoisotopic Mass	MS-DIAL score			RT / min	Rel. Abundance	
				[M+H]+	[M+2H]2+	[M+3H]3+		Mean	S.D
18	(NAG)(anNAM)-AelH	C39H59N9O20S	1005.35971		75.2		8.4	0.09%	0.07%
19	(NAG)(anNAM)-AelAA	C39H62N8O21S	1010.37502	65.4			10.1	0.63%	0.25%
20	(NAG)(NAM)-AelAG	C38H62N8O22S	1014.36994	63.9	67.7		7.3|8.0	4.68%	1.62%
21	(NAG)(NAM)-AelK	C39H66N8O21S	1014.40632		75.4		5.8|6.8	0.26%	0.12%
22	(NAG)(NAM)-AelH	C39H61N9O21S	1023.37027		66.3		5.8|6.8	0.22%	0.16%
23	(NAG)(anNAM)-AelR	C39H64N10O20S	1024.40191		65		8.7	0.09%	0.01%
24	(NAG)(NAM)-AelAA	C39H64N8O22S	1028.38559	65.6	66.9		8.0|8.7	17.42%	0.65%
25	(NAG)(NAM)-AelR	C39H66N10O21S	1042.41247		MS1		6.5|7.3	0.29%	0.04%
26	(NAG)(DAM)-AelAG	C40H64N8O23S	1056.3805	64.5	62.2		8.1|8.7	0.21%	0.35%
27	(NAG)(DAM)-AelAA	C41H66N8O23S	1070.39615	MS1	62.1		8.8|9.3	0.52%	0.76%
28	(NAG)(NAM)-AelAK	C42H71N9O22S	1085.44344	68.3	70.3		6.3|7.1	3.77%	0.19%
29	(NAG)(NAM)-AelAH	C42H66N10O22S	1094.40738	76.5	69.7		6.4|7.3	1.77%	1.03%
30	(NAG)(NAM)-AelAR	C42H71N11O22S	1113.44958	MS1	62.8		6.8|7.5|7.6	1.05%	0.63%
31	(NAG)(anNAM)-AelA; (3s-4)-(NAG)(anNAM)-AelA	C72H112N14O39S2	1860.66525		62.4		11.3	0.12%	0.02%
32	(NAG)(NAM)-AelA; (G-G)-(NAG)(NAM)-AelA	C72H116N14O41S2	1896.68638		75.4	74.7	8.7|9.0	5.00%	0.94%
33	(NAG)(NAM)-AelA; (3s-4)-(NAG)(NAM)-AelA	C72H116N14O41S2	1896.68638		75.6		9.7|10.1	2.70%	0.23%
34	(NAG)(DAM)-AelA; (G-G)-(NAG)(NAM)-AelG	C73H116N14O42S2	1924.6813		75.4		8.8|9.0	0.21%	0.06%
35	(NAG)(DAM)-AelA; (G-G)-(NAG)(NAM)-AelA	C74H118N14O42S2	1938.69695		67.8	75.7	9.2|9.4	9.02%	0.90%
36	(NAG)(NAM)-AelAG; (G-G)-(NAG)(NAM)-AelA	C74H119N15O42S2	1953.70784		74.5		8.5|8.8	0.91%	0.04%
37	(NAG)(NAM)-AelAG; (3s-4)-(NAG)(NAM)-AelA	C74H119N15O42S2	1953.70784		65.8		9.4|9.7	1.59%	0.41%
38	(NAG)(NAM)-AelAA; (G-G)-(NAG)(NAM)-AelA	C75H121N15O42S2	1967.72349		76.7	71.6	8.9|9.2	2.38%	0.58%
39	(NAG)(NAM)-AelAA; (3s-4)-(NAG)(NAM)-AelA	C75H121N15O42S2	1967.72349		68.8		9.8|10.1	3.55%	0.18%
40	(NAG)(DAM)-AelA; (G-G)-(NAG)(DAM)-AelA	C76H120N14O43S2	1980.70751		66.2	MS1	10.1|10.3	0.37%	0.37%
41	(NAG)(DAM)-AelA; (3s-4)-(NAG)(DAM)-AelA	C76H120N14O43S2	1980.70751		MS1		10.8|11.2	0.21%	0.24%

SN	Compound	Formula	Monoisotopic Mass	MS-DIAL score			RT / min	Rel. Abundance	
				[M+H]+	[M+2H]2+	[M+3H]3+		Mean	S.D
42	(NAG)(DAM)-AelAA; (G-G)-(NAG)(anNAM)-AelA	C77H121N15O42S2	1991.72349		62		11.5|11.6	0.21%	0.08%
43	(NAG)(DAM)-AelA; (G-G)-(NAG)(NAM)-AelAG	C76H121N15O43S2	1995.71841		71.2		8.9|9.2	0.79%	0.20%
44	(NAG)(DAM)-AelAA; (G-G)-(NAG)(NAM)-AelA	C77H123N15O43S2	2009.73406		74		9.3|9.6	1.93%	0.17%
45	(NAG)(NAM)-AelAG; (G-G)-(NAG)(NAM)-AelAG	C76H122N16O43S2	2010.72931		61.7		8.6	0.17%	0.07%
46	(NAG)(NAM)-AelAA; (G-G)-(NAG)(NAM)-AelAG	C77H124N16O43S2	2024.74496		67.8		8.7|9.0	0.60%	0.11%
47	(NAG)(NAM)-AelAK; (3s-4)-(NAG)(NAM)-AelA	C78H128N16O42S2	2024.78134		64.7		8.8|9.1	0.33%	0.06%
48	(NAG)(NAM)-AelAA; (G-G)-(NAG)(NAM)-AelAA	C78H126N16O43S2	2038.76061		67.3		9.0|9.4	1.80%	0.48%
49	(NAG)(DAM)-AelAA; (G-G)-(NAG)(anNAM)-AelAA	C80H126N16O43S2	2062.76061		MS1		11.7	0.25%	0.08%
50	(NAG)(DAM)-AelAA; (G-G)-(NAG)(NAM)-AelAA	C80H128N16O44S2	2080.77117		68		9.5|9.8	1.60%	0.07%
51	(NAG)(NAM)-AelA; (3s-4)-(NAG)(NAM)-AelA; (3s-4)-(NAG)(NAM)-AelA	C108H173N21O61S3	2836.02429			MS1	10.1	0.22%	0.05%

Table S11

Representative TIC and identified peptidoglycan in *B. adolescentis* ATCC15703.



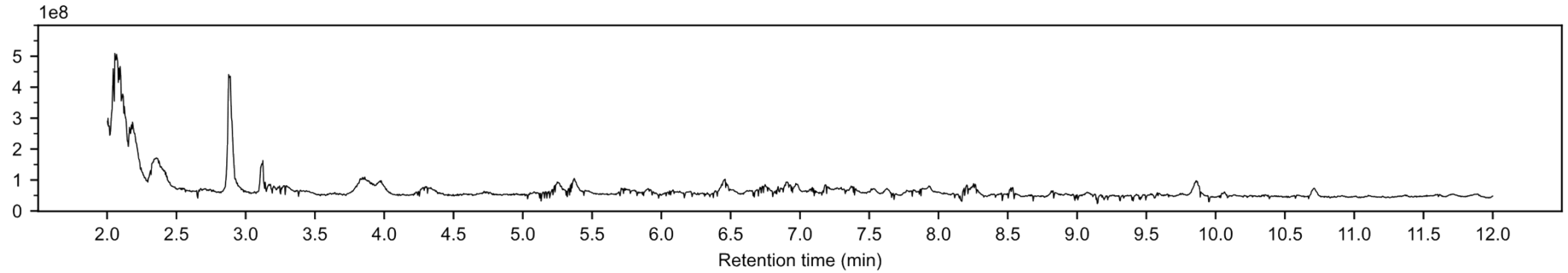
SN	Compound	Formula	Monoisotopic Mass	MS-DIAL score			RT / min	Rel. Abundance	
				[M+H]+	[M+2H]2+	[M+3H]3+		Mean	S.D
1	(NAG)(anNAM)-None	C19H30N2O12	478.17987	77.8			8.2	5.37%	1.24%
2	(NAG)(NAM)-None	C19H32N2O13	496.19044	79.5			5.9	1.24%	0.65%
3	None-AqKA[3-NH2-n]	C21H38N8O8	530.28126	65.2			3.2	0.41%	0.34%
4	(NAG)(anNAM)-A	C22H35N3O13	549.21699	74.6			10.7	4.59%	0.72%
5	(NAG)(NAM)-A	C22H37N3O14	567.22755	74.6			8.0|8.9	2.83%	0.32%
6	(NAG)(anNAM)-Aq	C27H43N5O15	677.27557	61.8			10.0	3.63%	0.86%
7	(NAG)(anNAM)-Ae	C27H42N4O16	678.25958	66.5			10.6	3.32%	1.63%
8	(NAG)(NAM)-Aq	C27H45N5O16	695.28613	66.1			7.4|8.2	3.90%	0.66%
9	(NAG)(NAM)-Ae	C27H44N4O17	696.27015	66.9			8.8	1.47%	0.69%
10	(NAG)(anNAM)-AqO	C32H53N7O16	791.35488	64.3			8.2	0.08%	0.04%
11	(NAG)(anNAM)-AqK	C33H55N7O16	805.37053		71.9		6.0	0.09%	0.08%
12	(NAG)(NAM)-AqO	C32H55N7O17	809.36544	57.3	46.2		6.4	0.08%	0.07%
13	(NAG)(NAM)-AqK	C33H57N7O17	823.38109	75.4	59.7		6.0|7.1	0.70%	0.59%
14	(NAG)(anNAM)-AqKA	C36H60N8O17	876.40764	74.5	71.3		9.4	1.11%	0.67%
15	(NAG)(NAM)-AqKA	C36H62N8O18	894.41821	73.6	69.2		7.1|7.9	1.11%	1.05%
16	(NAG)(anNAM)-AqO[3-NH2-n]	C36H59N9O18	905.39781	71.7	72.9		8.8	1.54%	1.56%
17	(NAG)(anNAM)-AeO[3-NH2-n]	C36H58N8O19	906.38182	63.5	74		9.0	0.25%	0.23%

SN	Compound	Formula	Monoisotopic Mass	MS-DIAL score			RT / min	Rel. Abundance	
				[M+H]+	[M+2H]2+	[M+3H]3+		Mean	S.D
18	(NAG)(anNAM)-AqK[3-NH2-n]	C37H61N9O18	919.41346	70	75.5		9.5	4.75%	1.49%
19	(NAG)(anNAM)-AeK[3-NH2-n]	C37H60N8O19	920.39747	68			9.7	0.50%	0.26%
20	(NAG)(NAM)-AqO[3-NH2-n]	C36H61N9O19	923.40837	69.4	63.6		6.5|7.3	2.03%	2.70%
21	(NAG)(NAM)-AeO[3-NH2-n]	C36H60N8O20	924.39239	58.5			7.0|7.7	0.16%	0.17%
22	(NAG)(NAM)-AqO[3-NH2-d]	C36H60N8O20	924.39239	59.3			6.8|7.6	0.09%	0.11%
23	(NAG)(NAM)-AqK[3-NH2-n]	C37H63N9O19	937.42402	72.4	63.6		7.5|8.1	6.61%	3.98%
24	(NAG)(NAM)-AeK[3-NH2-n]	C37H62N8O20	938.40804	70.8	57.7		7.9|8.5	3.16%	5.01%
25	(NAG)(NAM)-AqK[3-NH2-d]	C37H62N8O20	938.40804	73.7	73.7		7.7|8.4	0.80%	0.32%
26	(NAG)(NAM)-AqKAA	C39H67N9O19	965.45532	67.9	70.5		8.2	0.38%	0.32%
27	(NAG)(anNAM)-AqKG[3-NH2-n]	C39H64N10O19	976.43492	68.8	72.5		9.4	2.22%	0.54%
28	(NAG)(anNAM)-AqKG[3-NH2-d]	C39H63N9O20	977.41894	60.5	62.7		9.6	0.49%	0.12%
29	(NAG)(NAM)-AqOG[3-NH2-n]	C38H64N10O20	980.42983	65.8	39		6.5|7.5	0.30%	0.23%
30	(NAG)(anNAM)-AqKA[3-NH2-n]	C40H66N10O19	990.45057	70.7	73.4		10.0	9.98%	2.39%
31	(NAG)(anNAM)-AqKA[3-NH2-d]	C40H65N9O20	991.43459	70	76.1		10.2	2.33%	1.27%
32	(NAG)(NAM)-AqKG[3-NH2-n]	C39H66N10O20	994.44548	54.8	52.1		8.2	2.50%	1.83%
33	(NAG)(NAM)-AqOA[3-NH2-n]	C39H66N10O20	994.44548	66.5	55.9		7.3|8.0	0.80%	0.34%
34	(NAG)(NAM)-AqKA[3-NH2-n]	C40H68N10O20	1008.46113	72	63.4		8.0|8.7	13.25%	2.98%
35	(NAG)(NAM)-AqKA[3-NH2-d]	C40H67N9O21	1009.44515	70.6	73.3		8.3|8.9	4.53%	5.68%
36	(NAG)(anNAM)-AqKAA[3-NH2-n]	C43H71N11O20	1061.48768	67	69.1		10.1	1.05%	0.31%
37	(NAG)(NAM)-AqOAA[3-NH2-n]	C42H71N11O21	1065.4826	56.7	51.4		7.6|8.3	1.70%	1.09%
38	(NAG)(NAM)-AqKAA[3-NH2-n]	C43H73N11O21	1079.49825	68.3	73.9		8.2|8.8	5.55%	1.44%
39	(NAG)(NAM)-AqKAA[3-NH2-d]	C43H72N10O22	1080.48226	64.5			8.4|9.0	0.22%	0.22%
40	(NAG)(NAM)-AqKA[3-NH2-n]; (G-G)-(NAG)(NAM)-AqKA[3-NH2-n]	C80H134N20O39	1998.9117		73.4	61.7	8.8|9.1	2.23%	1.58%
41	(NAG)(NAM)-AqKA[3-NH2-n]; (3br-4)-(NAG)(NAM)-AqKA[3-NH2-n]	C80H134N20O39	1998.9117		59.8	MS1	9.7|10.0	1.23%	0.39%
42	(NAG)(NAM)-AqKA[3-NH2-d]; (G-G)-(NAG)(NAM)-AqKA[3-NH2-d]	C80H132N18O41	2000.87974		75.5		9.6	0.43%	0.30%
43	(NAG)(NAM)-AqKAA[3-NH2-n]; (3br-4)-(NAG)(NAM)-AqKA[3-NH2-n]	C83H139N21O40	2069.94882		62.1	MS1	9.7|10.0	0.43%	0.18%

SN	Compound	Formula	Monoisotopic Mass	MS-DIAL score			RT / min	Rel. Abundance	
				[M+H]+	[M+2H]2+	[M+3H]3+		Mean	S.D
44	(NAG)(NAM)-AqKAA[3-NH2-n]; (G-G)-(NAG)(NAM)-AqKAA[3-NH2-n]	C86H144N22O41	2140.98593		69.7		9.2	0.57%	0.54%

Table S12

Representative TIC and identified peptidoglycan in *B. bifidum* ATCC15696.



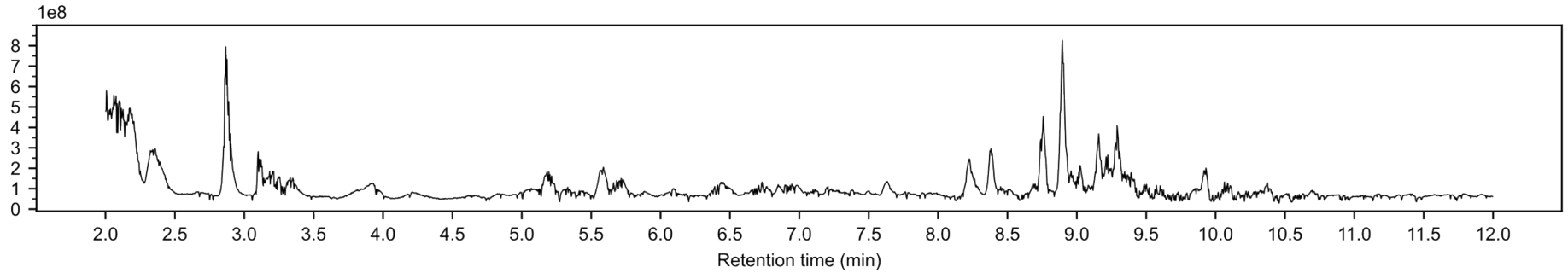
SN	Compound	Formula	Monoisotopic Mass	MS-DIAL score			RT / min	Rel. Abundance	
				[M+H]+	[M+2H]2+	[M+3H]3+		Mean	S.D
1	(NAG)(anNAM)-None	C19H30N2O12	478.17987	77.4			8.2	1.35%	0.50%
2	(NAG)(NAM)-None	C19H32N2O13	496.19044	79.9			5.9|6.8	0.63%	0.25%
3	(NAG)(MUR)-A	C20H35N3O13	525.21699	75.2			6.8|7.7	0.25%	0.09%
4	(DAG)(NAM)-None	C21H34N2O14	538.201	64.5			9.8	0.03%	0.01%
5	(NAG)(anNAM)-A	C22H35N3O13	549.21699	74.8			10.7	1.07%	0.42%
6	(NAG)(NAM)-A	C22H37N3O14	567.22755	73.2			8.0|8.9	2.26%	1.04%
7	(DAG)(NAM)-A	C24H39N3O15	609.23812	64.6			10.3|11.5	0.20%	0.13%
8	(NAG)(MUR)-Aq	C25H43N5O15	653.27557	65			5.9	0.06%	0.01%
9	(G)(NAM)-Aq	C25H43N5O15	653.27557	66.4			7.6	0.06%	0.03%
10	(NAG)(anNAM)-Aq	C27H43N5O15	677.27557	MS1			10.1	2.12%	0.70%
11	(NAG)(anNAM)-Ae	C27H42N4O16	678.25958	67.5			10.6	0.46%	0.18%
12	(NAG)(NAM)-Aq	C27H45N5O16	695.28613	67.2	68.5		7.4|8.3	9.84%	2.79%
13	(NAG)(NAM)-Ae	C27H44N4O17	696.27015	68.8			7.7|8.9	0.86%	0.36%
14	(DAG)(NAM)-Aq	C29H47N5O17	737.2967	64			9.4|10.6	0.78%	0.31%
15	(NAG)(DAM)-Aq	C29H47N5O17	737.2967	MS1			8.4|9.1	0.27%	0.09%
16	(NAG)(DAM)-Ae	C29H46N4O18	738.28071	MS1			9.7	0.03%	0.01%
17	(DAG)(NAM)-Ae	C29H46N4O18	738.28071	63.2			10.2|11.2	0.09%	0.03%

SN	Compound	Formula	Monoisotopic Mass	MS-DIAL score			RT / min	Rel. Abundance	
				[M+H]+	[M+2H]2+	[M+3H]3+		Mean	S.D
18	(NAG)(anNAM)-AqO[3-NH2-SD]	C39H63N9O21	993.41385		65.9		9.1	0.14%	0.10%
19	(NAG)(anNAM)-AeO[3-NH2-SD]	C39H62N8O22	994.39787	63.7	69.4		9.3	0.13%	0.02%
20	(NAG)(NAM)-AqO[3-NH2-SD]	C39H65N9O22	1011.42441	70.6	74.5		6.9|7.6	1.01%	0.37%
21	(NAG)(NAM)-AeO[3-NH2-SD]	C39H64N8O23	1012.40843	67.8	77.7		7.3|7.9	0.34%	0.24%
22	(G)(NAM)-AqOA[3-NH2-SD]	C40H68N10O22	1040.45096		66		6.9|7.7	0.22%	0.04%
23	(NAG)(anNAM)-AqOG[3-NH2-SD]	C41H66N10O22	1050.43531	MS1	70.5		9.1	1.12%	0.72%
24	(DAG)(NAM)-AqO[3-NH2-SD]	C41H67N9O23	1053.43498		MS1		9.4	0.05%	0.01%
25	(DAG)(NAM)-AeO[3-NH2-SD]	C41H66N8O24	1054.41899		75.1		9.7	0.03%	0.03%
26	(NAG)(anNAM)-AqOA[3-NH2-SD]	C42H68N10O22	1064.45096	MS1	MS1		9.6	3.81%	1.80%
27	(NAG)(anNAM)-AeOA[3-NH2-SD]	C42H67N9O23	1065.43498	MS1	68.4		9.9	0.73%	0.32%
28	(NAG)(NAM)-AqOG[3-NH2-SD]	C41H68N10O23	1068.44588	66.7	70.5		6.8|7.7	2.25%	0.69%
29	(NAG)(NAM)-AqOA[3-NH2-SD]	C42H70N10O23	1082.46153	63.1	75.5		7.5|8.2	22.54%	2.60%
30	(NAG)(NAM)-AeOA[3-NH2-SD]	C42H69N9O24	1083.44554	68.4	71.8		7.9|8.5	2.27%	0.49%
31	(NAG)(NAM)-AqKA[3-NH2-SD]	C43H72N10O23	1096.47718	65.3	74.4		8.2|8.8	2.07%	0.92%
32	(NAG)(NAM)-AeKA[3-NH2-SD]	C43H71N9O24	1097.46119		MS1		8.5|9.1	0.20%	0.06%
33	(NAG)(anNAM)-AqOAG[3-NH2-SD]	C44H71N11O23	1121.47243		66.2		9.3	0.13%	0.06%
34	(NAG)(DAM)-AqOA[3-NH2-SD]	C44H72N10O24	1124.47209		MS1		8.3|8.9	0.67%	0.27%
35	(DAG)(NAM)-AqOA[3-NH2-SD]	C44H72N10O24	1124.47209	MS1	66.2		9.9	0.67%	0.07%
36	(NAG)(DAM)-AeOA[3-NH2-SD]	C44H71N9O25	1125.45611		66.8		9.1	0.05%	0.01%
37	(NAG)(anNAM)-AqOAA[3-NH2-SD]	C45H73N11O23	1135.48808	MS1	67.7		9.9	1.58%	0.85%
38	(NAG)(anNAM)-AeOAA[3-NH2-SD]	C45H72N10O24	1136.47209		MS1		10.2	0.13%	0.08%
39	(NAG)(NAM)-AqOAG[3-NH2-SD]	C44H73N11O24	1139.48299	MS1	67.6		7.2|7.9	5.51%	2.56%
40	(NAG)(NAM)-AeOAG[3-NH2-SD]	C44H72N10O25	1140.46701		68.1		7.6|8.2	0.51%	0.17%
41	(NAG)(NAM)-AqOAA[3-NH2-SD]	C45H75N11O24	1153.49864	62.9	67.8		7.9|8.5	17.59%	2.12%
42	(NAG)(NAM)-AeOAA[3-NH2-SD]	C45H74N10O25	1154.48266	62.1	64.7		8.2|8.8	3.00%	1.34%
43	(NAG)(NAM)-AqKAA[3-NH2-SD]	C46H77N11O24	1167.51429		71.6		8.4|9.0	1.61%	0.93%
44	(NAG)(NAM)-AeKAA[3-NH2-SD]	C46H76N10O25	1168.49831		69.4		8.7|9.3	0.24%	0.16%
45	(NAG)(DAM)-AqOAA[3-NH2-SD]	C47H77N11O25	1195.50921		62.6		8.6|9.2	0.41%	0.27%

SN	Compound	Formula	Monoisotopic Mass	MS-DIAL score			RT / min	Rel. Abundance	
				[M+H]+	[M+2H]2+	[M+3H]3+		Mean	S.D
46	(DAG)(NAM)-AqOAA[3-NH2-SD]	C47H77N11O25	1195.50921		MS1		10.2	0.12%	0.02%
47	(NAG)(NAM)-AqOAN[3-NH2-SD]	C46H76N12O25	1196.50446		68.4		6.9|7.6	0.23%	0.11%
48	(NAG)(NAM)-AqOAD[3-NH2-SD]	C46H75N11O26	1197.48847		69.1		7.3|8.0	1.57%	0.34%
49	(G)(NAM)-AqOA[3-NH2-SD]; (G-G)-(NAG)(NAM)-AqOA[3-NH2-SD]	C82H136N20O44	2104.90193		64.8	74.7	8.0|8.3	0.53%	0.54%
50	(NAG)(NAM)-AqOG[3-NH2-SD]; (3br-4)-(NAG)(NAM)-AqOA[3-NH2-SD]	C83H136N20O45	2132.89684		MS1		9.0	0.14%	0.17%
51	(NAG)(NAM)-AqOA[3-NH2-SD]; (3br-4)-(NAG)(NAM)-AqOA[3-NH2-SD]	C84H138N20O45	2146.91249		61.9	MS1	9.3	0.71%	0.67%
52	(NAG)(NAM)-AqOA[3-NH2-SD]; (G-G)-(NAG)(NAM)-AqOA[3-NH2-SD]	C84H138N20O45	2146.91249		69.3	MS1	8.5|8.8	2.03%	0.99%
53	(NAG)(NAM)-AqOA[3-NH2-SD]; (3br-4)-(NAG)(NAM)-AeOA[3-NH2-SD]	C84H137N19O46	2147.89651		62.4	61.2	9.5	0.22%	0.23%
54	(G)(NAM)-AqOAA[3-NH2-SD]; (G-G)-(NAG)(NAM)-AqOA[3-NH2-SD]	C85H141N21O45	2175.93904			MS1	8.1|8.5	0.17%	0.17%
55	(NAG)(DAM)-AqOA[3-NH2-SD]; (G-G)-(NAG)(NAM)-AqOA[3-NH2-SD]	C86H140N20O46	2188.92306		73.2	74.4	9.2	0.59%	0.17%
56	(NAG)(NAM)-AqOAA[3-NH2-SD]; (G-G)-(NAG)(NAM)-AqOA[3-NH2-SD]	C87H143N21O46	2217.94961			67.6	9.0	0.40%	0.20%
57	(NAG)(NAM)-AqOAA[3-NH2-SD]; (3br-4)-(NAG)(NAM)-AqOA[3-NH2-SD]	C87H143N21O46	2217.94961		MS1	61.2	9.1|9.5	1.37%	0.65%
58	(G)(NAM)-AqOAA[3-NH2-SD]; (G-G)-(NAG)(NAM)-AqOAA[3-NH2-SD]	C88H146N22O46	2246.97616			68.1	8.3|8.6	0.15%	0.12%
59	(NAG)(NAM)-AqOAA[3-NH2-SD]; (G-G)-(NAG)(NAM)-AqOAA[3-NH2-SD]	C90H148N22O47	2288.98672		61.4	68.2	8.8|9.1	2.20%	1.22%
60	(NAG)(DAM)-AqOAA[3-NH2-SD]; (G-G)-(NAG)(NAM)-AqOAA[3-NH2-SD]	C92H150N22O48	2330.99728			72.1	9.5	0.21%	0.11%

Table S13

Representative TIC and identified peptidoglycan in *B. infantis* ATCC15697.



SN	Compound	Formula	Monoisotopic Mass	MS-DIAL score			RT / min	Rel. Abundance	
				[M+H]+	[M+2H]2+	[M+3H]3+		Mean	S.D
1	(NAG)(NAM)-None	C19H32N2O13	496.19044	80.1			5.9	0.13%	0.03%
2	(NAG)(anNAM)-A	C22H35N3O13	549.21699	74.6			10.7	0.31%	0.05%
3	(NAG)(NAM)-A	C22H37N3O14	567.22755	72.9			8.0|8.9	0.47%	0.09%
4	(NAG)(anNAM)-Aq	C27H43N5O15	677.27557	64			10.1	0.44%	0.12%
5	(NAG)(anNAM)-Ae	C27H42N4O16	678.25958	67.3			10.6	0.07%	0.01%
6	(NAG)(NAM)-Aq	C27H45N5O16	695.28613	MS1			7.4	0.16%	0.01%
7	(NAG)(NAM)-Ae	C27H44N4O17	696.27015	69.6			8.9	0.07%	0.02%
8	(NAG)(NAM)-AqO[3-NH2-SATA]	C45H77N11O23	1139.51938	61.4	70.9		7.7|8.4|8.5	0.73%	0.05%
9	(NAG)(anNAM)-AqOG[3-NH2-SATA]	C47H78N12O23	1178.53028	MS1	68.9		9.4	0.35%	0.12%
10	(NAG)(anNAM)-AqOA[3-NH2-SATA]	C48H80N12O23	1192.54593		65.4		9.9	3.64%	0.29%
11	(NAG)(anNAM)-AeOA[3-NH2-SATA]	C48H79N11O24	1193.52994	65	68.6		10.2	0.53%	0.04%
12	(NAG)(NAM)-AqOG[3-NH2-SATA]	C47H80N12O24	1196.54084	MS1	63.9		7.6|8.3	0.58%	0.18%
13	(NAG)(NAM)-AqOA[3-NH2-SATA]	C48H82N12O24	1210.55649	MS1	69.7		8.2|8.7	19.81%	1.92%
14	(NAG)(NAM)-AeOA[3-NH2-SATA]	C48H81N11O25	1211.54051	61.1	71.8		8.5|9.0	2.26%	0.45%
15	(NAG)(NAM)-AqKA[3-NH2-SATA]	C49H84N12O24	1224.57214	61.5	69.1		8.9|9.4	1.63%	0.65%
16	(NAG)(NAM)-AeKA[3-NH2-SATA]	C49H83N11O25	1225.55616		72		9.6	0.12%	0.09%
17	(NAG)(DAM)-AqOA[3-NH2-SATA]	C50H84N12O25	1252.56706		63.2		8.9|9.4	0.37%	0.31%

SN	Compound	Formula	Monoisotopic Mass	MS-DIAL score			RT / min	Rel. Abundance	
				[M+H]+	[M+2H]2+	[M+3H]3+		Mean	S.D
18	(NAG)(anNAM)-AqOAA[3-NH2-SATA]	C51H85N13O24	1263.58304	MS1	61.6		10.1	0.80%	0.12%
19	(NAG)(anNAM)-AeOAA[3-NH2-SATA]	C51H84N12O25	1264.56706		61.8		10.4	0.06%	0.01%
20	(NAG)(NAM)-AqOAG[3-NH2-SATA]	C50H85N13O25	1267.57796	MS1	62.8		8.4	0.57%	0.23%
21	(NAG)(NAM)-AqOAA[3-NH2-SATA]	C51H87N13O25	1281.59361	MS1	63.4		8.4|8.9	29.01%	2.73%
22	(NAG)(NAM)-AeOAA[3-NH2-SATA]	C51H86N12O26	1282.57762	MS1	66.4		8.7|9.2	2.72%	0.38%
23	(NAG)(NAM)-AqKAA[3-NH2-SATA]	C52H89N13O25	1295.60926		61.3		8.9	0.36%	0.03%
24	(NAG)(DAM)-AqOAA[3-NH2-SATA]	C53H89N13O26	1323.60417		MS1		9.5	0.07%	0.08%
25	(NAG)(NAM)-AqO[3-NH2-SATA]; (3br-4)-(NAG)(NAM)-AqOA[3-NH2-SATA]	C93H157N23O46	2332.0653			MS1	9.0	0.12%	0.05%
26	(NAG)(NAM)-AqOA[3-NH2-SATA]; (G-G)-(NAG)(NAM)-AqOA[3-NH2-SATA]	C96H162N24O47	2403.10242		69.1	66	8.9|9.2	7.41%	1.76%
27	(NAG)(NAM)-AqOA[3-NH2-SATA]; (3br-4)-(NAG)(NAM)-AqOA[3-NH2-SATA]	C96H162N24O47	2403.10242		MS1	MS1	10.3	1.09%	0.99%
28	(NAG)(NAM)-AqOA[3-NH2-SATA]; (G-G)-(NAG)(NAM)-AeOA[3-NH2-SATA]	C96H161N23O48	2404.08643		70.4	63.2	9.3	0.99%	0.27%
29	(NAG)(NAM)-AqOA[3-NH2-SATA]; (G-G)-(NAG)(NAM)-AqKA[3-NH2-SATA]	C97H164N24O47	2417.11807		66.8	70.1	9.3|9.5	1.37%	0.33%
30	(NAG)(NAM)-AqOA[3-NH2-SATA]; (G-G)-(NAG)(DAM)-AqOA[3-NH2-SATA]	C98H164N24O48	2445.11298			71.8	9.2|9.5	0.24%	0.09%
31	(NAG)(NAM)-AqOAA[3-NH2-SATA]; (3br-4)-(NAG)(NAM)-AqOA[3-NH2-SATA]	C99H167N25O48	2474.13953		MS1	MS1	10.1|10.4	2.94%	2.14%
32	(NAG)(NAM)-AqOAA[3-NH2-SATA]; (G-G)-(NAG)(NAM)-AqOA[3-NH2-SATA]	C99H167N25O48	2474.13953		66.3	67.4	9.0|9.2	5.19%	1.63%
33	(NAG)(NAM)-AeOAA[3-NH2-SATA]; (G-G)-(NAG)(NAM)-AqOA[3-NH2-SATA]	C99H166N24O49	2475.12355		65.6		9.4	0.29%	0.06%
34	(NAG)(NAM)-AqOA[3-NH2-SATA]; (G-G)-(NAG)(NAM)-AqKAA[3-NH2-SATA]	C100H169N25O48	2488.15518		MS1	64.4	9.3|9.6	0.74%	0.18%
35	(NAG)(NAM)-AqOAG[3-NH2-SATA]; (G-G)-(NAG)(NAM)-AqOAA[3-NH2-SATA]	C101H170N26O49	2531.161			60.1	9.1	0.17%	0.04%
36	(NAG)(NAM)-AqOAA[3-NH2-SATA]; (G-G)-(NAG)(NAM)-AqOAA[3-NH2-SATA]	C102H172N26O49	2545.17665		65.8	61.7	9.0|9.3	9.53%	3.89%
37	(NAG)(NAM)-AqOAA[3-NH2-SATA]; (G-G)-(NAG)(NAM)-AeOAA[3-NH2-SATA]	C102H171N25O50	2546.16066		68.5	66	9.3|9.5	3.59%	1.49%
38	(NAG)(NAM)-AqOAA[3-NH2-SATA]; (G-G)-(NAG)(NAM)-AqKAA[3-NH2-SATA]	C103H174N26O49	2559.1923			62.2	9.3|9.6	0.91%	0.24%
39	(NAG)(NAM)-AeOAA[3-NH2-SATA]; (G-G)-(NAG)(DAM)-AqOAA[3-NH2-SATA]	C104H173N25O51	2588.17123			61.1	9.6	0.13%	0.03%

Table S14

Collated results from running BLAST on UniProt for AslIfm (H8L902) and asparagine synthase (Q9CDJ2).[17] Blast was restricted to four species: *E. faecium* (omitted from the first search), *L. lactis* (omitted from the second search), *B. adolescentis, B. bifidum*, *B. infantis*. Only the top 10 results are shown.

Hit	Accession	Organism	Length	Score (Bits)	Identities(%)	Positives(%)	E()
<Target>	H8L902	*E. faecium*					
1	Q9CDJ3	*Lactococcus lactis subsp. lactis*	408	1311	59.7	75.5	0
2	A1A2D4	*Bifidobacterium adolescentis*	414	1154	54.1	71.6	7.50E-158
3	Q9CFV2	*Lactococcus lactis subsp. lactis*	1064	93	20.5	36.8	0.00021
4	Q9CHQ5	*Lactococcus lactis subsp. lactis*	848	65	32.7	61.5	0.45
5	B7GPW2	*Bifidobacterium longum subsp. infantis*	1127	65	20.8	42.1	0.47
6	Q9CJF7	*Lactococcus lactis subsp. lactis*	246	63	25.4	38.6	0.56
7	Q9CGE3	*Lactococcus lactis subsp. lactis*	349	61	27.8	48.1	1.1
8	Q9CIN5	*Lactococcus lactis subsp. lactis*	286	60	22.2	57.1	1.4
9	Q9CH82	*Lactococcus lactis subsp. lactis*	385	60	45.2	52.4	1.6
10	Q9CDR9	*Lactococcus lactis subsp. lactis*	136	54	34.1	46.3	4.9
<Target>	Q9CDJ2	*Lactococcus lactis subsp. lactis*					
1	A1A2C5	*Bifidobacterium adolescentis*	628	2210	65.8	80.7	0
2	A1A0R9	*Bifidobacterium adolescentis*	504	98	31.4	44.2	3.80E-05
3	A1A0M1	*Bifidobacterium adolescentis*	630	90	24.8	37.9	0.00037
4	A1A129	*Bifidobacterium adolescentis*	520	64	32.7	51.9	0.45
5	A1A284	*Bifidobacterium adolescentis*	256	59	24	58	1.4
6	A1A2S5	*Bifidobacterium adolescentis*	690	59	26	56	1.9
7	A0ZZN5	*Bifidobacterium adolescentis*	814	57	24.6	44.7	3.3
8	A1A307	*Bifidobacterium adolescentis*	415	55	32	50	5.1
9	A1A086	*Bifidobacterium adolescentis*	241	54	30.4	46.4	5.7
10	A0ZZG4	*Bifidobacterium adolescentis*	355	53	29.4	61.8	8.5

Table S15

Primers used and generated in this study.

Name	Description	Sequence
BaMltG FWD	*B. adolescentis* MltG (BaMltG) forward primer with NheI site	aacttgGCTAGCAAACATCTCAGGCAGATGAACGCC
BaMltG REV	*B. adolescentis* MltG (BaMltG) reverse primer with XhoI site	cagaatCTCGAGTCAGTTGGCATTCGCGTTGTTG
BbMltG FWD	*B. bifidum* MltG (BbMltG) forward primer with NheI site	aacttgGCTAGCGCCATCAGGGACCGCAACG
BbMltG REV	*B. bifidum* MltG (BbMltG) reverse primer with XhoI site	cagaatCTCGAGTTAGTTCGCGCTCTTGTTGTTGTCCTTG
BiMltG FWD	*B. infantis* MltG (BiMltG) forward primer with NheI site	aacttgGCTAGCAAGCTCATCGCATGGCGCG
BiMltG REV	*B. infantis* MltG (BiMltG) reverse primer with XhoI site	cagaatCTCGAGCTACTGGTTTTCGGCTTGCCACT
BaRpfB-Truncated FWD	*B. adolescentis* truncated RpfB homologue (BaRpfB-Truncated) forward primer with NheI site	gctagcGCCACCGCGGAGAAGTC
BaRpfB-Truncated REV	*B. adolescentis* truncated RpfB homologue (BaRpfB-Truncated) reverse primer with XhoI site	ctcgagTCAGTAGCAGTGGTTGGCGAG
BaRpfB-FL FWD	*B. adolescentis* RpfB homologue (BaRpfB-FL) forward primer with NheI site	ggaatcgctagcCGCAAATCCGTGGCATTG
BaRpfB-FL REV	*B. adolescentis* RpfB homologue (BaRpfB-FL) reverse primer with XhoI site	ggaatcctcgagTCAGTACCATCCGGTCTGTTC
BaMltG_E200A FWD	*B. adolescentis* MltG (BaMltG) forward primer for E200A mutagenesis	CGCCGAATCCgcgGCATGCAATC
BaMltG_E200A REV	*B. adolescentis* MltG (BaMltG) reverse primer with E200A mutagenesis	ATGGACGCGATGTTCATGATACG
β-actin FWD	*β*-actin forward primer for qPCR	CACTGTCGAGTCGCGTCC
β-actin REV	*β*-actin reverse primer for qPCR	TCATCCATGGCGAACTGGTG
IL-1β FWD	*IL-1β* forward primer for qPCR	TGCCACCTTTTGACAGTGATG
IL-1β REV	*IL-1β* reverse primer for qPCR	TGATGTGCTGCTGCGAGATT
TNF-α FWD	*TNF-α* forward primer for qPCR	GTCCCCAAAGGGATGAGAAGTT
TNF-α REV	*TNF-α* reverse primer for qPCR	CTCCTCCACTTGGTGGTTTG
IL-6 FWD	*IL-6* forward primer for qPCR	CGGCCTTCCCTACTTCACAA
IL-6 REV	*IL-6* reverse *primer for qPCR*	TGCCATTGCACAACTCTTTTC

Table S16

Plasmids used and generated in this study.

Name	Description
pET28a(+)	IPTG-inducible protein expression vector; Kanamycin resistant
pET28a(+)-BaMltG	His_6-BaMltG expression vector, Kanamycin resistant
pET28a(+)-BbMltG	His_6-BbMltG expression vector, Kanamycin resistant
pET28a(+)-BiMltG	His_6-BiMltG expression vector, Kanamycin resistant
pET28a(+)-BaMltG_E200A	His_6-BaMltG_E200A expression vector, Kanamycin resistant
pET28a(+)-BaRpfB-Truncated	His_6-BaRpfB-Truncated expression vector, Kanamycin resistant
pET28a(+)-BaRpfB-FL	His_6-BaRpfB-FL expression vector, Kanamycin resistant

Table S17

Strains used and generated in this study.

Strains	
Name	Description
E. coli MG1655	Model organism for peptidoglycan composition.
E. faecium COM15	Model organism for peptidoglycan composition.
E. faecalis OG1RF	Model organism for peptidoglycan composition.
L. plantarum BAA-793	Model organism for peptidoglycan composition.
S. aureus RN450	Model organism for peptidoglycan composition. Gift from Asst. Prof. John Chen (NUS)
S. aureus MW2 (MRSA)	Model organism for peptidoglycan composition.
A. muciniphila DSM 22959	Gut bacterium
B. adolescentis ATCC 15703	Gut bacterium
B. bifidum ATCC 15696	Gut bacterium
B. infantis ATCC 15697	Gut bacterium
F. nucleatum ATCC 25586	Gut bacterium
E. coli DH5*α*	Competent cells for transformation
E. coli BL21 (DE3)-RIPL	Competent cells for transformation
E. coli BL21 (DE3)-RIPL - BaMltG	:: pET28a(+)-BaMltG
E. coli BL21 (DE3)-RIPL - BbMltG	:: pET28a(+)-BbMltG
E. coli BL21 (DE3)-RIPL - BiMltG	:: pET28a(+)-BiMltG
E. coli BL21 (DE3)-RIPL - BaMltG_E200A	:: pET28a(+)-BaMltG_E200A
E. coli BL21 (DE3)-RIPL - BaRpfB-Truncated	:: pET28a(+)-BaRpfB-Truncated
E. coli BL21 (DE3)-RIPL - BaRpfB-FL	:: pET28a(+)-BaRpfB-FL

Table S18

Conditions for recombinant protein overexpression and purification.

Strains	MW / kDa	Buffer B	Induction Conditions
BaMltG	34	50 mM HEPES pH 7.5 150 mM NaCl 1% Triton X-100 10% glycerol	OD 0.7, 0.5 mM IPTG, 15 hr incubation at 20°C
BbMltG	35		
BiMltG	35		
BaRpfB-Truncated	30	50 mM NaH_2PO_4 pH 8.0, 300 mM NaCl	OD 0.8, 0.25 mM IPTG, 16 hr incubation at 16°C
BaRpfB-FL	50		

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