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Biotic and abiotic transformation of amino acids in cloud water: experimental studies and atmospheric implications

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Table S1: Composition of the artificial cloud medium used for biotic and abiotic transformation of amino acids in microcosms.

Chemical species	Concentration (µM)	Chemical species	Concentration (µM)
Acetate	100	cysteine	1
Formiate	72.5	glutamic acid	1
Succinate	7.5	glycine	1
Oxalate	15	histidine	1
Cl^-	200	isoleucine	1
NO_3^-	400	lysine	1
SO_4^{2-}	25	methionine	1
Na^+	1000	phenylalanine	1
NH_4^+	400	proline	1
K^+	25	serine	1
Mg^{2+}	50	threonine	1
Ca^{2+}	200	tryptophan	1
alanine	1	tyrosine	1
arginine	1	valine	1
asparagine	1	glutamine	1
aspartic acid	1	pH[a]	6

[a] The pH of the artificial cloud medium was adjusted to 6 (a few drops of NaOH at 1.38 M and of 0.39M H_2SO_4) and the medium was sterilized by filtration on a polyethersulphone (PES) membrane of 0.20 µm porosity (Fisher Scientific) before use. Note that cysteine was present in the medium but it could not be assayed by LC-HRMS, neither leucine that cannot be distinguished from isoleucine by LC-HRMS.

Table S2: Retention times, exact masses and LODs and LOQs measured for the 18 AA measured by UPLC-HRMS

Amino acid	Molecular formula	Retention time (min)	m/z [M+H]	LOD (μM)	LOQ (μM)
ALA	$C_3H_7NO_2$	4.25	90.0550	0.237	0.474
ARG	$C_6H_{14}N_4O_2$	7.41	175.1190	0.072	0.143
ASN	$C_4H_8N_2O_3$	5.57	133.0608	0.143	0.286
ASP	$C_4H_7NO_4$	5.09	134.0448	0.148	0.295
GLN	$C_5H_{10}N_2O_3$	5.35	147.0764	0.234	0.468
GLU	$C_5H_9NO_4$	4.8	148.0526	0.111	0.222
GLY	$C_2H_5NO_2$	4.6	76.0393	0.242	0.483
HIS	$C_6H_9N_3O_2$	7.47	156.0768	0.094	0.188
ILE	$C_6H_{13}NO_2$	2.59	160.1081	0.179	0.359
LYS	$C_6H_{14}N_2O_2$	7.62	147.1128	0.069	0.139
MET	$C_5H_{11}NO_2S$	2.69	150.0584	0.072	0.144
PHE	$C_9H_{11}NO_2$	2.64	166.0863	0.061	0.122
PRO	$C_5H_9NO_2$	3.05	116.0706	0.140	0.281
SER	C_3H7NO_3	5.36	106.0499	0.135	0.268
THR	$C_4H_9NO_3$	4.87	120.0655	0.163	0.327
TRP	$C_{11}H_{12}N_2O_2$	2.65	205.0972	0.058	0.117
TYR	$C_9H_{11}NO_3$	7.46	182.0812	0.072	0.143
VAL	$C_5H_{11}NO_2$	2.61	118.0863	0.237	0.475

Table S3: Relative standard deviation (RSD = Standard deviation/mean) for each AA based on calibration curves (3 technical replicates).

	Relative Standard Deviation (RSD = Standard deviation/mean)		
Amino acid	**0.1 µM (n = 3)**	**0.5 µM (n = 3)**	**1 µM (n = 3)**
ALA		**0.71%**	**3.61%**
ARG	**0.83%**	**1.96%**	**1.56%**
ASN	**5.23%**	**4.92%**	**3.63%**
ASP		**10.77%**	**5.96%**
GLN	**4.19%**	**4.37%**	**3.20%**
GLU	**3.77%**	**2.89%**	**3.92%**
GLY			**21.39%**
HIS	**0.62%**	**0.89%**	**1.22%**
ILE	**4.48%**	**0.48%**	**0.59%**
LYS	**6.64%**	**1.96%**	**1.50%**
MET	**4.49%**	**4.35%**	**6.38%**
PHE	**4.63%**	**1.68%**	**1.02%**
PRO	**11.67%**	**5.08%**	**1.28%**
SER	**14.34%**	**3.06%**	**3.20%**
THR	**14.15%**	**3.67%**	**1.06%**
TRP	**7.00%**	**1.67%**	**1.75%**
TYR	**0.94%**	**1.81%**	**1.15%**
VAL	**17.94%**	**2.98%**	**11.41%**

Table S4: Rate constants for 18 amino acids for the OH, O_3 and $^1O_2$ reactions. As most rate constants are only available at or near room temperature, we chose this temperature for all constants.

	k_{OH} / $M^{-1} s^{-1}$	Reference	k_{O3} / $M^{-1} s^{-1}$	Reference	k_{1O2} / $M^{-1} s^{-1}$	Reference
ALA	$7.7 \cdot 10^7$	(Scholes et al., 1965)	$2.5 \cdot 10^1$	(Ignatenko and Cherenkevich, 1985)	$2 \cdot 10^6$	(Matheson and Lee, 1979)
ARG	$3.5 \cdot 10^9$	(Buxton et al., 1988)	$2.8 \cdot 10^2$	(Ignatenko and Cherenkevich, 1985)	$< 1 \cdot 10^6$	(Kraljić and Sharpatyi, 1978)
ASN	$4.9 \cdot 10^7$	(MASUDA et al., 1973)	$7.0 \cdot 10^1$	(Ignatenko and Cherenkevich, 1985)		
ASP	$4.9 \cdot 10^7$	(MASUDA et al., 1973)	$5.0 \cdot 10^1$	(Ignatenko and Cherenkevich, 1985)		
GLN	$5.4 \cdot 10^8$	(MASUDA et al., 1973)	$8.0 \cdot 10^1$	(Ignatenko and Cherenkevich, 1985)		
GLU	$1.6 \cdot 10^8$	(Scholes et al., 1965)	$2 \cdot 10^{-1}$	(Ignatenko and Cherenkevich, 1985)	$5.0 \cdot 10^5$	(McGregor and Anastasio, 2001)
GLY	$1.7 \cdot 10^7$	(Scholes et al., 1965)	$2.1 \cdot 10^1$	(Ignatenko and Cherenkevich, 1985)	$< 1 \cdot 10^5$	(Michaeli and Feitelson, 1994)
HIS	$5 \cdot 10^9$	(Motohashi and Saito, 1993)	$3.9 \cdot 10^3$	(Ignatenko and Cherenkevich, 1985)	$6 \cdot 10^7$	(McGregor and Anastasio, 2001)
ILE	$1.8 \cdot 10^9$	(MASUDA et al., 1973)				
LYS	$3.5 \cdot 10^8$	(MASUDA et al., 1973)	$1.2 \cdot 10^2$	(Ignatenko and Cherenkevich, 1985)		
MET	$8.5 \cdot 10^9$	(Adams et al., 1965)	$4\ 10^6$	(Pryor et al., 1984)	$2.1 \cdot 10^7$	(Miskoski and García, 1993)
PHE	$6.5 \cdot 10^9$	(Buxton et al., 1988)	$1.3 \cdot 10^3$	(Ignatenko and Cherenkevich, 1985)	$7 \cdot 10^5$	(Michaeli and Feitelson, 1994)
PRO	$6.5 \cdot 10^8$	(MASUDA et al., 1973)	$4.8 \cdot 10^2$	(Ignatenko and Cherenkevich, 1985)		
SER	$2.5 \cdot 10^8$	(Scholes et al., 1965)	$1.8 \cdot 10^2$	(Ignatenko and Cherenkevich, 1985)		
THR	$5.1 \cdot 10^8$	(MASUDA et al., 1973)	$2.6 \cdot 10^2$	(Ignatenko and Cherenkevich, 1985)		

TRP	$1.3 \cdot 10^{10}$	(Buxton et al., 1988)	$5.6 \cdot 10^{4}$	(Ignatenko and Cherenkevich, 1985)	$4.1 \cdot 10^{7}$	(McGregor and Anastasio, 2001)
TYR	$1.3 \cdot 10^{10}$	(Solar et al., 1984)	$4.8 \cdot 10^{3}$	(Ignatenko and Cherenkevich, 1985)	$5 \cdot 10^{6}$	(McGregor and Anastasio, 2001)
VAL	$8.5 \cdot 10^{8}$	(Prütz and Vogel, 1976)	$4 \cdot 10^{1}$	(Ignatenko and Cherenkevich, 1985)		

Table S5: Selected experimental studies of amino acid oxidation by various oxidants. Note that the experimental conditions were not necessarily atmospherically-relevant. Products are only listed to demonstrate the wide variety of possible reaction pathways and products.

Amino acid	Oxidant	Main product(s)	Reference
ARG	Fenton chemistry	glutamic semialdehyde	(Stadtman, 1993; Stadtman and Levine, 2003) and reference therein
ASP	OH	NH_3,Malonic, oxalic and formic acids	(Marion et al., 2018)
CYS	Fenton chemistry	-S-S-disulfide cross-links	(Stadtman, 1993; Stadtman and Levine, 2003)
GLY	OH	Oxalic, formic, oxamic acids	(Berger et al., 1999)
GLY	O_3	Nitrate, nitrites	(Berger et al., 1999)
GLY-ALA-VAL-PRO peptides	OH	Hydroperoxides, alcohols	(Morgan et al., 2012)
HIS	O_3	PRO	(Mudd et al., 1969)
HIS	Fenton chemistry	ASP, ASN, 2-oxoimidazoline	(Stadtman, 1993; Stadtman and Levine, 2003) and references therein
LEU	OH	Isovaleric acid and other carbonyl compounds	
LYS	Fenton chemistry	2-amino-adipicsemialdehyde	(Stadtman, 1993; Stadtman and Levine, 2003) and references therein
MET	O_3	Methionine sulfoxide	(Mudd et al., 1969)
PHE	ROS	TYR	(Stadtman, 1993; Stadtman and Levine, 2003)
PHE	Direct UV absorption	TYR	(Pattison et al., 2012)
PRO	Fenton chemistry	GLU, pyroglutamate, Cis/trans-4-hydroxyproline, 2-pyrrolidone, glutamic semialdehyde	(Stadtman, 1993; Stadtman and Levine, 2003) and reference therein
SER	OH	carbonyl and carboxylic acid	
THR	Fenton chemistry	2-amino-3-ketobutyric acid	(Stadtman, 1993; Stadtman and Levine, 2003) and references therein
THR	O_3	Dihydroxyphenylalanine	(Mudd et al., 1969)
TRP	OH	Formic and acetic acids, many aromatic	(Bianco et al., 2016)

		intermediates	
TRP	$^1O_2$	3α-hydroxypyrroloindole; *N*-formylkynurenine, kynurenine, 3α-dihydroxypyrroloindole.	
TYR	OH	Enedial	(Prasse et al., 2018)
TYR	Fenton chemistry	Tyr-Tyr cross-links	(Stadtman, 1993; Stadtman and Levine, 2003) and references therein
TYR	$^1O_2$ or direct UV absorption	3a-hydroxy-6-oxo-2,3,3a,6,7,7a-hexahydro-1H-indol-2-carboxylic acid	

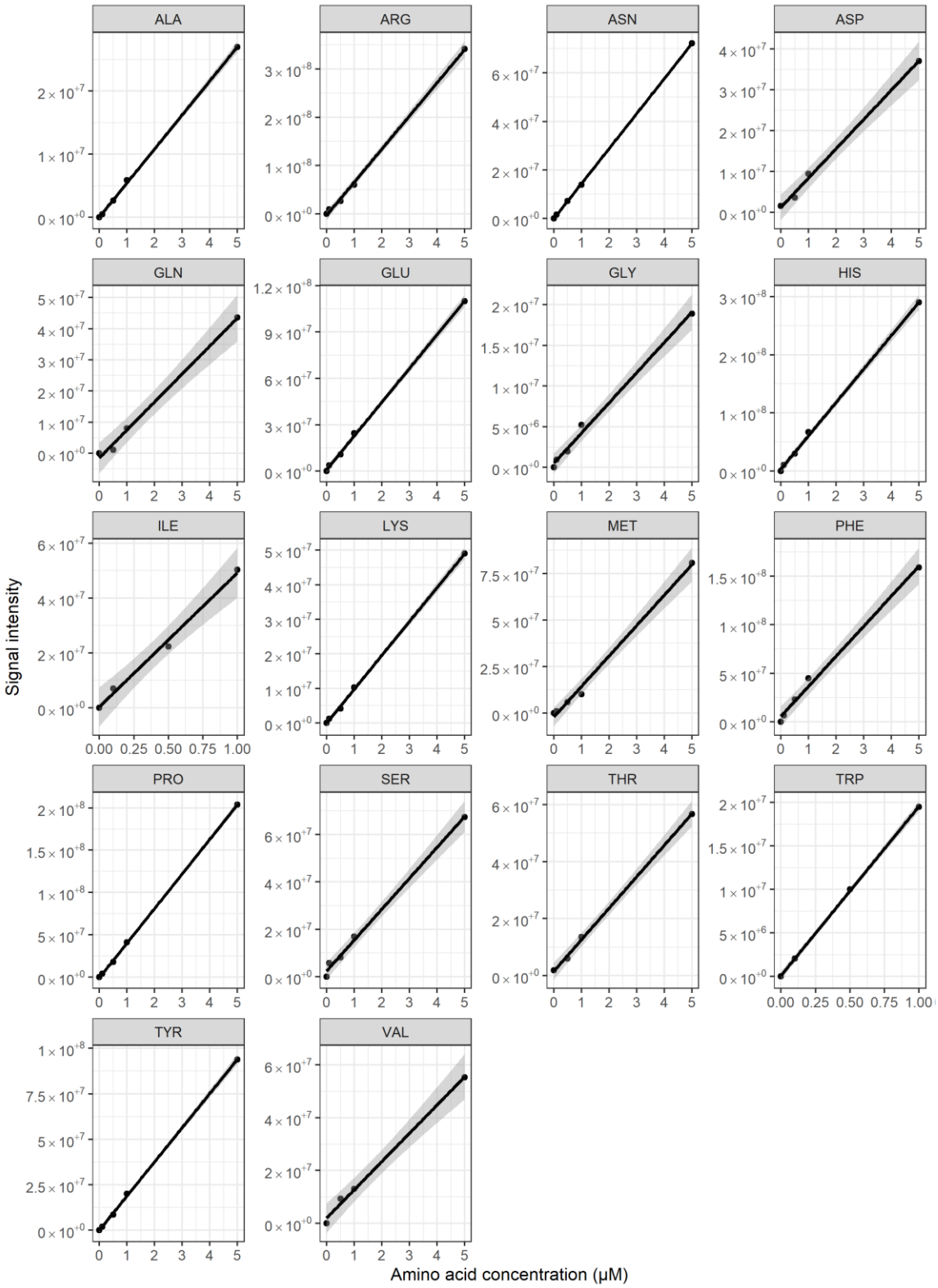

Figure S1: Calibration curves for LC-HRMS experiments

Example of biodegradation rate calculation:

Bacterial degradation of amino acids follows a first order decay equation as $C_t = C_0 . e^{-kt}$ with t the incubation time, C_0 and C_t the initial concentration and concentration at t respectively and k the first order decay constant.

First, concentration of each amino acid is followed through time (Figure S2A) by LC-HRMS as described in the Materials and Methods section. Values are converted to determine k corresponding to the slope of $Ln(C_t/C_0) = f(t)$ (Figure S2B)

Biodegration rates are then calculated as follows: $V_b = \frac{k \times C_0}{N_{cell}}$ with V_b the biodegradation rate (in mol h^{-1} $cell^{-1}$), k the first order decay constant (in h^{-1}), C_0 the initial concentration in aminoacid (in mol L^{-1}) and N_{cell} the bacterial concentration (in cell L^{-1}).

In this example, N_{cell} = 4 10^8 cell L^{-1} and C_0 = 1.16 10^{-6} mol L^{-1} so V_b = 8.88 10^{-16} mol h^{-1} $cell^{-1}$

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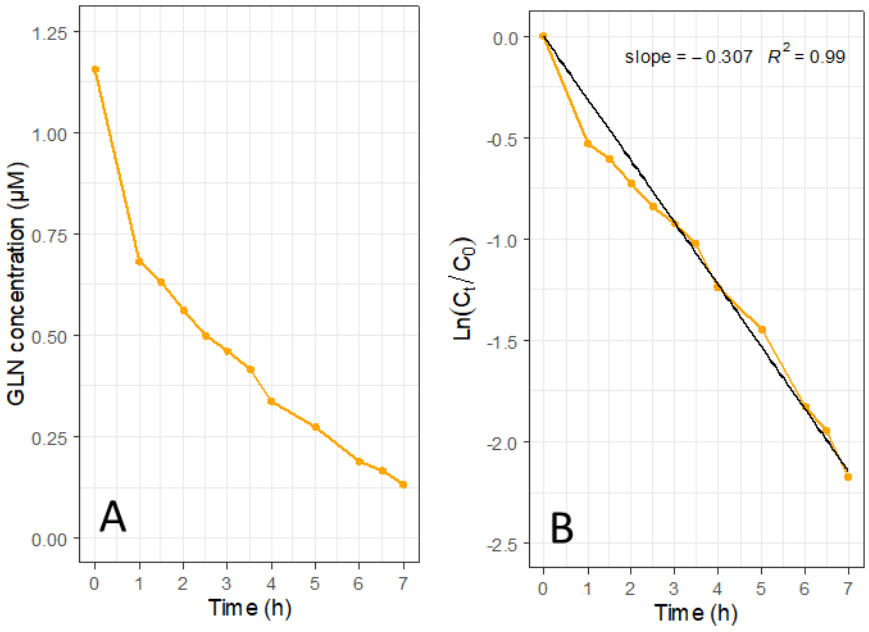

Figures S2: Example of calculation of the biodegradation rate of GLN. A) time dependence of GLN concentration with time measured by LC-HRMS. B) ln(Ct/C0)=f(t), degradation rates are calculated from the slope at the origin.

C_t: GLN measured concentration at time = t , C_0 :GLN measured concentration at time zero.

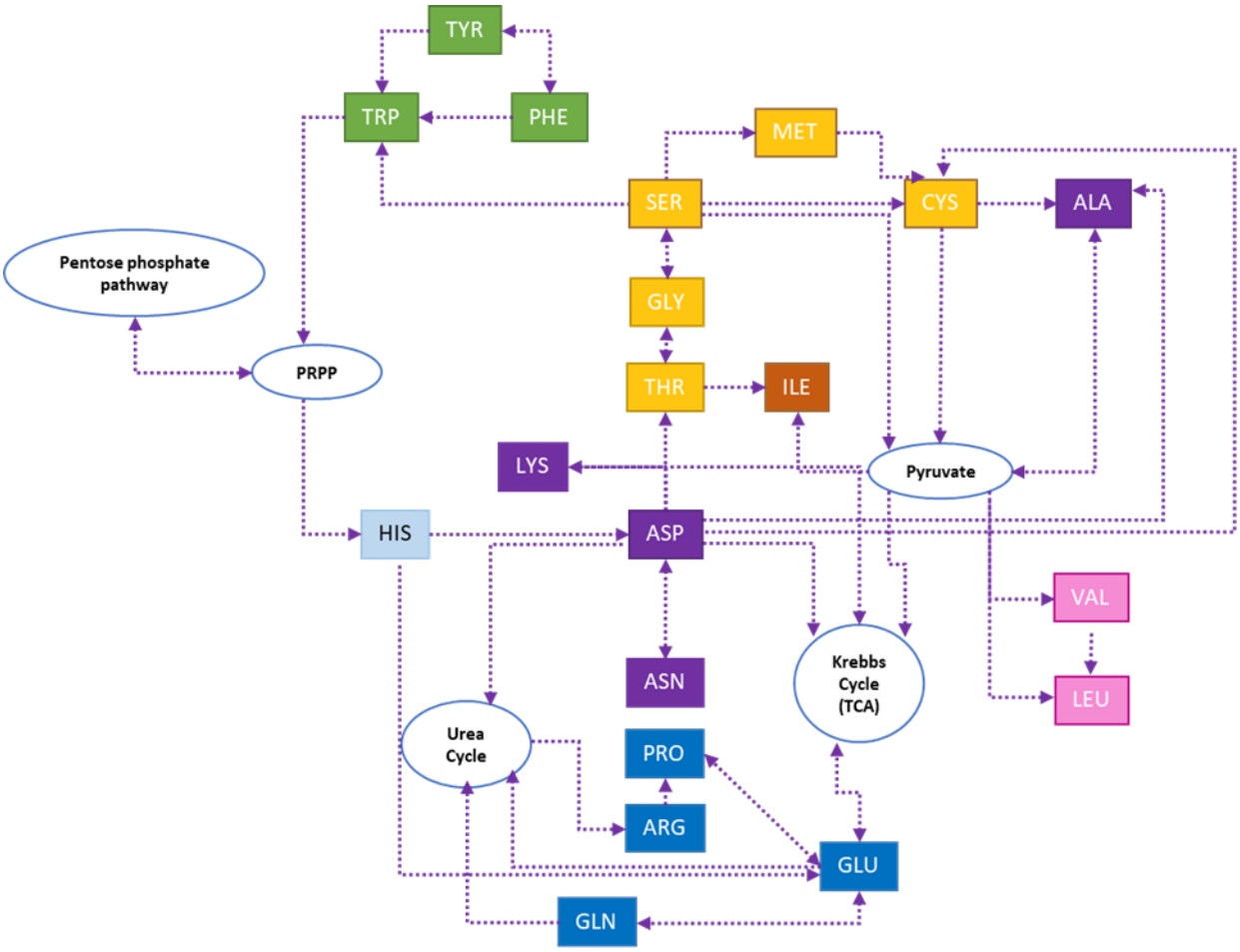

Figure S3: Main metabolic routes for AA metabolism according to (KEGG pathway database, n.d.)

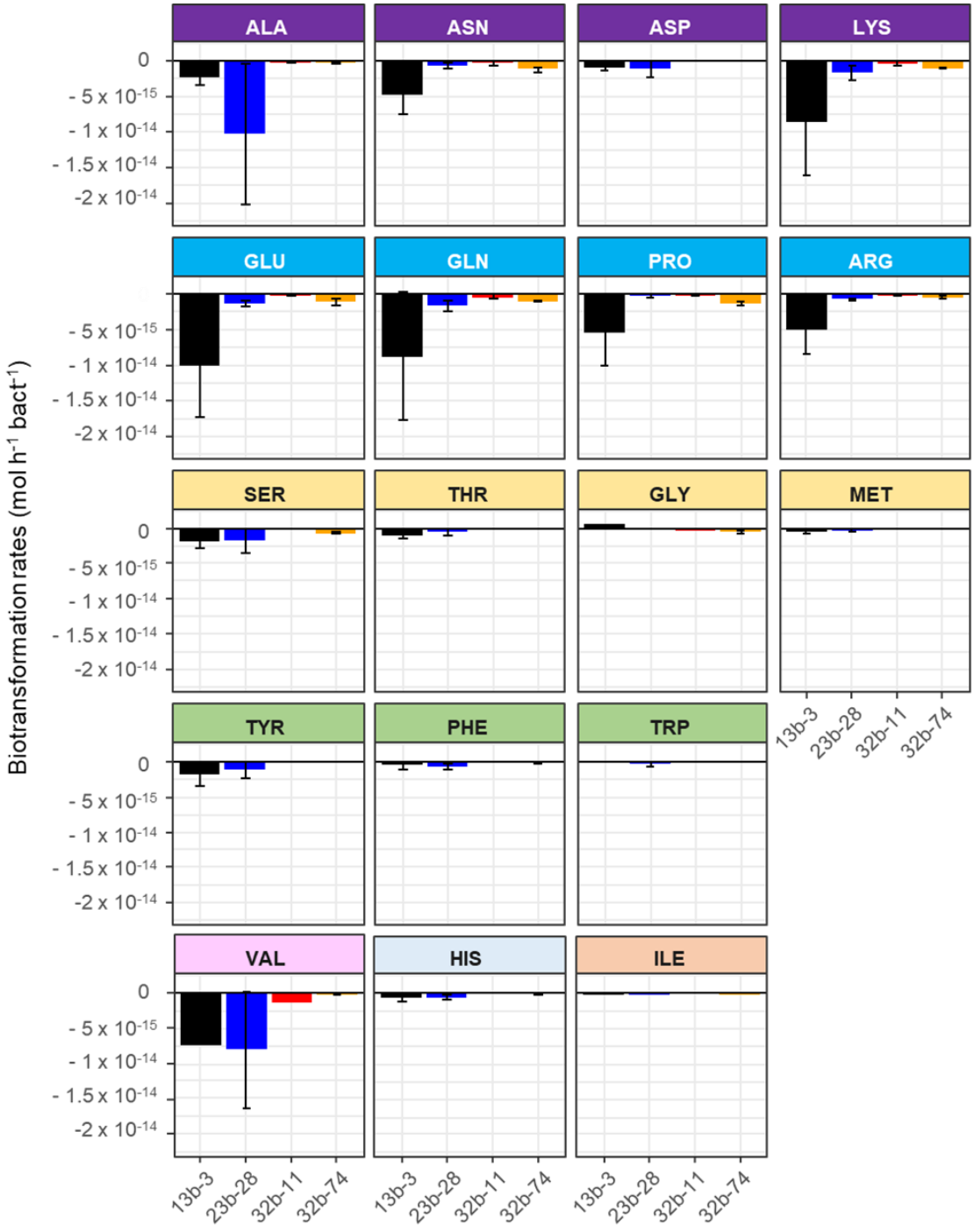

Figure S4: Biotransformation rates (mol $bact^{-1}$ h^{-1}) of AA by the four bacterial strains grouped according the metabolic pathways of the AA (see Figure S3). *Pseudomonas graminis* PDD-13b-3 in black, *Rhodococcus enclensis* PDD-23b-28 in blue, *Sphingomonas* sp. PDD-32b-11 in red and *Pseudomonas syringae* PDD-32b-74 in orange). The standard error bars reflect the rather important biological variability measured from 3 triplicates (independent incubations). Positive values correspond to a biosynthesis process, negative values to a biodegradation process.

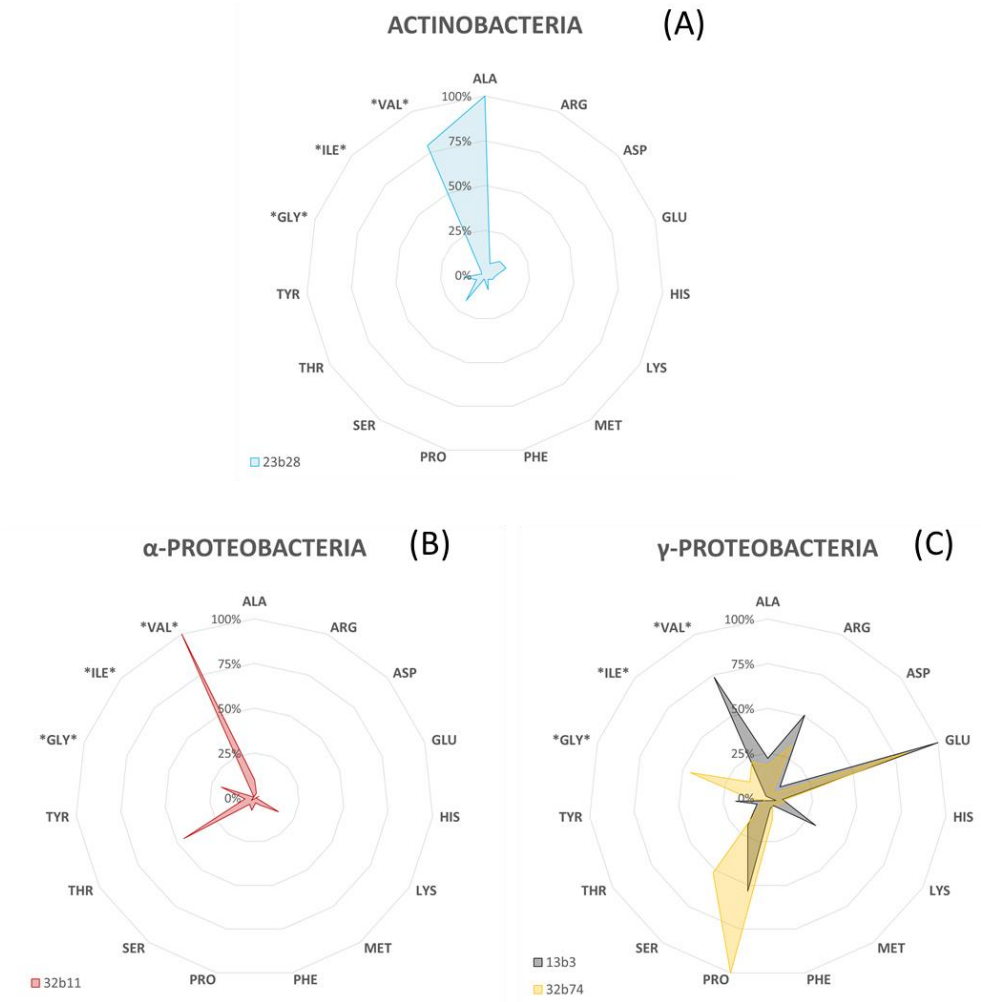

Figure S5: Comparison of the ability of the different strains to metabolize amino acids according to their phylogeny: (A) Actinobacteria (*Rhodococcus enclensis* PDD-23b-28 in blue), B) α-Proteobacteria (*Sphingomonas* sp PDD-32b-11 in red), (C) γ-Proteobacteria (*Pseudomonas graminis* PDD-13b-3 in black and *Pseudomonas syring*ae PDD-32b-74 in yellow). The rates of biodegradation (average from 3 replicates) are presented as a % for each amino acid in the form of a radar plot. For each strain the highest rate is put at 100%. (* only one replicate value was available).

An example of phylogenetic classification is given bellow

Phylum--→Class→Genus→species→strain number

Proteobacteria→γ-Proteobacteria→*Pseudomonas*→*graminis*→ PDD-13b-3

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