Supplementary Materials

Genome-based characterization of a plasmid-associated micrococcin P1 biosynthetic gene cluster and virulence factors in *Mammaliicoccus sciuri* IMDO-S72

David Van der Veken[1], Charlie Hollanders[2], Marko Verce[1], Chris Michiels[3], Steven Ballet[2], Stefan Weckx[1] and Frédéric Leroy[1*]

[1]Research Group of Industrial Microbiology and Food Biotechnology (IMDO), Faculty of Sciences and Bioengineering Sciences, Vrije Universiteit Brussel, Brussels, Belgium

[2]Research Group of Organic Chemistry (ORGC), Faculty of Sciences and Bioengineering Sciences, Vrije Universiteit Brussel, Brussels, Belgium

[3]Laboratory of Food Microbiology and Leuven Food Science and Nutrition Research Centre (LFoRCe), KU Leuven, Leuven, Belgium

Content

SUPPLEMENTARY METHODS

Growth experiments and kinetic modelling

The meat simulation medium (MSM) contained the following ingredients (expressed per L): 8.8 g Lab-Lemco (Oxoid), 11.0 g bacteriological peptone (Oxoid), 2.2 g yeast extract (Oxoid), 1 mL Tween 80 (Sigma), 0.038 g $MnSO_4.5H_2O$ (VWR International, Radnor, PA, USA), 40.0 g NaCl (VWR International), 2.96 g arginine (Oxoid), 13.0 g glucose (VWR International), 100 mg oleic acid (Merck), 50 mg palmitic acid (Merck), 50 mg linoleic acid (Merck) and 25 mg stearic acid (Merck). All compounds were dissolved in ultrapure water and sterilized *in situ* at 121°C for 20 min, except for glucose and fatty acids which were sterilized separately and then aseptically added to the bioreactor.

Inoculum build-up of *M. sciuri* IMDO-S72 was achieved through three subcultures. A single colony from MSA was transferred into 10 mL of BHI broth and incubated for 8 h at 30°C. From this first subculture, 1 mL was transferred to 100 mL of MSM (pH 6.5) and incubated for 24 h at 30°C. The third subculture was obtained by transferring 1 mL of the second subculture into 100 mL of MSM (pH 6.5) followed by an incubation of 16 h at 30°C and was used as final inoculum. The MSM used in the inoculum build-up was free of arginine and fatty acids.

Experiments were performed for 46 h at a constant pH of 5.7 through automated addition of 1 M of NaOH and HCl solutions, and at a temperature of 25°C. Aerobic conditions were assumed by dispersing an airflow of 2.0 L min^{-1} through the cultivation medium at an agitation rate of 200 rpm. Microaerobic conditions were achieved without airflow and agitation rate of 200 rpm. To avoid excessive foam formation, 10 mL of Antifoam 204 (Merck) was added to aerobic fermentations. Samples were taken at regular time intervals, after which the sampling valve was steam-sterilized during 15 min. For microbial enumeration, appropriate dilutions were made in PBS and plated on MSA followed by a 24 h incubation at 30°C. Supernatant was obtained by immediate centrifugation (4696 x *g* for 20 min at 4°C) of the culture liquid after sampling and stored at -20°C until further analysis.

Semi-quantitative determination of the antibacterial activity was performed using an agar spot test against *Staphylococcus carnosus* IMDO-S5 as susceptible indicator strain. For each time point, a two-fold dilution series of cell-free supernatant in PBS was made after heat treatment for 15 min at 80°C. Ten µL of each dilution was spotted onto BHI agar and overlayed with BHI soft agar (0.7%, w/v) inoculated with 100 µL of an overnight grown indicator strain, followed by an incubation of 15 h at 30°C.

SUPPLEMENTARY TABLES S1-S3

Supplementary Table S1. Sequence alignment (blastp) output for the functional comparison of the thiopeptide gene clusters of *Mc. caseolyticus* 115, *M. sciuri* IMDO-S72 and *B. cereus* ATCC 14579 as depicted in Figure 1.

Locus tags of pIMDO-S72-2	***Macrococcus caseolyticus* 115**		***Bacillus cereus* ATCC 14579**		**Predicted enzyme function**	**Functional classification**	**Color**
	Protein homolog	**qcovs/evalue/bitscore/pident/ppos***	**Protein homolog**	**qcovs/evalue/bitscore/pident/ppos***			
SSCS72_02882	-	-	BC_RS25430	13/1.3/18.5/31.25/75	*M. sciuri*: replication initiator protein *Mc. caseolyticus*: - *B. cereus*: -	Plasmid replication	Orange
SSCS72_02883	-	-	-	-	*M. sciuri*: - *Mc. caseolyticus*: - *B. cereus*: -	-	-
SSCS72_02884	-	-	-	-	*M. sciuri*: transposase *Mc. caseolyticus*: - *B. cereus*: -	DNA recombination	Rose
SSCS72_02885	AIU53931.1	26/1.6/19.2/31.818/45.45	-	-	*M. sciuri*: - *Mc. caseolyticus*: ATPase *B. cereus*: -	-	-
SSCS72_02886	AIU53928.1	19/1.5/18.9/36.111/44.44	BC_RS25370	11/0.18/23.1/41.176/82.35	*M. sciuri*: invertase *Mc. caseolyticus*: integrase *B. cereus*: -	DNA recombination	Rose
SSCS72_02887	AIU53929.1	9/0.98/20.0/32.143/53.57	-	-	*M. sciuri*: transposase *Mc. caseolyticus*: - *B. cereus*: -	DNA recombination	Rose
SSCS72_02888	AIU53951.1 (*tclU*)	70/3.35E-10/44.3/32.5/53.75	BC_RS25310 (*tclU*)	57/4.86E-10/44.3/36.842/61.84	*M. sciuri*: transcriptional regulator *Mc. caseolyticus*: transcriptional regulator *B. cereus*: transcriptional regulator	Transcriptional regulation	Brown
SSCS72_02889	AIU53950.1 (*tclP*)	100/7.49E-83/238/55.932/69.49	BC_RS25335 (*tclP*)	73/1.72E-58/177/51.613/68.28	*M. sciuri*: dehydrogenase *Mc. caseolyticus*: decarboxylase *B. cereus*: dehydrogenase	Dehydrogenation	Green
SSCS72_02890	AIU53949.1 (*tclN*)	98/1.10E-125/363/44.045/63.82	BC_RS25345 (*tclN*)	97/5.32E-17/72.8/24.225/41.09	*M. sciuri*: - *Mc. caseolyticus*: cyclodehydratase *B. cereus*: dehydrogenase	Heterocyclization	Blue
SSCS72_02891	AIU53948.1 (*tclM*)	92/1.36E-62/189/43.725/63.16	BC_RS25350 (*tclM*)	71/9.69E-25/91.7/29.31/48.71	*M. sciuri*: - *Mc. caseolyticus*: - *B. cereus*: -	Macrocyclization	Purple
SSCS72_02892	AIU53947.1 (*tclL*)	98/1.55E-67/201/42.857/63.53	BC_RS25355 (*tclL*)	94/1.16E-32/112/30.263/48.03	*M. sciuri*: dehydratase *Mc. caseolyticus*: dehydratase *B. cereus*: dehydrogenase	Dehydration	Red
SSCS72_02893	AIU53946.1	99/0/564/41.065/62.01	BC_RS25360	89/4.98E-58/203/26.289/47.04	*M. sciuri*: dehydratase	Dehydration	Red

	(*tclK*)		(*tclK*)		*Mc. caseolyticus*: dehydratase *B. cereus*: dehydratase		
SSCS72_02894	AIU53945.1 (*tclJ*)	99/1.81E-170/486/47.602/65.72	BC_RS25365 (*tclJ*)	76/2.30E-82/261/35.838/50.67	*M. sciuri*: cyclodehydratase *Mc. caseolyticus*: cyclodehydratase *B. cereus*: cyclodehydratase	Heterocyclization	Blue
SSCS72_02895	AIU53944.1 (*tclI*)	98/4.57E-33/111/36.111/54.76	- (*tclI* gene of BC has best hit with SSCS72_02886)	-	*M. sciuri*: - *Mc. caseolyticus*: thiopeptide biosynthesis protein *B. cereus*: -	Heterocyclization	Blue
SSCS72_02896	AIU53943.1 (*tclQ*)	100/7.55E-76/213/72.34/87.94	BC_RS25315 (*tclQ*) BC_RS25330 (*tclT*)	99/6.00E-60/172/56.429/76.43 99/6.00E-60/172/56.429/76.43	*M. sciuri*: 50S ribosomal protein L11 *Mc. caseolyticus*: 50S ribosomal protein L11 *B. cereus*: 50S ribosomal protein L11	Immunity	Yellow
SSCS72_02897	AIU53940.1	92/4.30E-13/54.3/29.221/48.7	BC_RS25435	13/0.079/24.3/27.143/42.86	*M. sciuri*: - *Mc. caseolyticus*: - *B. cereus*: thiol cytolysin	Unknown	Gray
SSCS72_02898	AIU53941.1 (*tclS*)	100/8.83E-37/119/39.207/55.51	BC_RS25320 (*tclS*)	53/4.72E-14/59.3/31.746/50	*M. sciuri*: dehydrogenase *Mc. caseolyticus*: decarboxylase *B. cereus*: dehydrogenase	Dehydrogenation	Green
SSCS72_02899	AIU53942.1 (*tclE*)	86/0.024/20.4/66.667/83.33	BC_RS25375 (*tclE*) BC_RS25380 (*tclF*) BC_RS25385 (*tclG*) BC_RS25390 (*tclH*)	13/9/14.2/57.143/85.71 13/9/14.2/57.143/85.71 13/9/14.2/57.143/85.71 13/9/14.2/57.143/85.71	*M. sciuri*: precursor peptide *Mc. caseolyticus*: precursor peptide *B. cereus*: precursor peptide	Precursor peptide	Black
SSCS72_02900	-	-	-	-	*M. sciuri*: - *Mc. caseolyticus*: - *B. cereus*: -	-	-
SSCS72_02901	-	-	-	-	*M. sciuri*: membrane associated *Mc. caseolyticus*: - *B. cereus*: -	-	-
SSCS72_02902	AIU53934.1	42/0.027/21.6/40.741/66.67	-	-	*M. sciuri*: membrane associated *Mc. caseolyticus*: - *B. cereus*: -	-	-
SSCS72_02903	-	-	BC_RS25440	19/0.11/22.7/32.653/46.94	*M. sciuri*: membrane associated *Mc. caseolyticus*: - *B. cereus*: ABC transporter	-	-
SSCS72_02904	-	-	-	-	*M. sciuri*: membrane associated *Mc. caseolyticus*: - *B. cereus*: -	-	-
SSCS72_02905	-	-	-	-	*M. sciuri*: - *Mc. caseolyticus*: - *B. cereus*: -	-	-

SSCS72_02906	-	-	-	-	*M. sciuri*: - *Mc. caseolyticus*: - *B. cereus*: -	-	-
SSCS72_02907	AIU53937.1	97/3.30E-37/124/26.563/54.3	BC_RS25295	2/2.8/19.2/62.5/75	*M. sciuri*: partitioning protein *Mc. caseolyticus*: plasmid-replication associated protein *B. cereus*: transporter	Plasmid replication	Orange
SSCS72_02908	-	-	-	-	*M. sciuri*: membrane associated *Mc. caseolyticus*: - *B. cereus*: -	-	-

*qcovs, Query coverage per subject; evalue, Expect value; bitscore, Bit score; pident, Percentage of identical matches; ppos, Percentage of positive-scoring matches.

Supplementary Table S2. Dataset of the comparative pathogenomic output generated by VFanalyzer and used for plotting of the heatmap represented in Figure 5. Numbers 0 and 1 represent ‘no detection of a homologue gene’ and ‘detection of at least one homologue gene’, respectively. Gene_ID represents the *M. sciuri* IMDO-S72 locus tags that were detected as homologues of known virulence factors. The data in the column of *M. sciuri* IMDO-S72 is the result of a detection by either VFanalyzer and/or manual sequence alignment (blastp) as indicated by the last three columns (output for the other strains is solely based on VFanalyzer). Reference strains whose preanalyzed genomes were used by VFanalyzer to construct orthologous groups are shaded in orange, while strains whose genomes were queried are shaded in blue.

Virulence_Class	**Virulence_Factors**	**Related_Genes**	**Gene_ID**	***M. sciuri* IMDO-S72**	***S.aureus* subsp. *aureus* JH1**	***S. aureus* subsp. *aureus* MRSA252**	***S. aureus* subsp. *aureus* MW2**	***S. aureus* subsp. *aureus* USA300_TCH1516**	***S. epidermidis* RP62A**	***S. haemolyticus* JCSC1435**	***S. saprophyticus* subsp. *saprophyticus* ATCC 15305**	***S. carnosus* TM300**	***S. xylosus* HKUOPL8**	***S. equorum* KS1039**	**VFanalyzer**	**BLASTP**	**Detected by both methods**
Adherence	Autolysin	*atl*	SSCS72_02005, SSCS72_02030, SSCS72_01175, SSCS72_00583, SSCS72_02003	1	1	1	1	1	1	1	1	0	1	1	0	1	0
Adherence	Cell wall associated fibronectin binding protein	*ebh*	SSCS72_01534	1	1	1	1	1	1	0	0	0	0	0	0	1	0
Adherence	Clumping factor A	*clfA*		0	1	1	1	1	0	0	0	0	0	0	0	0	0
Adherence	Clumping factor B	*clfB*	SSCS72_00342	1	1	1	1	1	0	0	0	0	0	0	1	0	0
Adherence	Collagen adhesion	*cna*		0	0	1	1	0	0	0	0	0	0	0	0	0	0
Adherence	Elastin binding protein	*ebp*	SSCS72_01488	1	1	1	1	1	1	1	1	0	1	1	0	1	0
Adherence	Extracellular adherence protein/MHC analogous protein	*eap/map*		0	1	1	1	1	0	0	0	0	0	0	0	0	0
Adherence	Fibrinogen binding protein	*efb*		0	1	1	1	1	0	0	0	0	0	0	0	0	0
Adherence	Fibronectin binding proteins	*fnbA*		0	1	1	1	1	0	0	0	0	1	0	0	0	0
Adherence	Fibronectin binding proteins	*fnbB*		0	1	0	1	1	0	0	0	0	0	0	0	0	0

Adherence	Intercellular adhesin	*icaA*	SSCS72_02865	1	1	1	1	1	1	0	0	0	0	0	1	1	1
Adherence	Intercellular adhesin	*icaB*	SSCS72_02863	1	1	1	1	1	1	0	0	0	1	0	1	1	1
Adherence	Intercellular adhesin	*icaC*	SSCS72_02862, SSCS72_00185, SSCS72_01996	1	1	1	1	1	1	0	0	0	1	0	1	1	1
Adherence	Intercellular adhesin	*icaD*	SSCS72_02864	1	1	1	1	1	1	0	0	0	0	0	1	1	1
Adherence	Intercellular adhesin	*icaR*	SSCS72_02866, SSCS72_00184	1	1	1	1	1	1	0	0	0	1	0	1	1	1
Adherence	Ser-Asp rich fibrinogen-binding proteins	*sdrC*	SSCS72_00382	1	1	1	1	1	0	0	0	0	0	0	1	1	1
Adherence	Ser-Asp rich fibrinogen-binding proteins	*sdrD*		0	1	0	1	1	0	0	0	0	0	0	0	0	0
Adherence	Ser-Asp rich fibrinogen-binding proteins	*sdrE*		0	1	1	1	1	0	0	0	0	0	1	0	0	0
Adherence	Ser-Asp rich fibrinogen-binding proteins	*sdrF*		0	0	0	0	0	1	0	0	0	0	0	0	0	0
Adherence	Ser-Asp rich fibrinogen-binding proteins	*sdrG*		0	0	0	0	0	1	0	0	0	0	0	0	0	0
Adherence	Ser-Asp rich fibrinogen-binding proteins	*sdrH*		0	0	0	0	0	1	0	0	0	0	0	0	0	0
Adherence	Staphylococcal protein A	*spa*		0	1	1	1	1	0	0	0	0	0	0	0	0	0
Enzyme	Cysteine protease	*sspB*		0	1	1	1	1	1	0	0	0	0	0	0	0	0
Enzyme	Cysteine protease	*sspC*		0	1	1	1	1	0	0	0	0	0	0	0	0	0
Enzyme	Hyaluronate lyase	*hysA*		0	1	1	1	1	0	0	0	0	0	0	0	0	0
Enzyme	Lipase	*geh*		0	1	1	1	1	1	0	1	1	1	0	0	0	0
Enzyme	Lipase	*lip*		0	1	1	1	1	1	1	1	1	1	0	0	0	0
Enzyme	Serine V8 protease	*sspA*	SSCS72_01332, SSCS72_02553	1	1	1	1	1	1	0	1	0	1	1	1	1	1
Enzyme	Serine protease	*splA*		0	1	0	1	1	0	0	0	0	0	0	0	0	0
Enzyme	Serine protease	*splB*		0	1	0	1	1	0	0	0	0	0	0	0	0	0

Enzyme	Serine protease	*splC*		0	1	1	1	1	0	0	0	0	0	0	0	0	0
Enzyme	Serine protease	*splD*		0	1	1	0	1	0	0	0	0	0	0	0	0	0
Enzyme	Serine protease	*splE*		0	0	1	0	1	0	0	0	0	0	0	0	0	0
Enzyme	Serine protease	*splF*		0	0	1	1	1	0	0	0	0	0	0	0	0	0
Enzyme	Staphylocoagulase	*coa*		0	1	1	1	1	0	0	0	0	0	0	0	0	0
Enzyme	Staphylokinase	*sak*		0	1	1	1	1	0	0	0	0	0	0	0	0	0
Enzyme	Thermonuclease	*nuc*	SSCS72_02216	1	1	1	1	1	1	1	1	1	1	1	0	1	0
Immune evasion	AdsA	*adsA*		0	1	1	1	1	0	0	0	1	0	0	0	0	0
Immune evasion	CHIPS	*chp*		0	1	1	0	1	0	0	0	0	0	0	0	0	0
Immune evasion	Capsule	Undetermined	SSCS72_00443, SSCS72_00779, SSCS72_01982, SSCS72_00350, SSCS72_00562	1	1	1	1	1	0	1	1	1	1	1	1	1	1
Immune evasion	SCIN	*scn*		0	1	1	1	1	0	0	0	0	0	0	0	0	0
Immune evasion	Sbi	*sbi*		0	1	1	1	1	0	0	0	0	0	0	0	0	0
Secretion system	Type VII secretion system	*esaA*		0	1	1	1	1	0	0	0	0	0	0	0	0	0
Secretion system	Type VII secretion system	*esaB*		0	1	1	1	1	0	0	0	0	0	0	0	0	0
Secretion system	Type VII secretion system	*esaD*		0	1	0	1	1	0	0	0	0	0	0	0	0	0
Secretion system	Type VII secretion system	*esaE*		0	1	0	1	1	0	0	0	0	0	0	0	0	0
Secretion system	Type VII secretion system	*esaG*		0	1	1	1	1	0	0	0	0	0	0	0	0	0
Secretion system	Type VII secretion system	*essA*		0	1	1	1	1	0	0	0	0	0	0	0	0	0
Secretion system	Type VII secretion system	*essB*		0	1	1	1	1	0	0	0	0	0	0	0	0	0
Secretion system	Type VII secretion system	*essC*		0	1	1	1	1	0	0	0	0	0	0	0	0	0
Secretion system	Type VII secretion system	*esxA*		0	1	1	1	1	0	0	0	0	0	0	0	0	0
Secretion system	Type VII secretion system	*esxB*		0	1	0	1	1	0	0	0	0	0	0	0	0	0

Secretion system	Type VII secretion system	*esxC*		0	1	0	1	1	0	0	0	0	0	0	0	0	0
Secretion system	Type VII secretion system	*esxD*		0	1	0	1	1	0	0	0	0	0	0	0	0	0
Toxin	Alpha hemolysin	*hly/hla*		0	1	1	1	1	0	0	0	0	0	0	0	0	0
Toxin	Beta hemolysin	*hlb*		0	1	1	1	1	1	0	0	0	0	0	0	0	0
Toxin	Delta hemolysin	*hld*		0	1	1	1	1	1	0	0	0	0	0	0	0	0
Toxin	Enterotoxin A	*sea*		0	0	1	1	0	0	0	0	0	0	0	0	0	0
Toxin	Enterotoxin B	*seb*		0	0	0	0	0	0	0	0	0	0	0	0	0	0
Toxin	Enterotoxin C	*sec*		0	0	0	1	0	0	0	0	0	0	0	0	0	0
Toxin	Enterotoxin D	*sed*		0	0	0	0	0	0	0	0	0	0	0	0	0	0
Toxin	Enterotoxin E	*see*		0	0	0	0	0	0	0	0	0	0	0	0	0	0
Toxin	Enterotoxin G	*seg*		0	1	1	0	0	0	0	0	0	0	0	0	0	0
Toxin	Enterotoxin H	*seh*		0	0	0	1	0	0	0	0	0	0	0	0	0	0
Toxin	Enterotoxin I	*sei*		0	1	1	0	0	0	0	0	0	0	0	0	0	0
Toxin	Enterotoxin J	*sej*		0	0	0	0	0	0	0	0	0	0	0	0	0	0
Toxin	Enterotoxin Yent1	*yent1*		0	0	0	0	0	0	0	0	0	0	0	0	0	0
Toxin	Enterotoxin Yent2	*yent2*		0	0	0	0	0	0	0	0	0	0	0	0	0	0
Toxin	Enterotoxin-like K	*selk*		0	0	0	1	1	0	0	0	0	0	0	0	0	0
Toxin	Enterotoxin-like L	*sell*		0	0	0	1	0	0	0	0	0	0	0	0	0	0
Toxin	Enterotoxin-like M	*selm*		0	1	1	0	0	0	0	0	0	0	0	0	0	0
Toxin	Enterotoxin-like N	*seln*		0	1	1	0	0	0	0	0	0	0	0	0	0	0
Toxin	Enterotoxin-like O	*selo*		0	1	1	0	0	0	0	0	0	0	0	0	0	0
Toxin	Enterotoxin-like P	*selp*		0	0	0	0	0	0	0	0	0	0	0	0	0	0
Toxin	Enterotoxin-like Q	*selq*		0	0	0	1	1	0	0	0	0	0	0	0	0	0
Toxin	Enterotoxin-like R	*selr*		0	0	0	0	0	0	0	0	0	0	0	0	0	0
Toxin	Enterotoxin-like U	*selu*		0	0	1	0	0	0	0	0	0	0	0	0	0	0
Toxin	Exfoliative toxin type A	*eta*		0	1	1	1	1	0	0	0	0	0	0	0	0	0
Toxin	Exfoliative toxin type B	*etb*		0	0	0	0	0	0	0	0	0	0	0	0	0	0

Toxin	Exfoliative toxin type C	*etc*		0	0	0	0	0	0	0	0	0	0	0	0	0	0
Toxin	Exfoliative toxin type D	*etd*		0	0	0	0	0	0	0	0	0	0	0	0	0	0
Toxin	Exotoxin	set10		0	1	0	0	0	0	0	0	0	0	0	0	0	0
Toxin	Exotoxin	set11		0	1	0	0	0	0	0	0	0	0	0	0	0	0
Toxin	Exotoxin	set12		0	1	0	0	0	0	0	0	0	0	0	0	0	0
Toxin	Exotoxin	set13		0	1	0	0	0	0	0	0	0	0	0	0	0	0
Toxin	Exotoxin	set14		0	1	0	0	0	0	0	0	0	0	0	0	0	0
Toxin	Exotoxin	set15		0	1	0	0	0	0	0	0	0	0	0	0	0	0
Toxin	Exotoxin	set16		0	0	0	1	0	0	0	0	0	0	0	0	0	0
Toxin	Exotoxin	set17		0	0	0	1	0	0	0	0	0	0	0	0	0	0
Toxin	Exotoxin	set18		0	0	0	1	0	0	0	0	0	0	0	0	0	0
Toxin	Exotoxin	set19		0	0	0	1	0	0	0	0	0	0	0	0	0	0
Toxin	Exotoxin	set1		0	0	1	0	0	0	0	0	0	0	0	0	0	0
Toxin	Exotoxin	set20		0	0	0	1	0	0	0	0	0	0	0	0	0	0
Toxin	Exotoxin	set21		0	0	0	1	0	0	0	0	0	0	0	0	0	0
Toxin	Exotoxin	set22		0	0	0	1	0	0	0	0	0	0	0	0	0	0
Toxin	Exotoxin	set23		0	0	0	1	0	0	0	0	0	0	0	0	0	0
Toxin	Exotoxin	set24		0	0	0	1	0	0	0	0	0	0	0	0	0	0
Toxin	Exotoxin	set25		0	0	0	1	0	0	0	0	0	0	0	0	0	0
Toxin	Exotoxin	set26		0	0	0	1	0	0	0	0	0	0	0	0	0	0
Toxin	Exotoxin	set2		0	0	1	0	0	0	0	0	0	0	0	0	0	0
Toxin	Exotoxin	set30		0	0	0	0	1	0	0	0	0	0	0	0	0	0
Toxin	Exotoxin	set31		0	0	0	0	1	0	0	0	0	0	0	0	0	0
Toxin	Exotoxin	set32		0	0	0	0	1	0	0	0	0	0	0	0	0	0
Toxin	Exotoxin	set33		0	0	0	0	1	0	0	0	0	0	0	0	0	0
Toxin	Exotoxin	set34		0	0	0	0	1	0	0	0	0	0	0	0	0	0
Toxin	Exotoxin	set35		0	0	0	0	1	0	0	0	0	0	0	0	0	0
Toxin	Exotoxin	set36		0	0	0	0	1	0	0	0	0	0	0	0	0	0

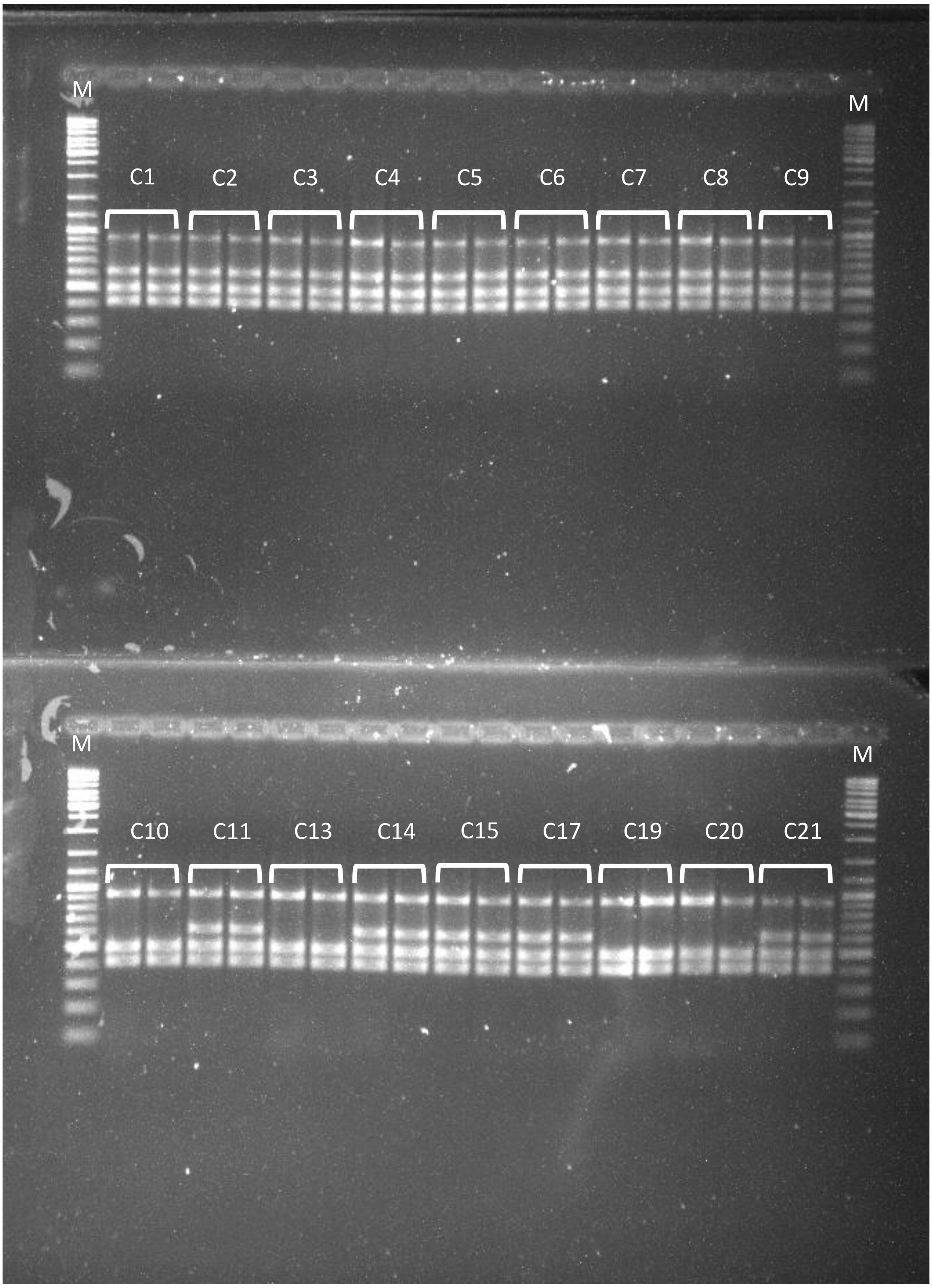
Toxin	Exotoxin	set37		0	0	0	0	1	0	0	0	0	0	0	0	0	0
Toxin	Exotoxin	set38		0	0	0	0	1	0	0	0	0	0	0	0	0	0
Toxin	Exotoxin	set39		0	0	0	0	1	0	0	0	0	0	0	0	0	0
Toxin	Exotoxin	set3		0	0	1	0	0	0	0	0	0	0	0	0	0	0
Toxin	Exotoxin	set40		0	0	0	0	1	0	0	0	0	0	0	0	0	0
Toxin	Exotoxin	set4		0	0	1	0	0	0	0	0	0	0	0	0	0	0
Toxin	Exotoxin	set5		0	0	1	0	0	0	0	0	0	0	0	0	0	0
Toxin	Exotoxin	set6		0	1	0	0	0	0	0	0	0	0	0	0	0	0
Toxin	Exotoxin	set7		0	1	0	0	0	0	0	0	0	0	0	0	0	0
Toxin	Exotoxin	set8		0	1	0	0	0	0	0	0	0	0	0	0	0	0
Toxin	Exotoxin	set9		0	1	0	0	0	0	0	0	0	0	0	0	0	0
Toxin	Gamma hemolysin	*hlgA*		0	1	1	1	1	0	0	0	0	0	0	0	0	0
Toxin	Gamma hemolysin	*hlgB*		0	1	1	1	1	0	0	0	0	0	0	0	0	0
Toxin	Gamma hemolysin	*hlgC*		0	1	1	1	1	0	0	0	0	0	0	0	0	0
Toxin	Leukocidin M	*lukF-like*		0	0	0	0	0	0	0	0	0	0	0	0	0	0
Toxin	Leukocidin M	*lukM*		0	0	0	0	0	0	0	0	0	0	0	0	0	0
Toxin	Leukotoxin D	*lukD*		0	1	1	1	1	0	0	0	0	0	0	0	0	0
Toxin	Leukotoxin E	*lukE*		0	1	1	1	1	0	0	0	0	0	0	0	0	0
Toxin	Panton-Valentine leukocidin	*lukF-PV*		0	0	0	1	1	0	0	0	0	0	0	0	0	0
Toxin	Panton-Valentine leukocidin	*lukS-PV*		0	0	0	1	1	0	0	0	0	0	0	0	0	0
Toxin	Toxic shock syndrome toxin	*tsst*		0	0	0	0	0	0	0	0	0	0	0	0	0	0
Phagosome arresting	Nucleoside diphosphate kinase	*ndk*	SSCS72_01502	1	0	0	0	0	0	0	0	0	0	0	1	1	1
Surface protein anchoring	Lipoprotein diacylglyceryl transferase	*lgt*	SSCS72_02264	1	0	0	0	0	0	0	0	0	0	0	1	1	1

Supplementary Table S3. Log values [log(CFU mL^{-1})] of raw count data with corresponding standard deviation (SD) and antibacterial activity values (AU) of supernatant obtained during aerobic and microaerobic growth experiments, which was used for modelling. In the aerobic case, values represent the average based on three biological independent replicates.

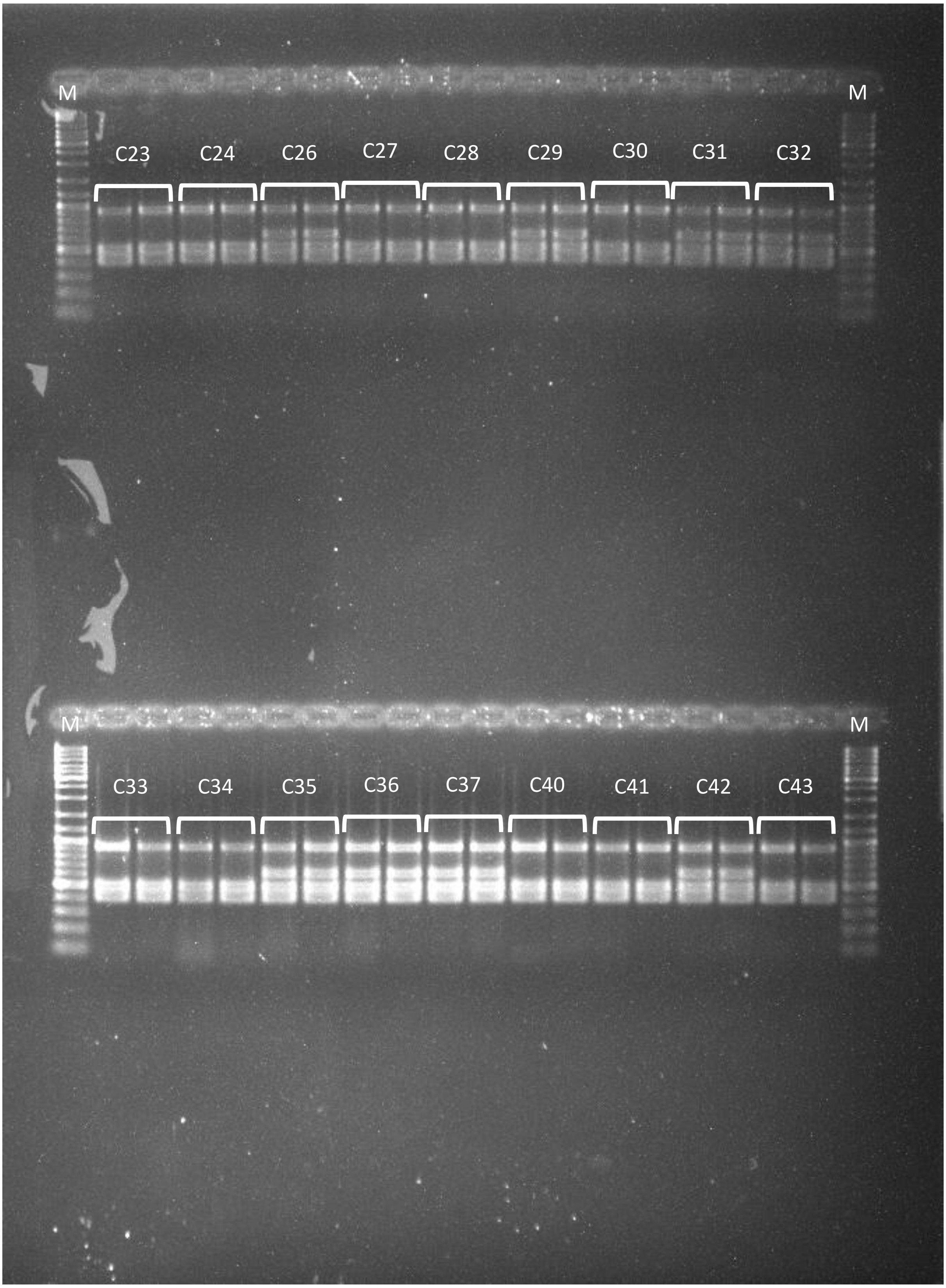
	Aerobic condition			**Microaerobic condition**	
Time (h)	Log(CFU mL^{-1})	SD	Antibacterial activity (AU)	Log(CFU mL^{-1})	Antibacterial activity (AU)
0	5.85	0.21	0	5.39	0
2	5.80	0.18	0	5.28	0
4	6.31	0.55	0	5.89	0
6	6.93	0.27	0	6.17	0
8	7.47	0.33	0	6.94	0
10	7.98	0.17	0	7.69	0
12	8.42	0.25	100	7.43	0
14	8.86	0.13	233	7.92	50
16	9.28	0.38	333	7.90	100
18	9.26	0.17	433	8.12	100
20	9.46	0.21	800	8.18	150
22	9.69	0.20	800	8.18	150
24	9.63	0.26	1600	8.06	150
26	9.65	0.21	2133	8.16	150
28	9.68	0.14	2400	8.12	200
30	9.55	0.13	2400	8.19	200
32			2933		100
34	9.98	0.15	3200	8.42	200
36			3200		150
38	10.01	0.09	3200	8.75	200
40			3200		200
42	10.28	0.32	3200	8.13	200
44			3200		300
46	9.87	0.10	3200	8.13	100

SUPPLEMENTARY FIGURES S1-S9

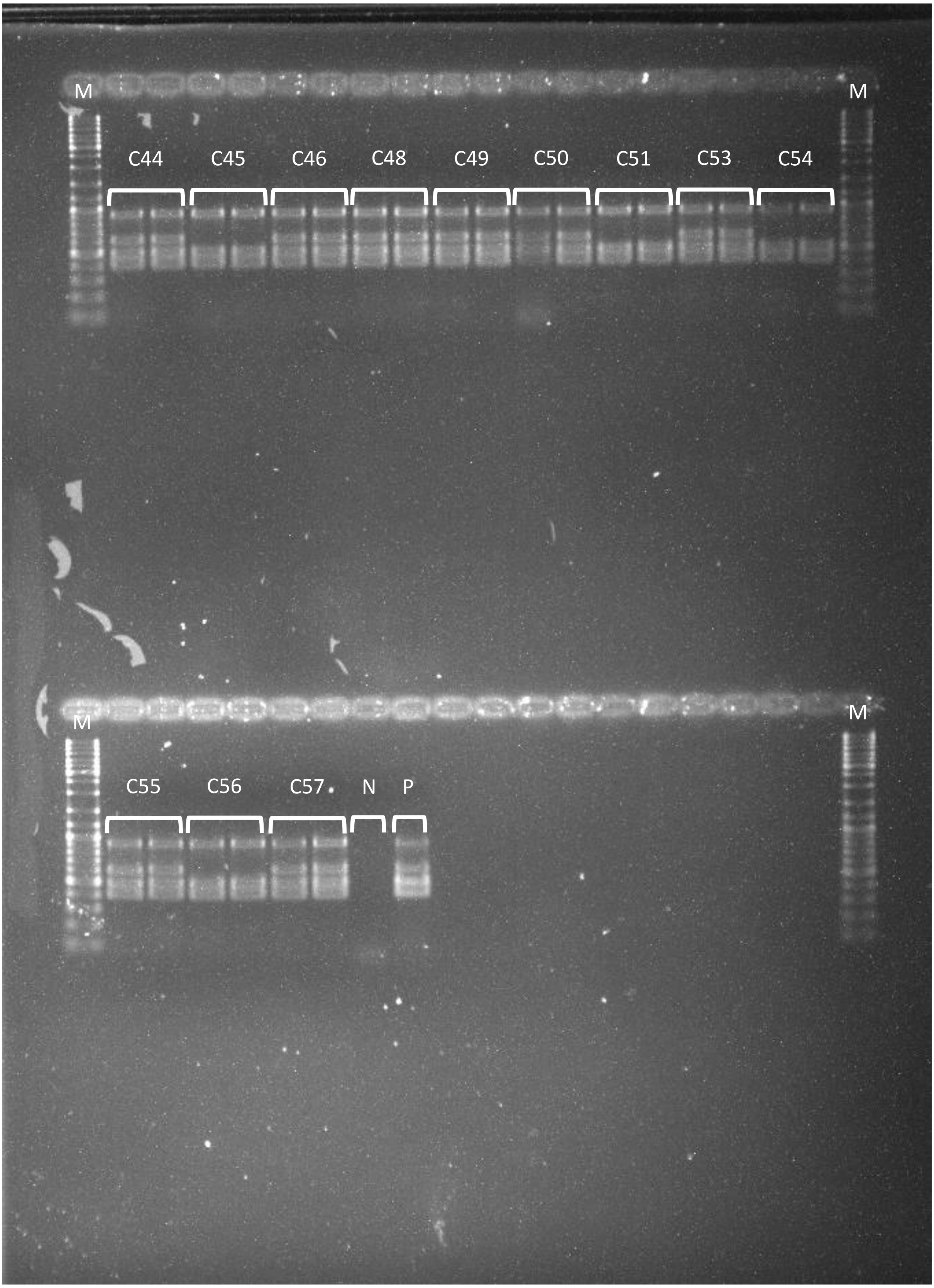
Supplementary Figure S1. PCR profiles of the heat-treated isolates used to determine the frequency of the plasmid genotypes found. Each isolate was screened in duplicate and is indicated with a C number above each two lanes [excluding the non-antibacterial isolates (C12, C16, C18, C22, C25, C38, C39, C47, C52, C61, C63, C76, C86, C87, C88, C94, C100, C117, C121, C127, C133, C154, C156, C167, C168 and C178), the profiles of which are shown in Supplementary Figure S2]. Lanes denoted with M represent molecular size markers, lanes denoted with N represent negative PCR controls and lanes denoted with P represent the PCR profile of the native *M. sciuri* IMDO-S72 strain.



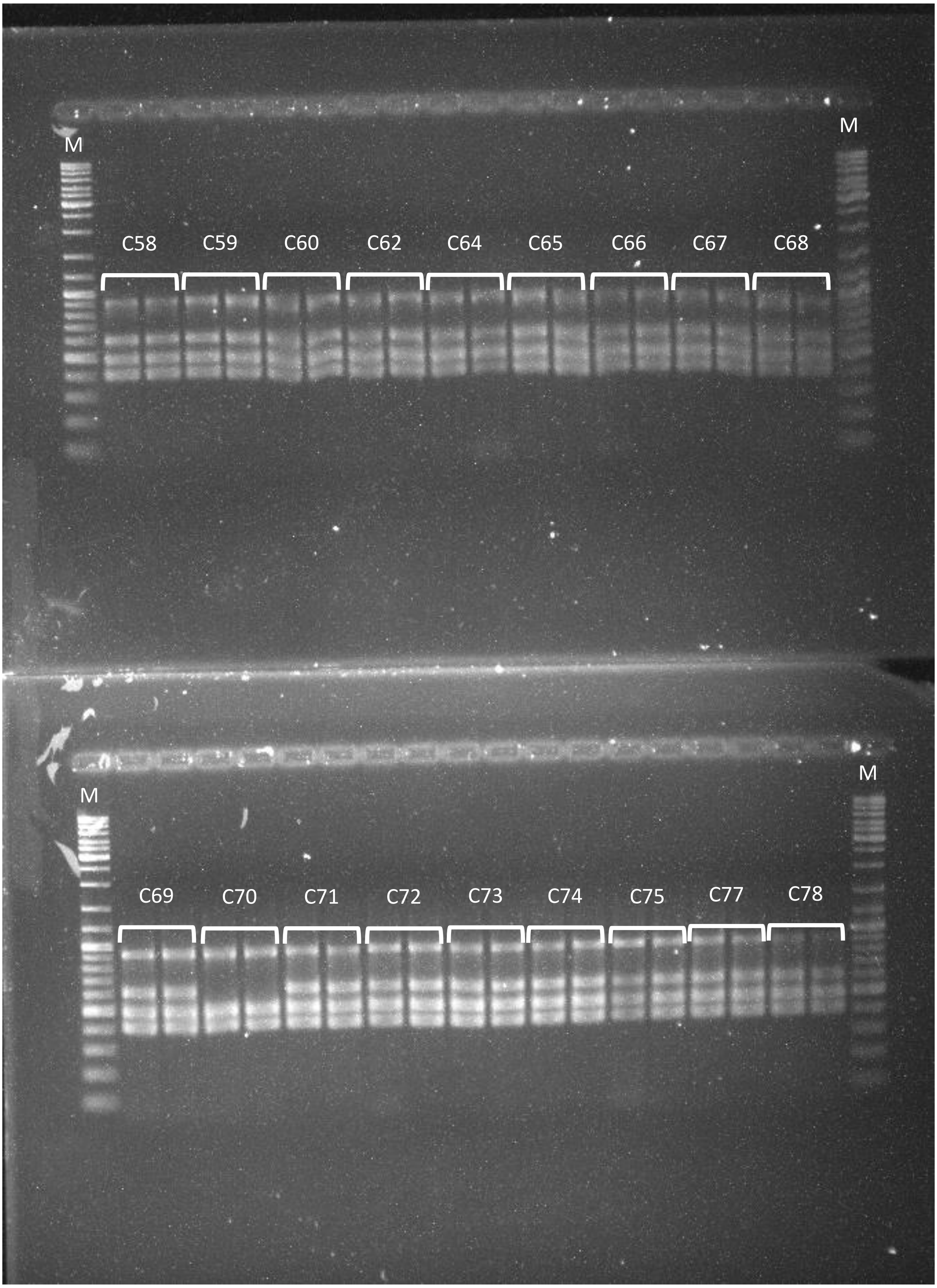
Supplementary Figure S1. *continued*



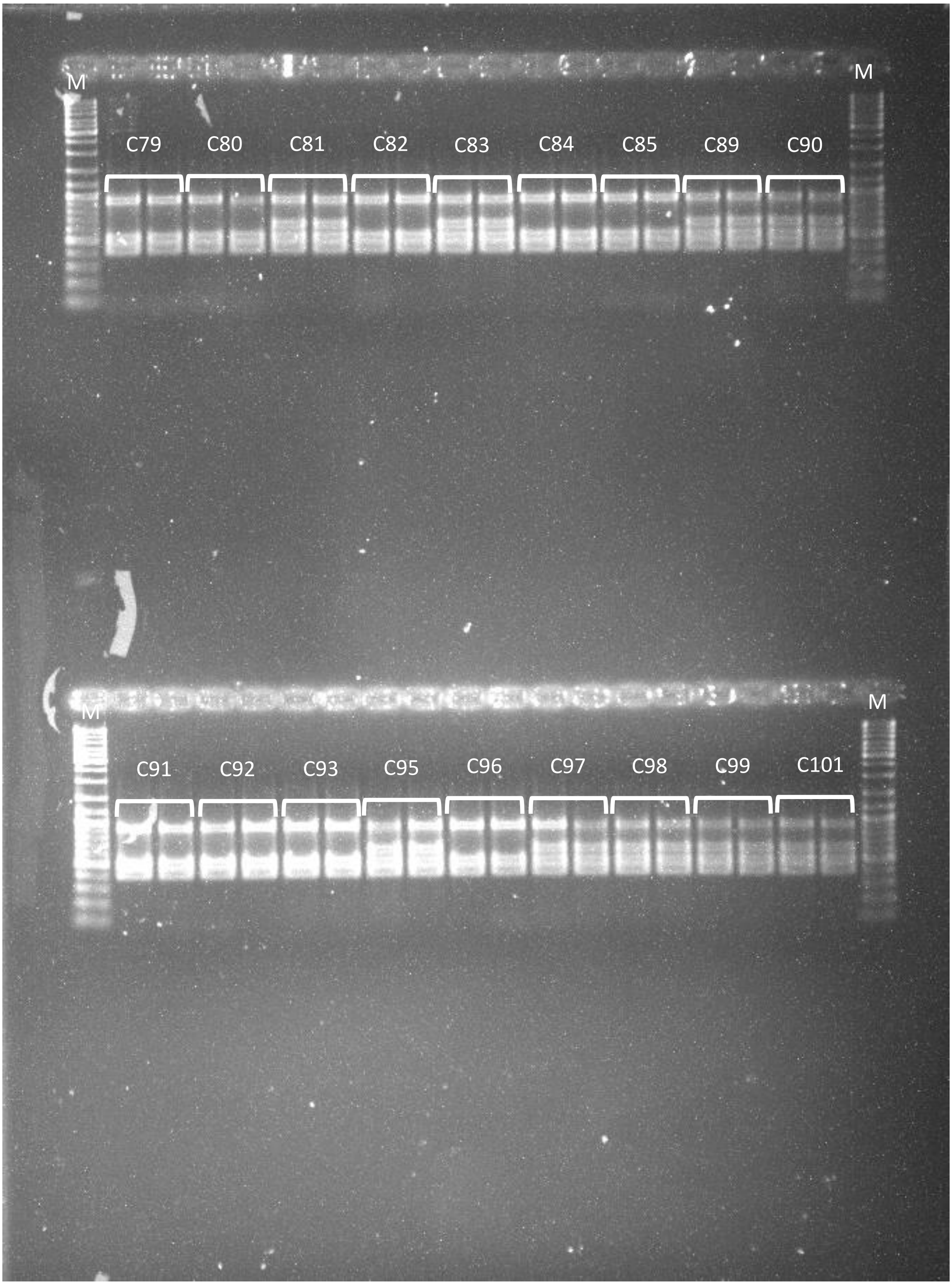
Supplementary Figure S1. *continued*



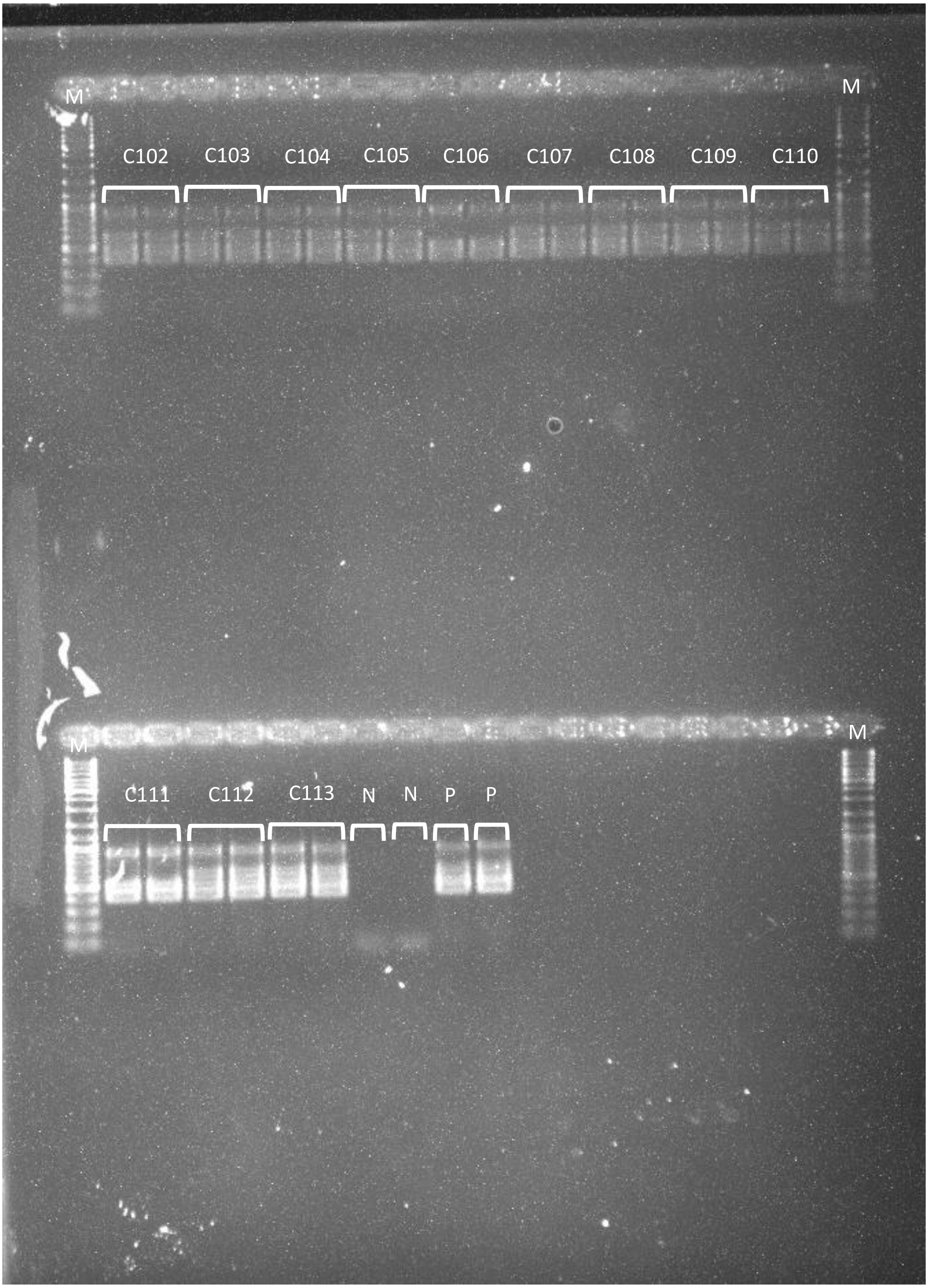
Supplementary Figure S1. *continued*



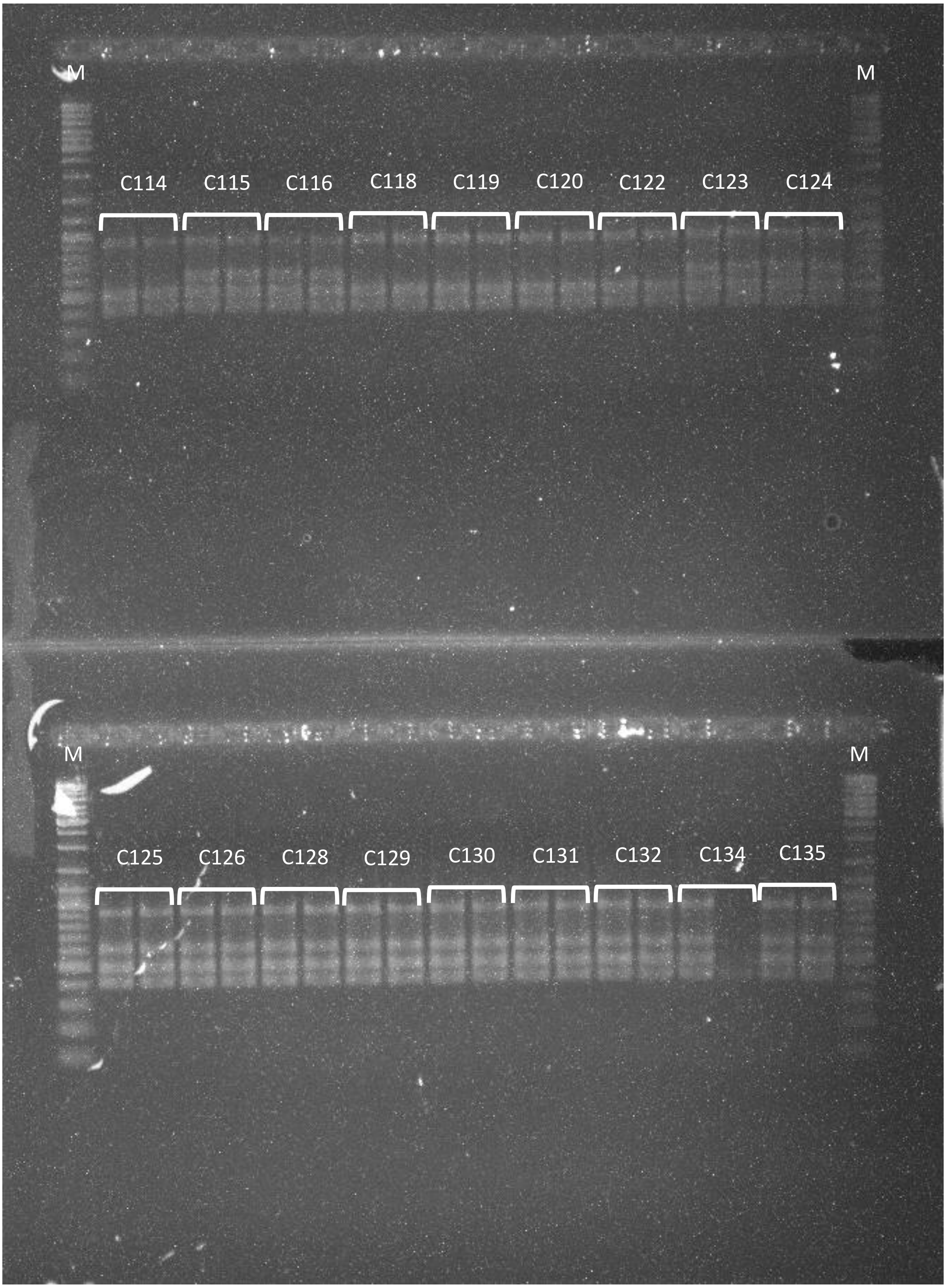
Supplementary Figure S1. *continued*



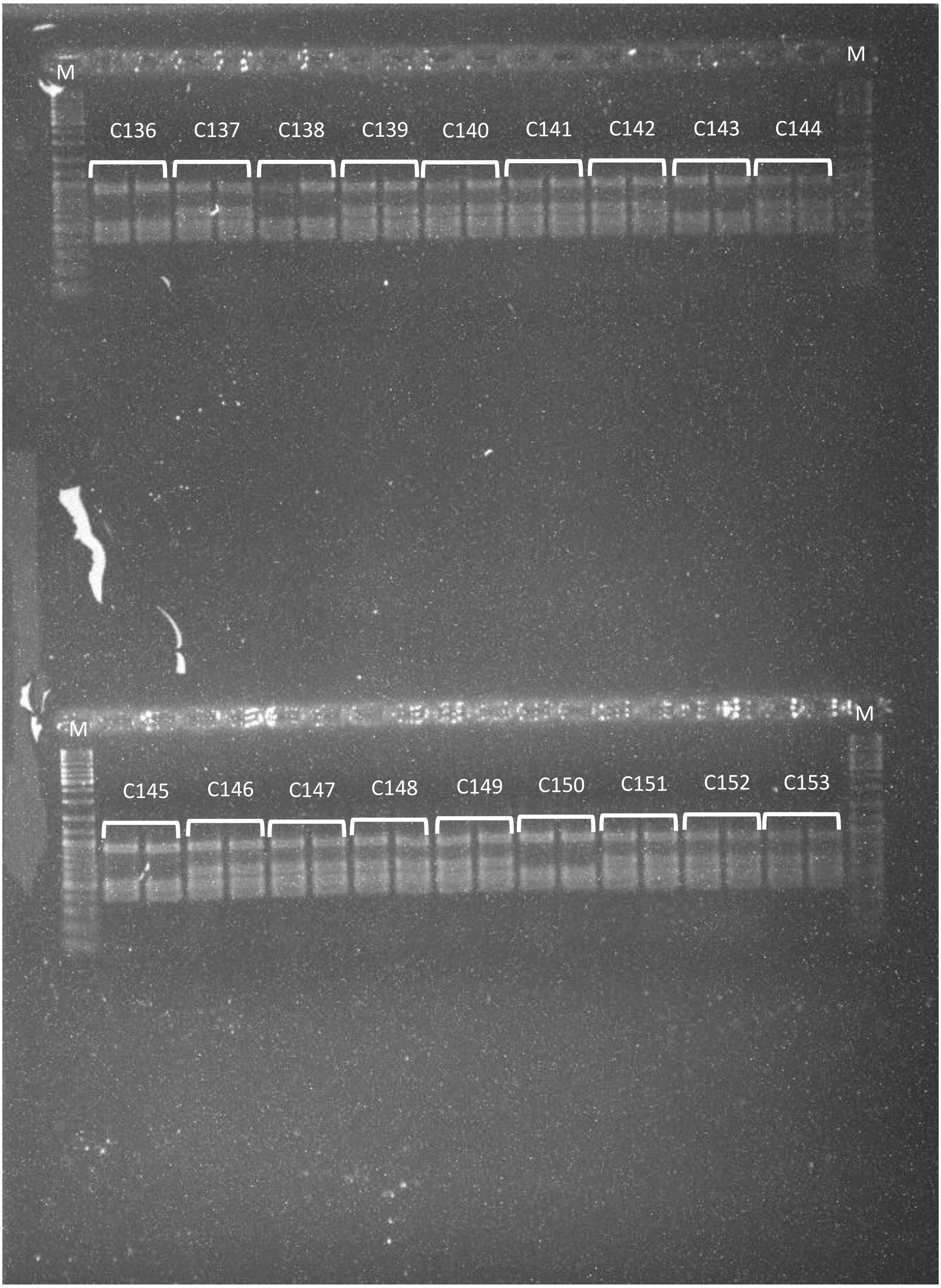
Supplementary Figure S1. *continued*



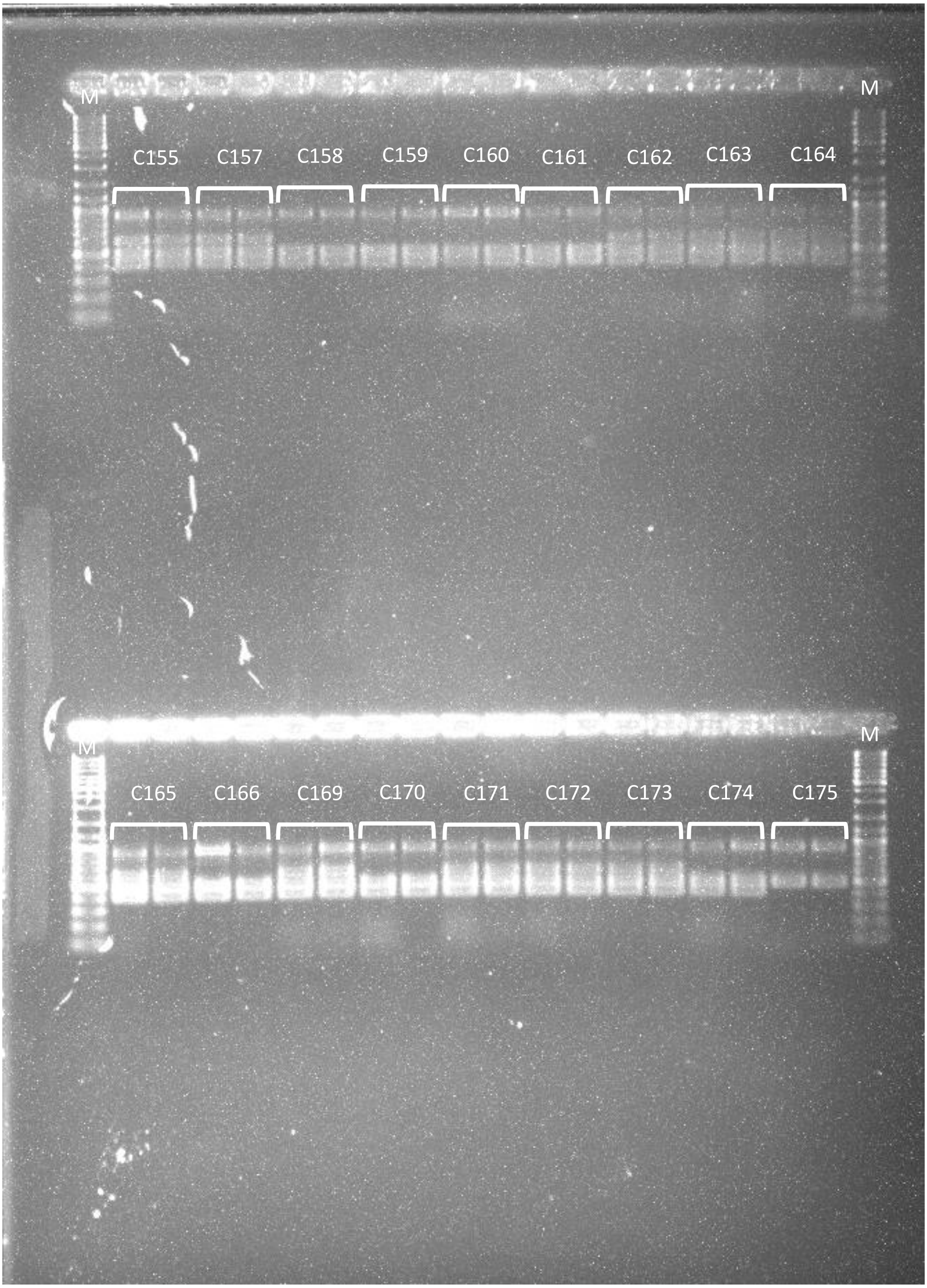
Supplementary Figure S1. *continued*



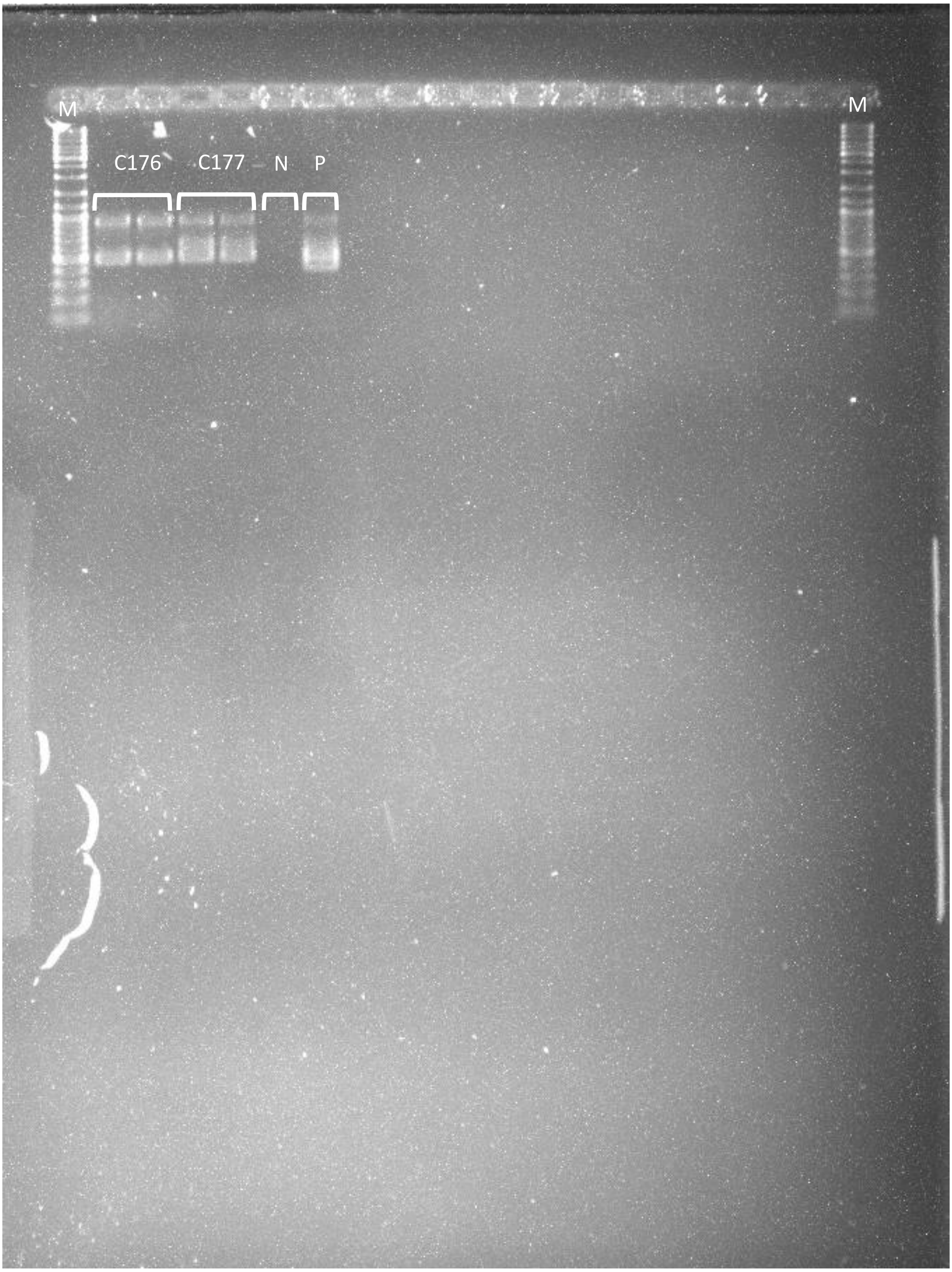
Supplementary Figure S1. *continued*



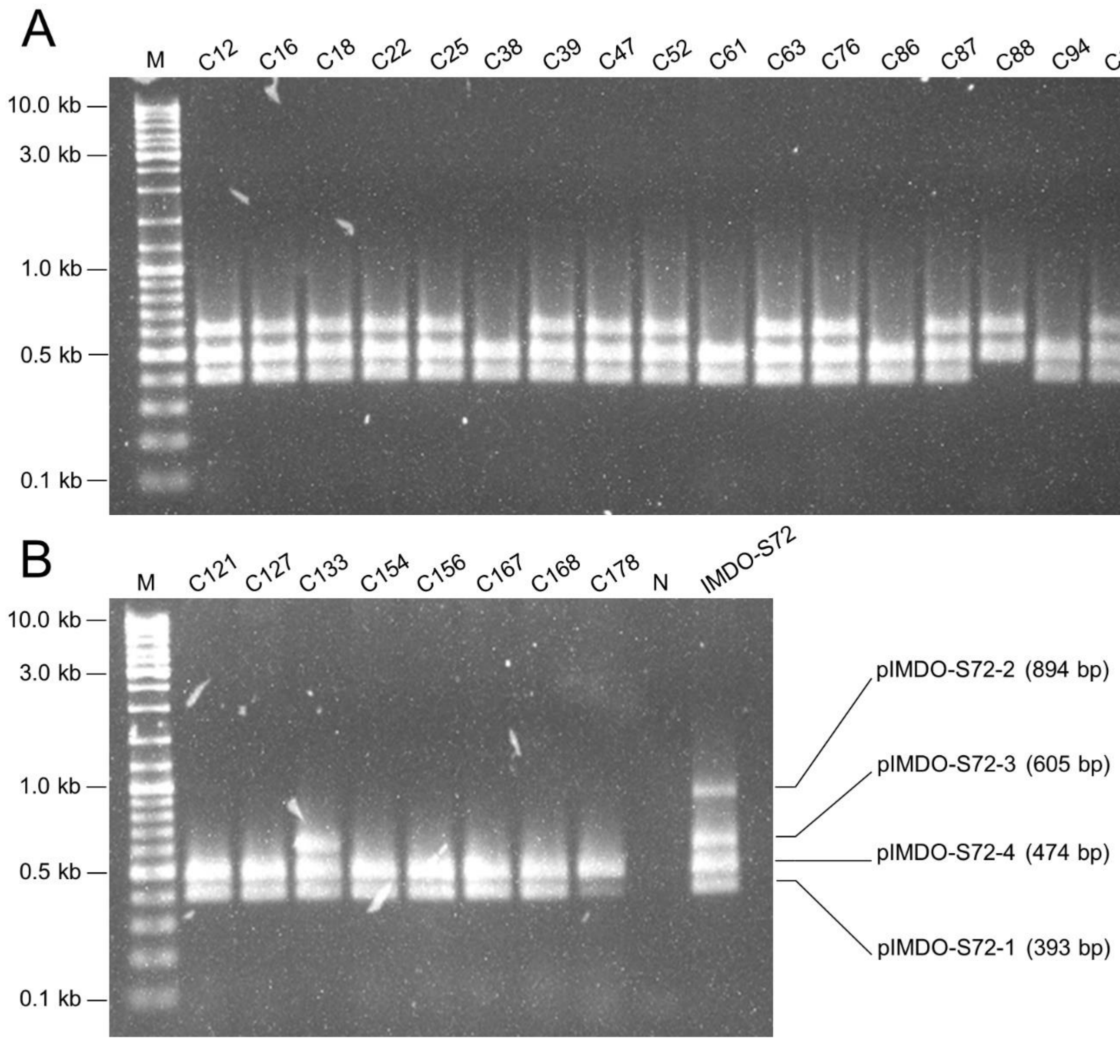
Supplementary Figure S1. *continued*



Supplementary Figure S1. *continued*

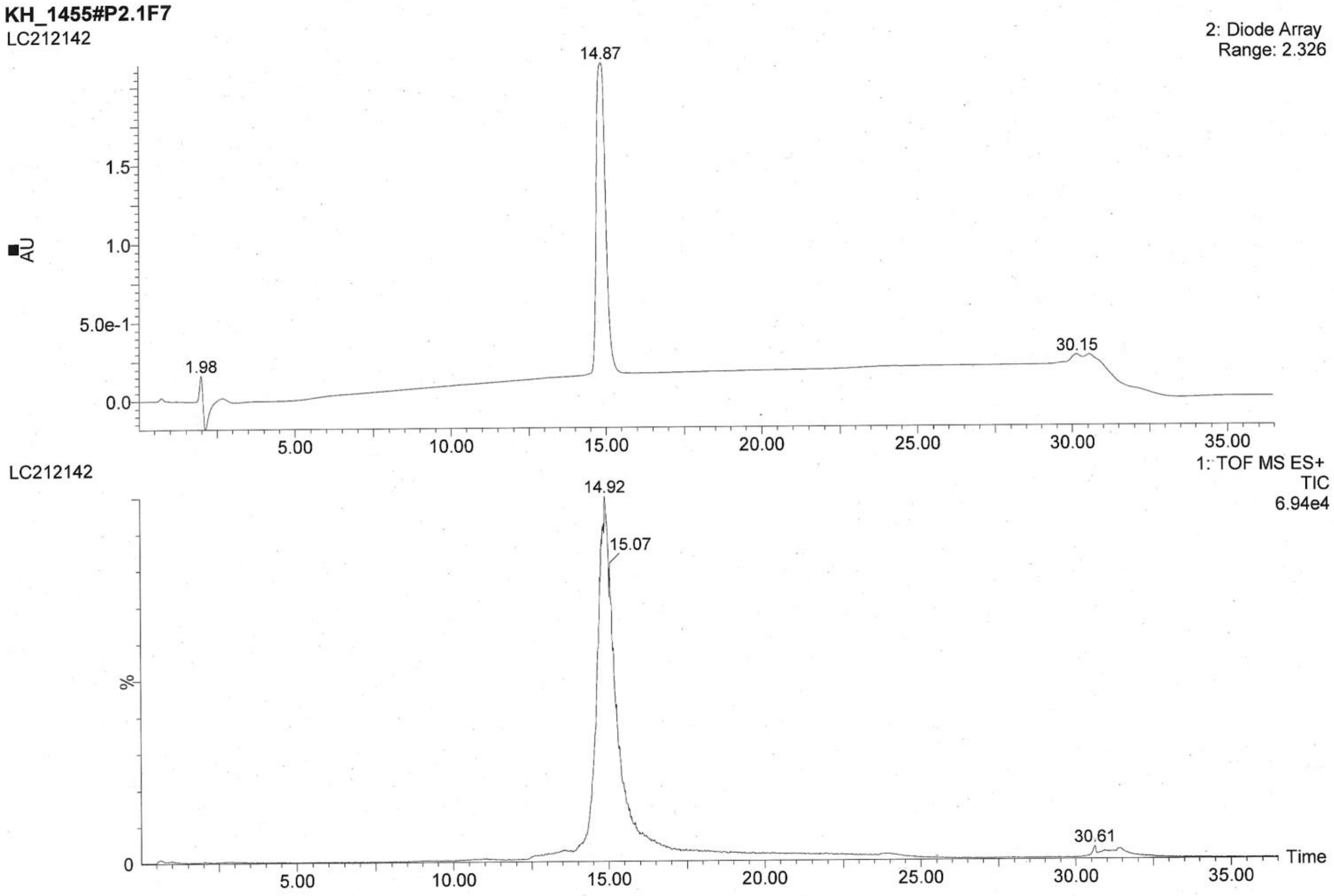


Supplementary Figure S2. PCR profiles of non-antibacterial isolates (indicated with C numbers for each lane) next to the profile of the native *M. sciuri* IMDO-S72. Profiles are based on multiplex PCR using extracted genomic DNA as input. Lanes denoted with M represent molecular size markers, the lane denoted with N represents a negative PCR control.



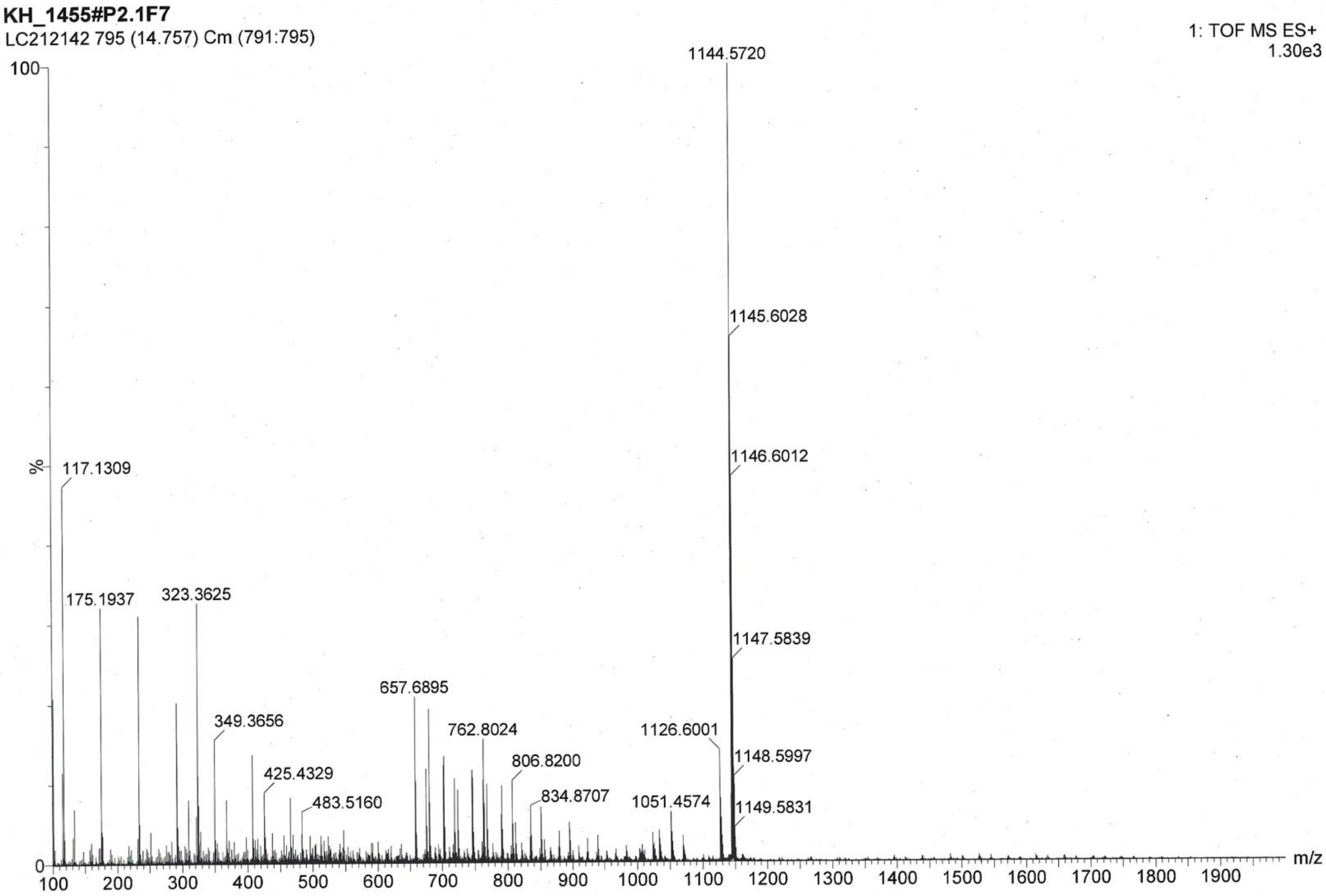
Supplementary Figure S3. Confirmation of the purified micrococcin P1 from fermentation supernatant with LC-MS. (A) Chromatogram showing the elution of micrococcin P1 around 15 min (upper graph) and the total ion count of the corresponding peak (lower graph). (B) Mass spectrum of the eluted fraction at 14.87 min, with an m/z ratio of 1144.572 approximating that of the protonated micrococcin P1 [M+H]$^+$.

A



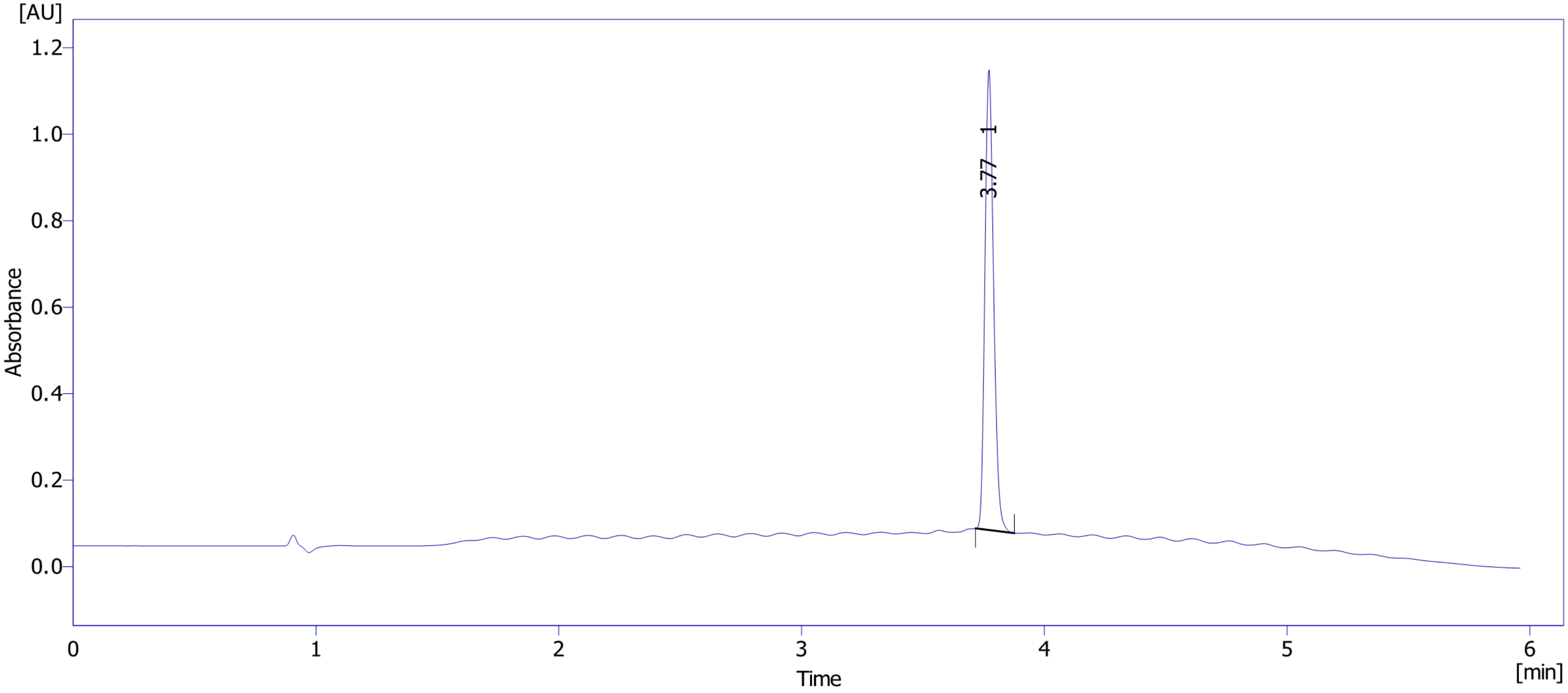
Supplementary Figure S3. *continued*

B



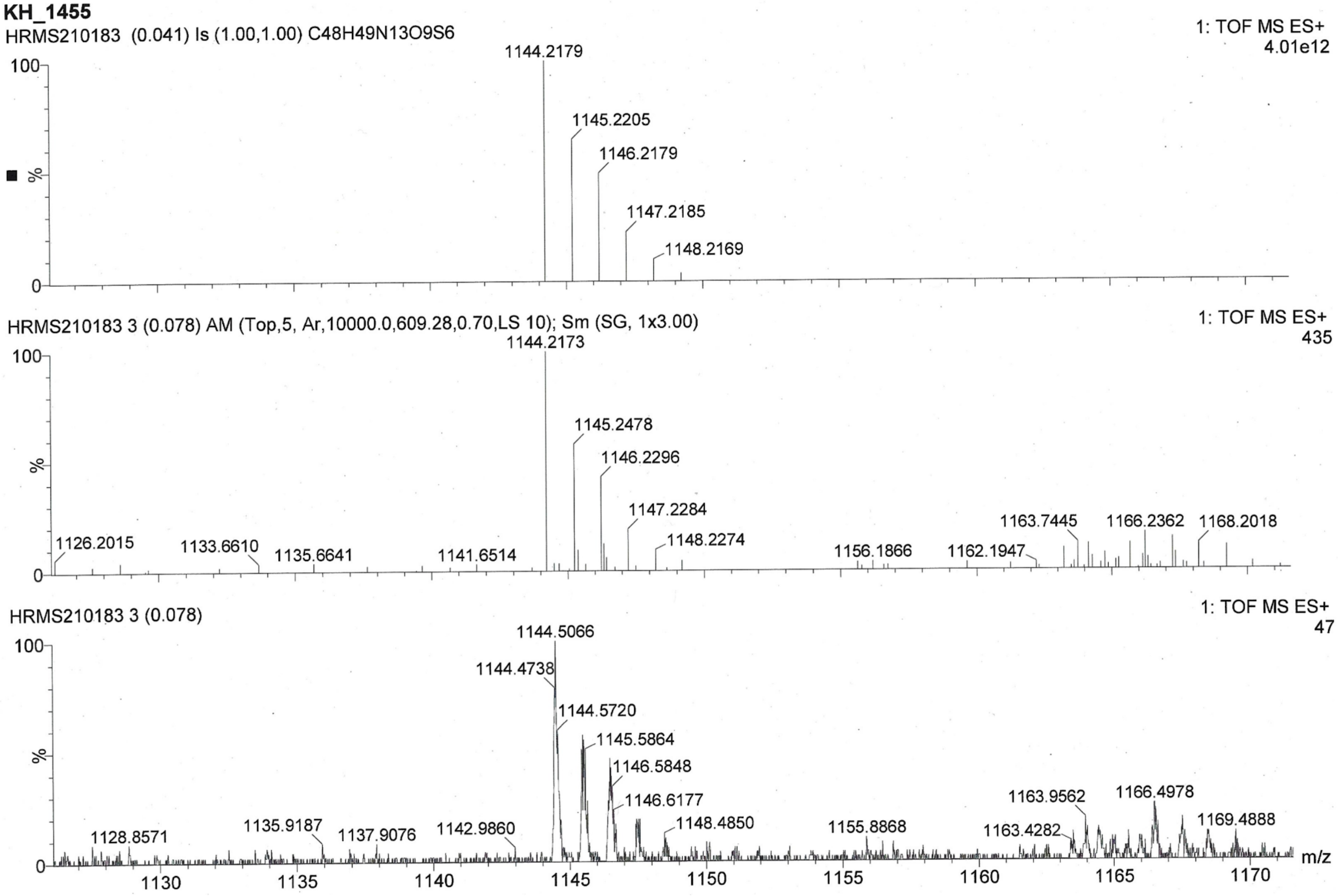
Supplementary Figure S4. Chromatogram of the purified micrococcin P1 extract based on reversed-phase HPLC (A) and its corresponding high-resolution mass spectrum based on HRMS (B).

A

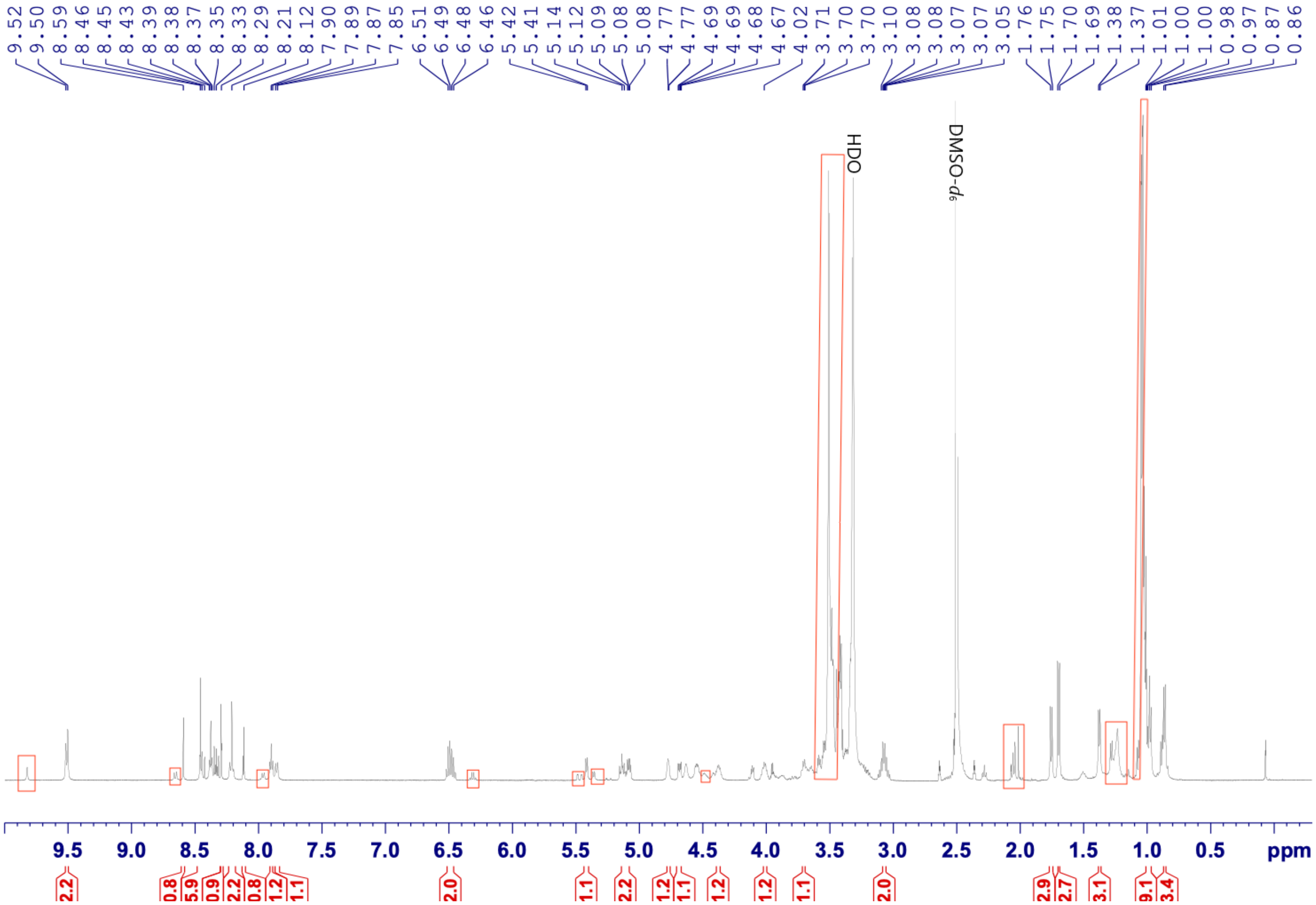


Supplementary Figure S4. *continued*

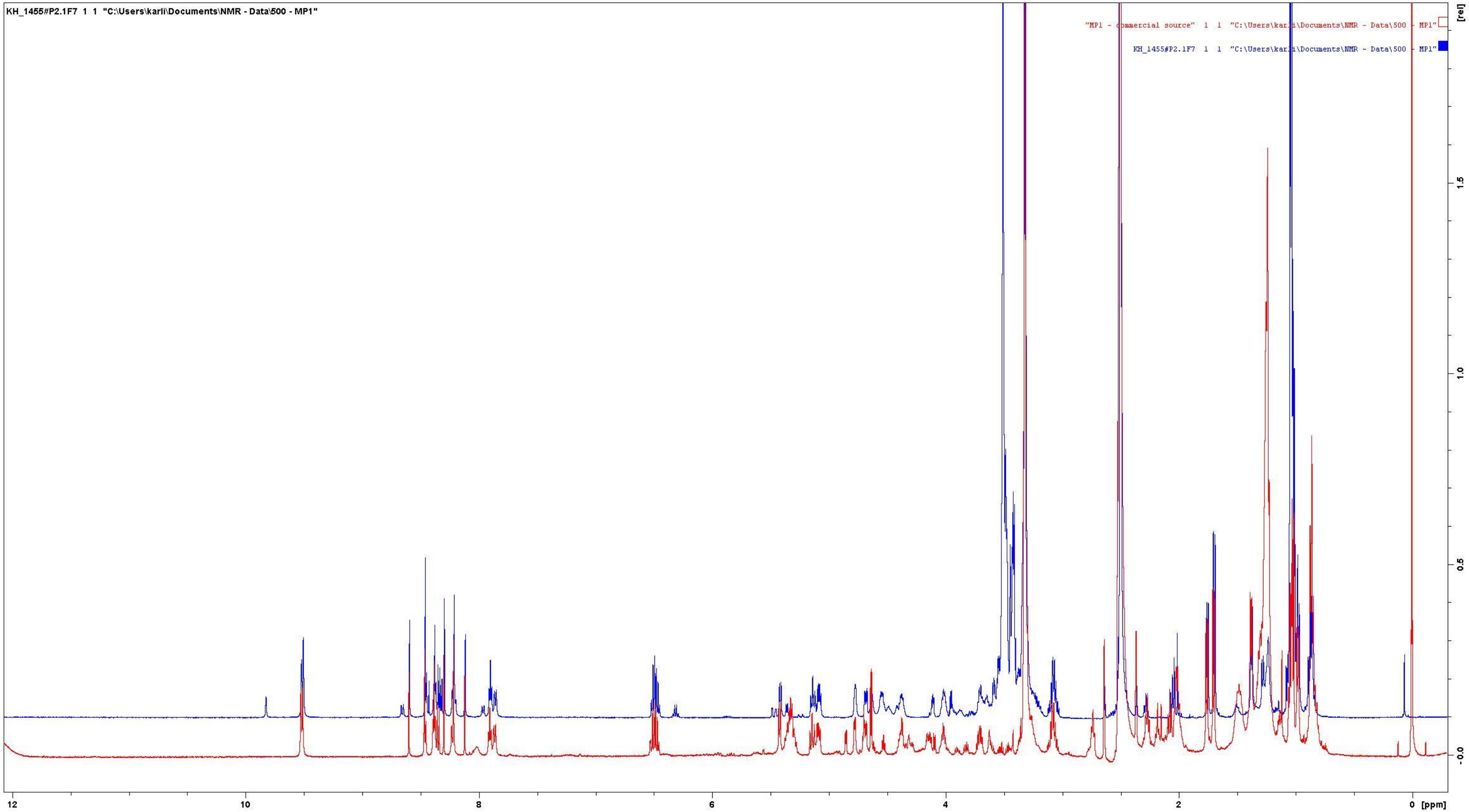
B



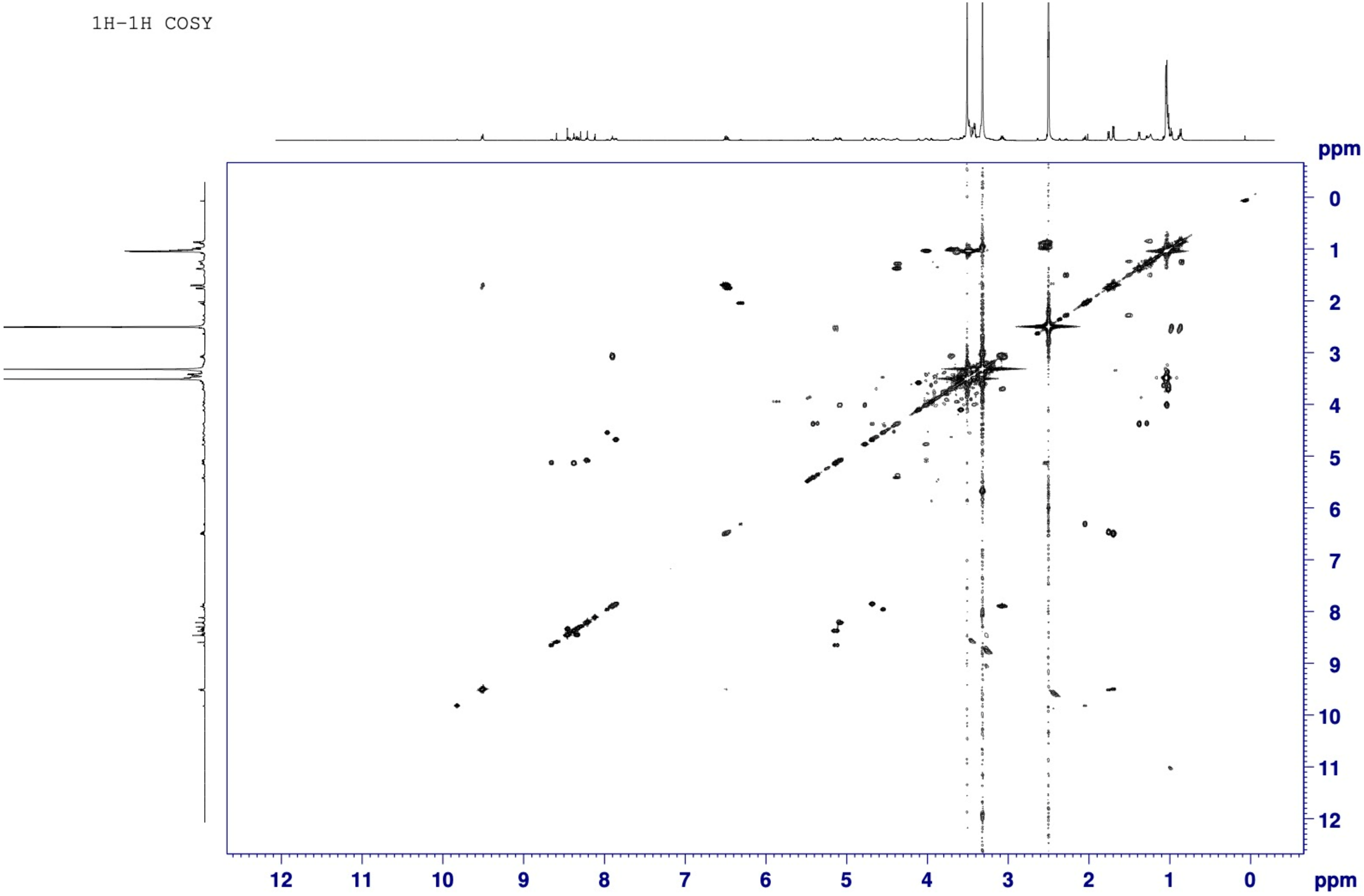
Supplementary Figure S5. ^{1}H NMR spectrum of the purified compound with the signals of micrococcin P1 indicated on top and peak integrations represented in red on the bottom. Signals related to impurities are indicated with a red frame. The sample was recorded in DMSO-*d6* at a field strength of 500 MHz.



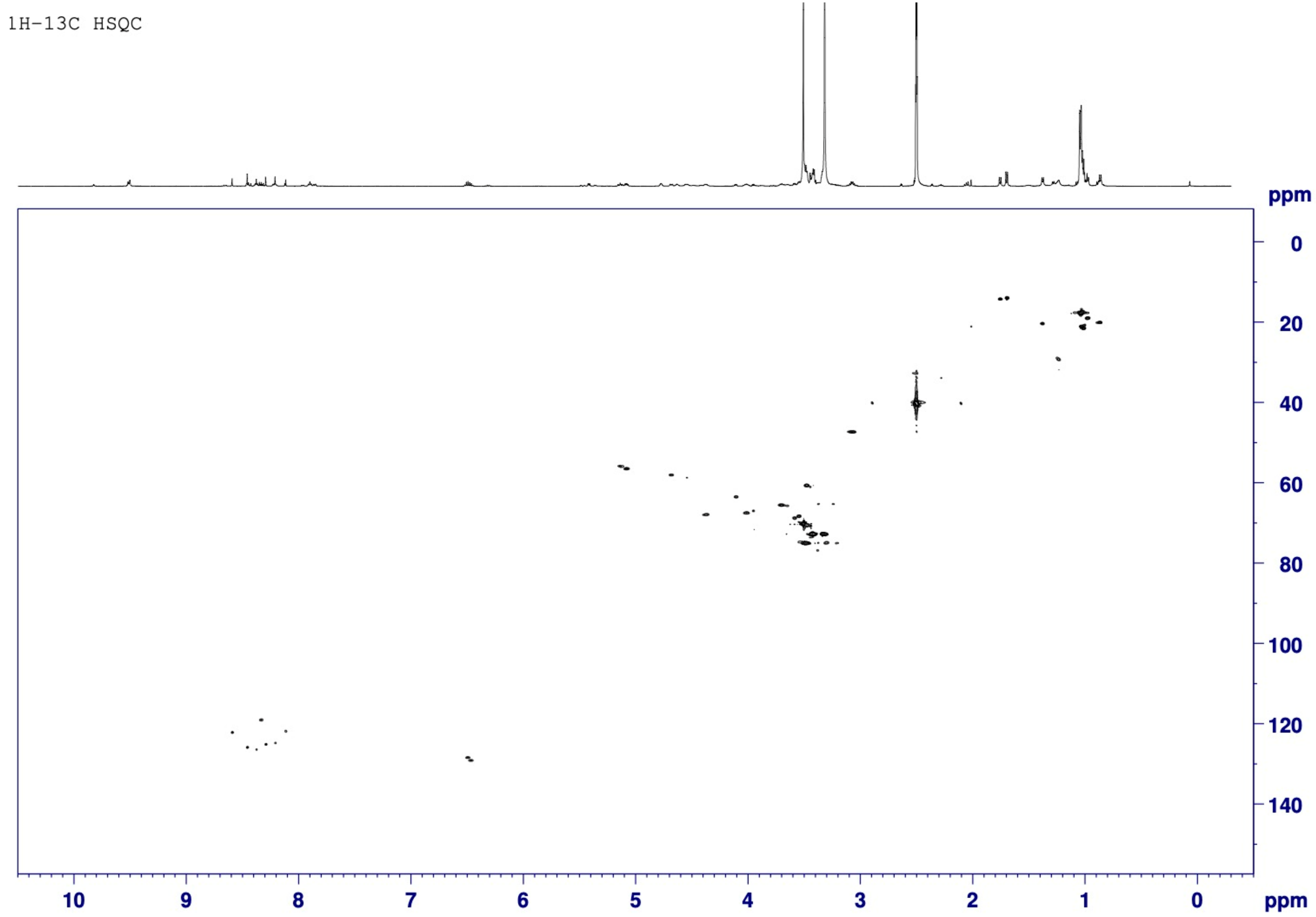
Supplementary Figure S6. ^{1}H NMR spectrum overlay of the purified compound (blue) with the commercially available micrococcin P1 (red) from Cayman Chemical with a purity of 95%. The samples were recorded in DMSO-*d6* at a field strength of 500 MHz.



Supplementary Figure S7. ^{1}H-^{1}H COSY spectrum of the purified compound recorded in DMSO-d_6 with a 500 MHz field strength.



Supplementary Figure S8. ^{1}H-^{13}C HSQC spectrum of the purified compound recorded in DMSO-d_6 with a 500 MHz field strength.



Supplementary Figure S9. ^{1}H-^{13}C HMBC spectrum of the purified compound recorded in DMSO-d_6 with a 500 MHz field strength.