

Supplementary materials

Journal name:

Applied Microbiology and Biotechnology

Title:

Novel recombinant aminoacylase from *Paraburkholderia monticola* capable of N-acyl-amino acid synthesis

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Supplementary

Protein sequence of PmAcy (N-terminal Strep-tag and linker underlined):

MWSHPQFEKSGMLSLVLPGLSQAQSTLPPAPPAPKPVLFNTNFRFLFDGKSMTLRDGLYMVVEGNSISQLG
QGQPASVEGKTLVDCGGKVMMPGLIDMHWALLAALPIQVILQSDIAFVHLAASAEARTLLRGFTTI
RDAGGPSFALKQAIDSGMISGPRIYPSGAMITTTGGHGDFRPLTDLPTSSQVTQGERDGGFAIADTE
DEMRVRVREQFIQGATQIKLVGSGGVSTPRSPDLMLTFTEKQLRAAVETAADWGTYYVLSHAYTPEAVQ
RSVAAGAQCIEHGHLMDDKTAALMAKNGTWLSTQPFISEEDVGPLAPQSREKFLEVAVAGTDNAFRLAR
KHGIKVAFGTDLLFSQAIATRQGTMLTHMKRWYSPAELGMATGTNGQLLALTGKRNPYPRLGVLEE
GAYADLLLVDGNPLENLDLIANPEQNLRIVMKDGKFKYKNTLKA

Codon -optimized sequence with N-terminal strep-tag (as ordered from GeneArt, Thermo Fisher); GenBank OR188138:

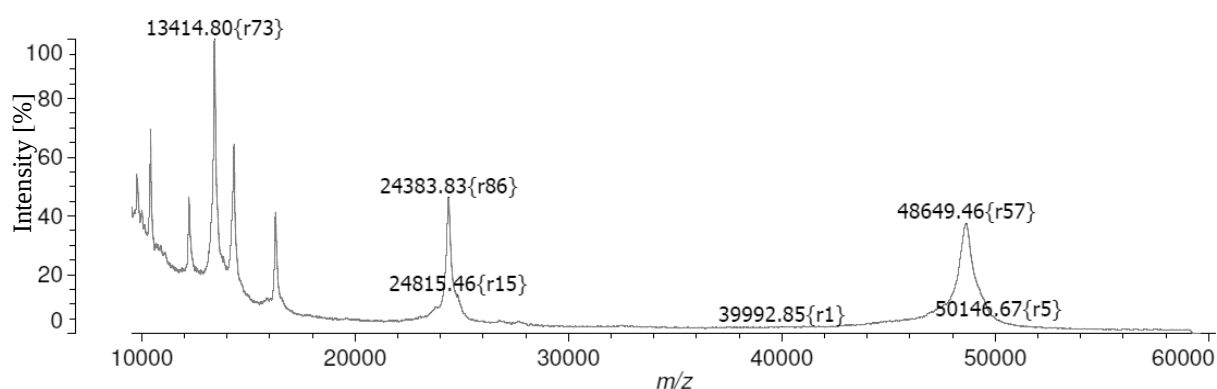
ATGTGGTCACATCCGCAGTTTGAAAAAGCGGTATGCTGAGCCTGGTTCTGCCTGGTCTGAG
CCAGGCACAGAGCACCTGCCTCCGGCACCGCCTGCAAAACCGGTTCTGTTTACCAATTTTC
GTCTGTTTGATGGCAAAAGCATGACCTGCGTGATGGTCTGTATATGGTTGTTGAAGGTAAT
AGCATTAGCCAGTTAGGTCAGGGTCAGCCTGCAAGCGTGGAAGGTAAAACCTGGTTGATTG
TGGTGGTAAAGTTATGATGCCAGGTCTGATTGATATGCATTGGCATGCACTGCTGGCAGCAC
TGCCGATTCAGGTTATTCTGCAGAGCGATATTGCATTTGTTTCATCTGGCAGCAAGCGCAGAA
GCAGAACGTACCCTGCTGCGTGGTTTTACCACAATTCGTGATGCCGGTGGTCCGAGCTTTGC
ACTGAAACAGGCAATTGATAGCGGTATGATTAGCGGTCCGCGTATTTATCCGAGCGGTGCAA
TGATTACCACAACCGGTGGTCATGGTGATTTTCGTCCGCTGACCGATCTGCCTCGTACCAGC
AGCCAGGTTACCCAGGGTGAACGTGATGGTGGTTTTGCAATTGCCGATACCGAAGATGAAAT
GCGTGTTCTGTGTGCGTGAACAGTTTATTCAGGGTGCAACACAGATTAAACTGGTTGGTAGCG
GTGGTGTTAGCACACCGCGTAGTCCGCTGGATATGCTGACCTTTACCGAAAAACAGCTGCGT
GCAGCAGTTGAAACCGCAGCAGATTGGGGCACCTATGTTCTGAGCCATGCATATACACCGGA
AGCAGTTCAGCGTAGCGTTGCAGCCGGTGCACAGTGTATTGAACATGGTCATCTGATGGATG
ATAAAACAGCAGCACTGATGGCCAAAAATGGCACCTGGCTGAGCACCCAGCCGTTTATTAGC
GAAGAAGATGTTGGTCCGCTGGCACCGCAGAGCCGTGAAAAATTTCTGGAAGTTGTTGCAGG
CACCGATAATGCATTTTCGTCTGGCACGTAAACATGGTATTAAAGTTGCATTTGGTACGGATC
TGCTGTTTAGCCAGGCCATTGCAACCCGTCAGGGCACCATGCTGACCCATATGAAACGTTGG
TATAGCCCTGCCGAAGCATTAGGTATGGCAACCGGCACCAATGGTCAGCTGCTGGCACTGAC
CGGTAAACGTAATCCGTATCCGCGTCGTCTGGGTGTTCTGGAAGAAGGTGCATACGCCGATT
TACTGCTGGTTGATGGTAACCCGCTGGAAAATCTGGATCTGATTGCAAATCCGGAACAGAAT
CTGCGTATTGTGATGAAAGATGGCAAGTTCTATAAGAACACCCTGAAAGCA**AGCGGTTGGAG**
CCATCCTCAGTTCGAGAAATGA

Primer sequences for cloning:

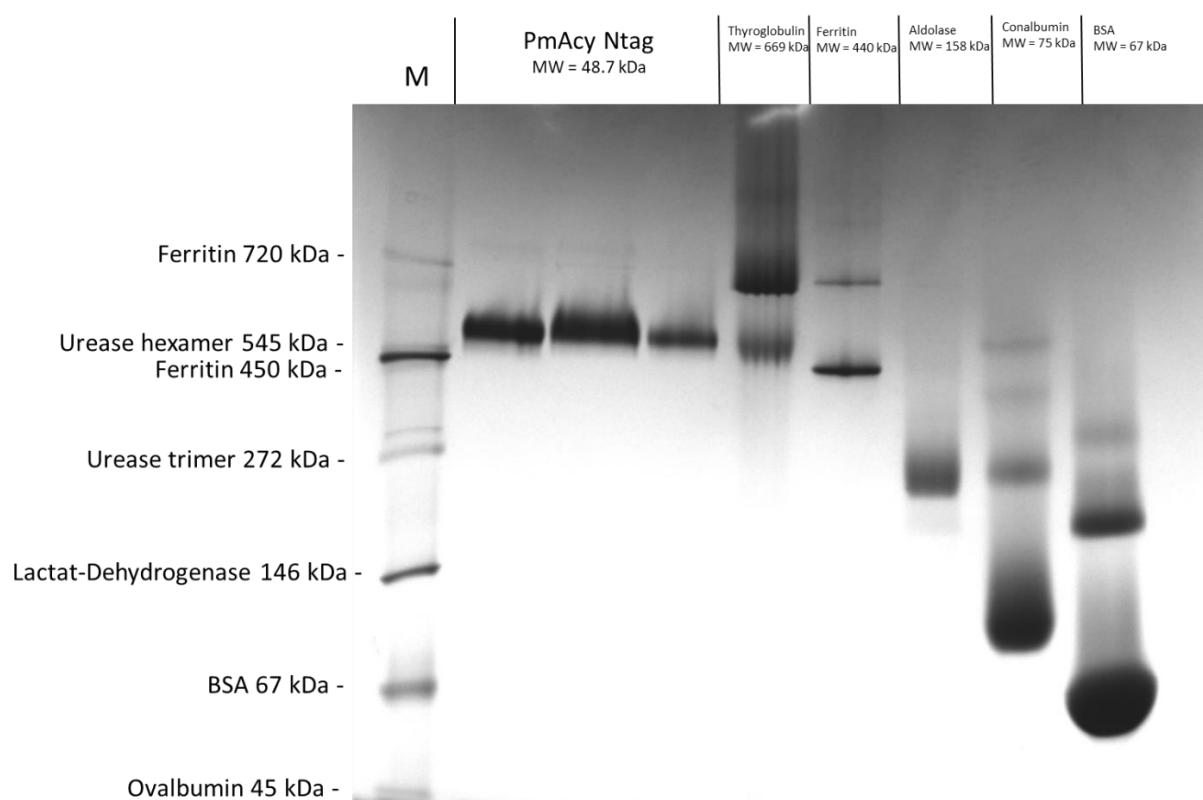
Table S1: Primer sequences used for amplification and cloning of *pmAcy Ntag*. BsaI-sites are highlighted in bold font.

No.	Name	Sequence 5'→3'
P1	NTag for:	GGTCTC CCCATGTGGTCACATCCGCAGTTTGAAAAAG
P2	noTag for:	GGTCTC CCCATGCTGAGCCTGGTTCTGCC
P3	Ctag rev:	GGTCTC TCTCATTTCTCGAACTGAGGATGGCTC
P4	noTag rev:	GGTCTC TCTCATGCTTTCAGGGTGTTCCTTATAGAACTTGC

MALDI-TOF analysis:



Native PAGE:



Michaelis-Menten plot:

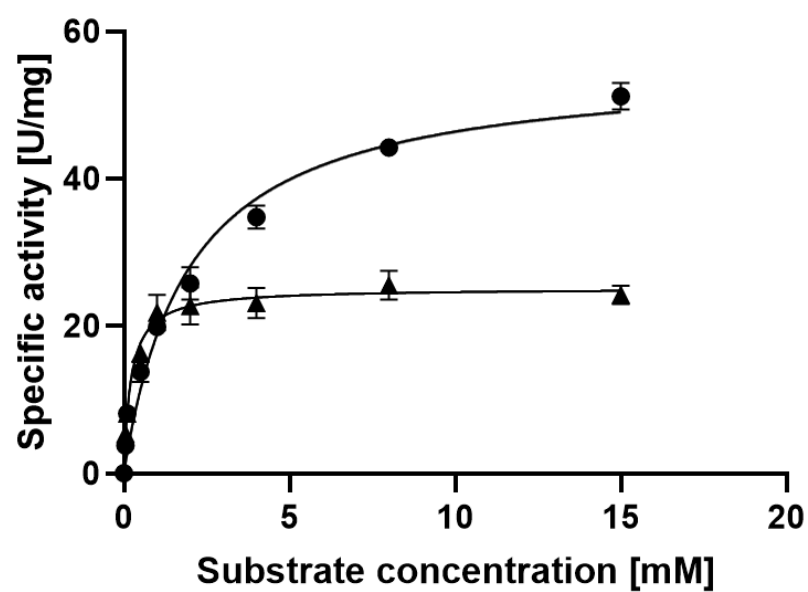
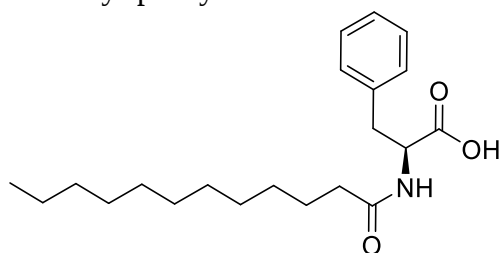


Figure S3: Michaelis-Menten hydrolysis kinetics of PmAcy NTag against lauroyl-alanine (●) and lauroyl-phenylalanine (▲). Non-linear fit to generate Michaelis-Menten plot was conducted with GraphPad Prism 8.

Analytical Data:
N-lauroyl-phenylalanine



LC-MS: (EI, 70 eV): m/z [%] = 695 (2M+H, 80), 411 (M+ACN+Na, 10), 389 (M+ACN+H, 100), 348 (M+H, 100).

$^1\text{H-NMR}$ (CDCl_3 , 400 MHz) δ = 0.88 (t, 3J = 7 Hz, 3H), 1.19 – 1.36 (m, 17 H), 1.48 – 1.59 (m, 2 H), 2.182 (dt, 2J = 3.2 Hz, 3J = 7.2 Hz, 2 H), 3.09 – 3.28 (m, 2 H), 4.88 (q, 3J = 5.9 Hz, 1 H), 6.09 (d, 3J = 7.5 Hz, 1 H), 7.13 – 7.18 (m, 2 H), 7.22 – 7.33 (m, 3 H).

$^{13}\text{C-NMR}$ (CDCl_3 , 100 MHz) δ = 14.14, 22.70, 25.59, 29.16, 29.32, 29.35, 29.48, 29.63, 29.93, 36.44, 37.27, 53.14, 127.22, 128.64, 129.38, 135.69, 174.14, 174.62.

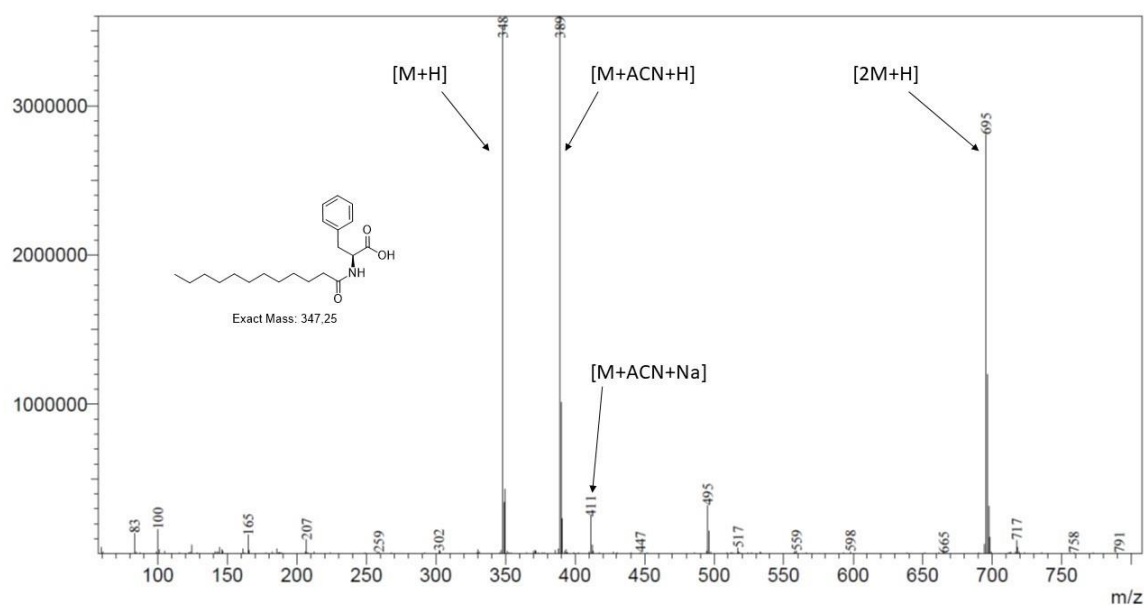


Figure S4: Mass-spectrum of N-lauroyl-phenylalanine, produced with PmAcy, isolated by acidic precipitation with 5 N HCl.

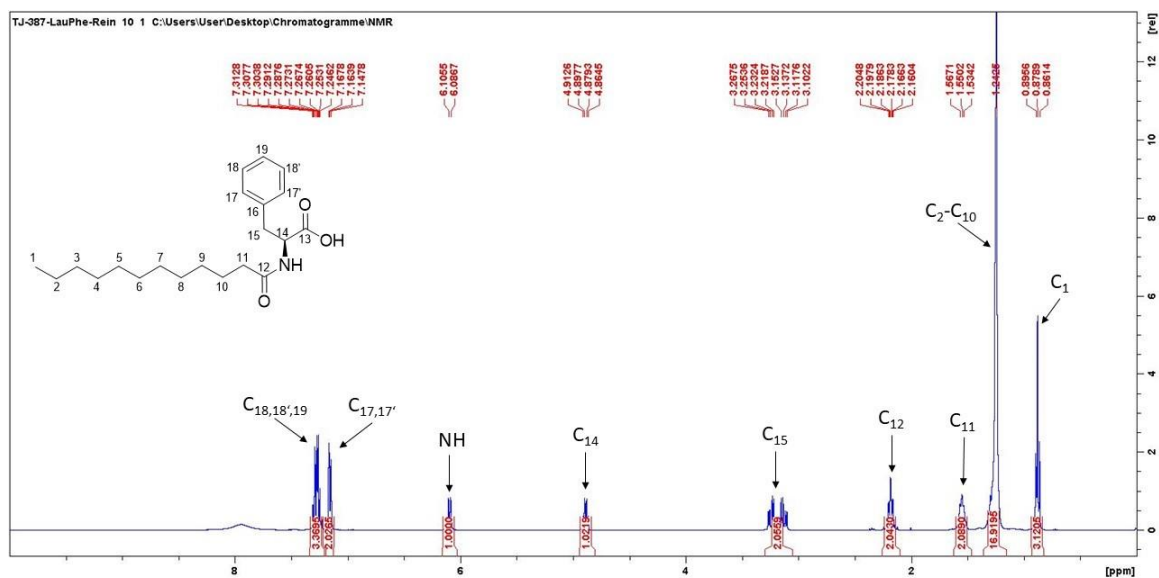


Figure S5: ^1H -NMR-spectrum of N-lauroyl-phenylalanine, produced with PmAcy, isolated by acidic precipitation with 5 N HCl.

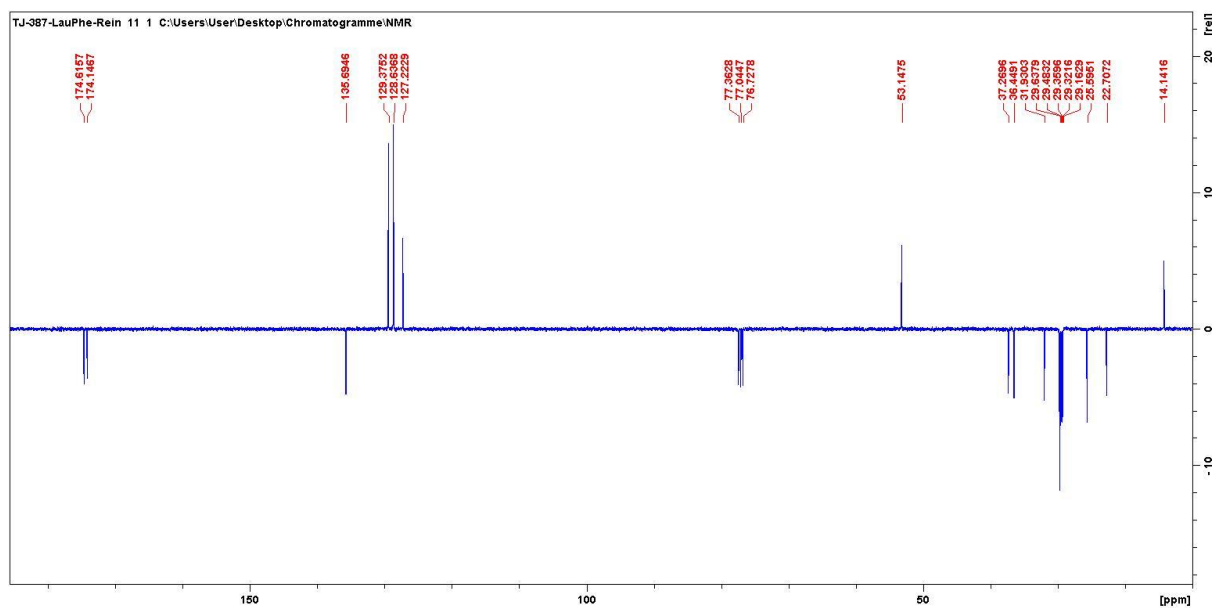


Figure S6: ^{13}C -NMR-spectrum of N-lauroyl-phenylalanine, produced with PmAcy, isolated by acidic precipitation with 5 N HCl.