

Actinomadura rubteroloni* sp. nov. and *Actinomadura macrotermitis* sp. nov., isolated from the gut of fungus growing-termite *Macrotermes natalensis

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Table S1. Similarities of 16S rDNA gene sequences between strain RB29^T and closest *Actinomadura*.

Strain	Similarity ^a [%]
RB29 ¹	100.00
<i>Actinomadura rayongensis</i> RY35 ¹ (AB889544)	99.15
<i>Actinomadura atramentaria</i> DSM 43919 ¹ (AJ420138)	98.68
<i>Actinomadura gamaensis</i> NEAU-Gz5 ¹ (KT989505.2)	97.86
<i>Actinomadura geliboluensis</i> A8036 ¹ (HQ157187)	97.64
<i>Actinomadura glauciflava</i> NBRC 14668 ¹ (AB184612.2)	97.61
<i>Actinomadura mexicana</i> DSM 44485 ¹ (AF277195)	97.60
<i>Actinomadura bangladeshensis</i> 3-46-b(3) ¹ (AB331652)	97.57
<i>Actinomadura vinacea</i> JCM 3325 ¹ (AF134070)	97.55
<i>Actinomadura formosensis</i> IMSNU 22194 ¹ (AJ293703)	97.54
<i>Actinomadura roseirufa</i> LMG30035 ¹ (LT996936)	97.47
<i>Actinomadura physcomitrii</i> LD22 ¹ (MH715905)	97.46
<i>Actinomadura madurae</i> DSM 43067 ¹ (X97889)	97.42
<i>Actinomadura adraensis</i> ACD12 ¹ (KU356942)	97.41
<i>Actinomadura hallensis</i> H647-1 ¹ (DQ076484)	97.39
<i>Actinomadura cremae</i> subsp. <i>cremae</i> DSM 43676 ¹ (AF134067)	97.38
<i>Actinomadura sputi</i> IMMIB L-889 ¹ (FM957483)	97.35
<i>Actinomadura meyeri</i> A288 ¹ (AY273787)	97.34
<i>Actinomadura sediminis</i> YIM M 10931 ¹ (JF272484)	97.33
<i>Actinomadura rupiterrae</i> CS5-AC15 ¹ (FM210337)	97.31
<i>Actinomadura viridis</i> DSM 43175T ¹ (AJ420141)	97.29
<i>Actinomadura algeriensis</i> ACD1 ¹ (KT259320)	97.28
<i>Actinomadura chokoriensis</i> JCM 13932 ¹ (AB331730)	97.25
<i>Actinomadura flavalba</i> DSM 45200 ¹ (FJ157185)	97.22
<i>Actinomadura montaniterrae</i> CYP1-1B ¹ (LC126428)	97.22
<i>Actinomadura jiaoheensis</i> NEAU-Jh1-3 ¹ (KM000835)	97.20
<i>Actinomadura sporangiiformans</i> NEAU-Jh2-5 ¹ (KM000834)	97.20
<i>Actinomadura livida</i> IMSNU 22191T (AJ293706)	97.14
<i>Actinomadura harenae</i> NEAU-Ht49 ¹ (MK203829)	97.14
<i>Actinomadura rudentiformis</i> HMC1 ¹ (DQ285420)	97.11
<i>Actinomadura oligospora</i> ATCC43269 ¹ (AF163118)	97.10
<i>Actinomadura napiensis</i> B60 ¹ (AY568292)	97.09
<i>Actinomadura miaoliensis</i> BC 44T-5 (EF116925)	97.07
<i>Actinomadura meridiana</i> DLS-45 ¹ (FN646663)	97.03
<i>Actinomadura keratinilytica</i> WCC-2265 ¹ (EU637009)	97.02
<i>Actinomadura hibisca</i> IMSNU 22185 ¹ (AJ293705)	97.00
<i>Actinomadura syzygii</i> strain GKU 157 ¹ (KF667496)	96.99
<i>Actinomadura verrucospora</i> IFO14100T (U49011)	96.93
<i>Actinomadura xylanilytica</i> BK147 ¹ (FR692101)	96.89
<i>Actinomadura pelletieri</i> IMSNU 22169 ¹ (AJ293710)	96.84
<i>Actinomadura darangshiensis</i> DLS-70 ¹ (FN646682)	96.83
<i>Actinomadura apis</i> JCM 16576 ¹ (AB557596)	96.82
<i>Actinomadura fulvescens</i> DSM 43923 ¹ (AJ420137)	96.81
<i>Actinomadura namibiensis</i> DSM 44197 ¹ (AJ420134)	96.78
<i>Actinomadura fibrosa</i> IMSNU 22177 ¹ (AJ293702)	96.74
<i>Actinomadura kijaniata</i> strain DSM 4376 ¹ (X97890)	96.73
<i>Actinomadura latina</i> DSM 43382 ¹ (AY035998)	96.73
<i>Actinomadura nitritigenes</i> DSM 44137 ¹ (AY035999)	96.71
<i>Actinomadura macra</i> DSM 43862 ¹ (U49009)	96.70
<i>Actinomadura yumaensis</i> ATCC 43060 ¹ (AF163122)	96.40
<i>Actinomadura viridilutea</i> DSM44433 ¹ (D86943)	96.23
<i>Actinomadura glomerata</i> IMSNU 22179 ¹ (AJ293704)	96.19
<i>Actinomadura chibensis</i> DSM 45038 ¹ (AB264086)	96.19
<i>Actinomadura longicatena</i> IMSNU 22180 ¹ (AJ293707)	96.18
<i>Actinomadura rubrobrunea</i> DSM 43750 ¹ (EU637008)	96.13
<i>Actinomadura rifamycini</i> IFO 14183 ¹ (U49003)	95.99
<i>Actinomadura catellatospora</i> JCM 10667 ¹ (AF154127)	95.77
<i>Actinomadura libanotica</i> DSM 43544 ¹ (U49007)	95.57
<i>Actinomadura alba</i> YIM 45681 ¹ (DQ985164)	95.13
<i>Actinomadura scrupuli</i> R-Ac121 ¹ (FM210339)	94.71

^a Sequence similarities were calculated using the method recommended by Meier-Kolthoff (2)

Table S2. Similarities of 16S rDNA gene sequences between strain RB68^T and closest *Actinomadura* strains.

Strain	Similarity ^a [%]
RB68 ^T	100.00
<i>Actinomadura hibisca</i> IMSNU 22185 ^T (AJ293705.1)	98.27
<i>Actinomadura gamaensis</i> NEAU-Gz5 ^T (KT989505.2)	98.13
<i>Actinomadura namibiensis</i> DSM 44197 ^T (AJ420134.1)	98.04
<i>Actinomadura montaniterrae</i> CYP1-1B ^T (LC126428.1)	98.04
<i>Actinomadura kijaniata</i> DSM 4376 ^T (X97890.1)	97.85
<i>Actinomadura physcomitrii</i> LD22 ^T (MH715905)	97.80
<i>Actinomadura nitritigenes</i> DSM 44137 ^T (AY035999.1)	97.75
<i>Actinomadura fibrosa</i> IMSNU 22177 ^T (AJ293702.1)	97.70
<i>Actinomadura harenae</i> NEAU-Ht49 ^T (MK203829)	97.68
<i>Actinomadura roseirufa</i> LMG30035T (LT996936)	97.54
<i>Actinomadura miaoliensis</i> BC 44T-5 ^T (EF116925.1)	97.54
<i>Actinomadura rudentiformis</i> HMC1 ^T (DQ285420.1)	97.54
RB29 ^T	97.53
<i>Actinomadura geliboluensis</i> A8036 ^T (HQ157187.1)	97.51
<i>Actinomadura fulvescens</i> DSM 43923 ^T (AJ420137.1)	97.44
<i>Actinomadura keratinilytica</i> WCC-2265 ^T (EU637009.1)	97.42
<i>Actinomadura xylanilytica</i> BK147 ^T (FR692101.1)	97.31
<i>Actinomadura oligospora</i> ATCC43269 ^T (AF163118.1)	97.31
<i>Actinomadura rayongensis</i> RY35 ^T (AB889544.1)	97.22
<i>Actinomadura rupiterrae</i> CS5-AC15 ^T (FM210337.1)	97.12
<i>Actinomadura atramentaria</i> DSM 43919 ^T (AJ420138)	97.10
<i>Actinomadura flavalba</i> DSM 45200 ^T (FJ157185.1)	97.09
<i>Actinomadura madurae</i> DSM 43067 ^T (X97889.1)	97.08
<i>Actinomadura vinacea</i> JCM 3325 ^T (AF134070.1)	97.07
<i>Actinomadura bangladeshensis</i> 3-46-b(3) ^T (AB331652.1)	96.97
<i>Actinomadura darangshiensis</i> DLS-70 ^T (FN646682.1)	96.96
<i>Actinomadura napierensis</i> B60 ^T (AY568292.1)	96.95
<i>Actinomadura jiaoheensis</i> NEAU-Jh1-3 ^T (KM000835.1)	96.94
<i>Actinomadura sporangiiformans</i> NEAU-Jh2-5 ^T (KM000834.1)	96.94
<i>Actinomadura viridis</i> DSM 43175 ^T (AJ420141.1)	96.88
<i>Actinomadura formosensis</i> IMSNU 22194 ^T (AJ293703.1)	96.87
<i>Actinomadura hallensis</i> H647-1 ^T (DQ076484.1)	96.86
<i>Actinomadura glomerata</i> IMSNU 22179 ^T (AJ293704.1)	96.80
<i>Actinomadura meyerae</i> A288 ^T (AY273787.1)	96.80
<i>Actinomadura sputi</i> IMMIB L-889 ^T (FM957483.1)	96.75
<i>Actinomadura glauciflava</i> NBRC 14668 ^T (AB184612.2)	96.74
<i>Actinomadura longicatena</i> IMSNU 22180 ^T (AJ293707.1)	96.73
<i>Actinomadura mexicana</i> DSM 44485 ^T (AF277195.1)	96.69
<i>Actinomadura chokoriensis</i> JCM 13932 ^T (AB331730.1)	96.65
<i>Actinomadura yumaensis</i> ATCC 43060 ^T (AF163122.1)	96.55
<i>Actinomadura syzygii</i> GKU 157 ^T (KF667496.1)	96.39
<i>Actinomadura libanotica</i> DSM 43544 ^T (U49007.1)	96.36
<i>Actinomadura macra</i> DSM 43862 ^T (U49009.1)	96.34
<i>Actinomadura viridilutea</i> DSM44433 ^T (D86943.1)	96.31
<i>Actinomadura rubrobrunea</i> DSM 43750 ^T (EU637008.1)	96.21
<i>Actinomadura apis</i> JCM 16576 ^T (AB557596.1)	96.16
<i>Actinomadura adrarensis</i> ACD12 ^T (KU356942.1)	96.06
<i>Actinomadura sediminis</i> YIM M 10931 ^T (JF272484.1)	96.02
<i>Actinomadura cremea</i> subsp. <i>cremea</i> DSM 43676 ^T (AF134067.1)	95.99
<i>Actinomadura algeriensis</i> ACD1 ^T (KT259320.1)	95.91
<i>Actinomadura latina</i> DSM 43382 ^T (AY035998.1)	95.85
<i>Actinomadura alba</i> YIM 45681 ^T (DQ985164.1)	95.83
<i>Actinomadura chibensis</i> DSM 45038 ^T (AB264086.1)	95.71
<i>Actinomadura meridiana</i> DLS-45 ^T (FN646663.1)	95.57
<i>Actinomadura rifamycini</i> IFO 14183 ^T (U49003.1)	95.45
<i>Actinomadura scrupuli</i> R-Ac121 ^T (FM210339.1)	95.36
<i>Actinomadura catellatospora</i> JCM 10667 ^T (AF154127.1)	95.28

^a Sequence similarities were calculated using the method recommended by Meier-Kolthoff (2)

Table S3. *In silico* DDH and ANI values of RB29^T and publically available *Actinomadura* genomes.

	NCBI number	Estimated dDDH ^b [%]	ANI ^b [%]	OrthoANI ^d [%]
RB68 ¹		22.50 (20.30-24.80)	78.16	83.51
<i>Actinomadura atramentaria</i> DSM43919 ¹	ARMS00000000.1	26.40 (24.10-28.90)	82.29	78.96
<i>Actinomadura bangladeshensis</i> DSM45347 ^T	SMJW00000000.1	22.50 [20.20-24.90]	77.39	78.83
<i>Actinomadura chibensis</i> NBRC106107 ¹	NZ_BCQP00000000.1	21.90 (19.70-24.40)	77.30	78.82
<i>Actinomadura darangshiensis</i> DSM45941 ^T	SMKY00000000.1	22.00 (19.80-24.50)	77.36	80.35
<i>Actinomadura flavalba</i> DSM45200 ¹	ARFO00000000.1	23.20 (20.90-25.60)	78.96	79.14
<i>Actinomadura fibrosa</i> LMG29177 ¹	CAACUY00000000.1	22.70 (20.40-25.10)	77.97	78.82
<i>Actinomadura formosensis</i> NBRC14204 ¹	BCQQ00000000.1	22.10 (19.80-23.60)	77.22	78.91
<i>Actinomadura geliboluensis</i> A8036 ¹	VCKZ00000000.1	22.50 (20.20-25.00)	77.76	78.94
<i>Actinomadura hallensis</i> DSM45043 ¹	VFPO00000000.1	22.90 (20.60-25.3)	77.34	78.74
<i>Actinomadura harenae</i> NEAU-Ht49 ¹	RFFG00000000.1	22.50 (20.20-24.90)	77.01	78.49
<i>Actinomadura hibisca</i> NBRC15177 ¹	BCRO00000000.1	22.40 (20.10-24.80)	77.74	78.55
<i>Actinomadura kijaniata</i> NBRC14229 ¹	BCQR00000000.1	22.00 (19.70-24.40)	77.11	78.60
<i>Actinomadura latina</i> NBRC106108 ¹	BCQS00000000.1	21.90 (19.70-24.40)	77.13	78.09
<i>Actinomadura macra</i> NBRC14102 ¹	BCQT00000000.1	(19.70-24.40)	76.92	78.74
<i>Actinomadura madurae</i> DSM43067 ¹	FOVH00000000.1	22.00 (19.70-24.40)	77.14	78.62
<i>Actinomadura mexicana</i> DSM44485 ¹	FZNP00000000.1	22.20 (19.90-24.60)	77.32	78.85
<i>Actinomadura meyeri</i> DSM44715 ¹	FZOR00000000.1	22.30 (19.80-24.50)	77.48	78.20
<i>Actinomadura montaniterrae</i> CYP1-1B ¹	WBMR00000000.1	22.90 (20.60-25.30)	77.89	79.17
<i>Actinomadura oligospora</i> ATCC43269 ¹	JADG00000000.1	22.10 (19.80-24.50)	76.85	73.55
<i>Actinomadura parvosata</i> subsp. <i>Kistnae</i> isolate1	OOHJ00000000.1	20.00 (17.80-22.40)	71.96	78.12
<i>Actinomadura pelletieri</i> DSM43383 ¹	RBWU00000000.1	22.00 (19.70-24.40)	76.61	83.51
<i>Actinomadura physcomitrii</i> LD22 ¹	WBMS00000000.2	22.60 (20.30-25.10)	81.27	79.15
<i>Actinomadura rayongensis</i> DSM102126 ¹	NZ_WUTW00000000	53.19 (50.60-55.90)	93.15	94.00
<i>Actinomadura rifamycini</i> DSM43936 ¹	AULB00000000.1	22.10 (19.90-24.60)	77.25	78.74
<i>Actinomadura roseirufa</i> LMG 30035 ¹	CAACVB00000000.1	22.80 (20.50-25.30)	77.35	78.88
<i>Actinomadura rubrobrunea</i> NBRC15275 ¹	BCQU00000000.1	22.40 (20.20-24.90)	77.01	78.26
<i>Actinomadura rudentiformis</i> HMC1 ¹	WBMT00000000.1	22.00 (19.80-24.50)	76.05	77.70
<i>Actinomadura syzygii</i> GKU157 ¹	VSFF00000000.1	21.80 (19.50-24.20)	77.03	78.45
<i>Actinomadura viridilutea</i> DSM44433 ¹	PVNI00000000.1	22.30 (20.10-24.80)	76.98	78.22

^b *in silico* DDH values were calculated using the GGDC web server available at <http://ggdc.dsmz.de/>, confidence values between parentheses (3)

^c ANI values were calculated using ANI calculator web server available at <http://jspecies.ribohost.com/jspeciesws> (4)

^d ANI values were calculated using EZBio cloudr web server available at <https://www.ezbiocloud.net/tools/ani> (5)

Table S4. Statistical parameters of sequenced genomes.

Parameter	RB29 ¹	RB68 ¹	<i>Actinomadura rayongensis</i> DSM 102126 ¹
Genome size [bp]	6.473.359	8.536.380	6.868.284
GC content mol%	73.09	73.12	73.06
Number of contigs	13	13	15
N50	2.134.329	1.947.532	1.778.705

Table S5. *In silico* DDH and ANI values of RB68^T and *Actinomadura* genomes available at NCBI server.

	NCBI number	Estimated dDDH ^e [%]	ANi ^f [%]	OrthoANI ^g [%]
<i>Actinomadura atramentaria</i> DSM43919 ¹	ARMS000000000.1	22.90 (20.60-25.30)	77.74	79.69
<i>Actinomadura bangladeshensis</i> DSM45347 ^T	SMJW000000000.1	23.30 (21.00-25.80)	78.29	80.02
<i>Actinomadura chibensis</i> NBRC106107 ¹	BCQP000000000.1	22.60 (20.40-25.10)	77.78	79.58
<i>Actinomadura darangshiensis</i> DSM45941 ¹	SMKY000000000.1	23.00 (20.70-25.40)	78.29	79.77
<i>Actinomadura flavalba</i> DSM45200 ¹	ARFO000000000.1	23.80 (20.70-25.40)	77.78	79.89
<i>Actinomadura fibrosa</i> LMG29177 ¹	CAACUY000000000.1	23.00 (21.50-26.30)	79.01	80.22
<i>Actinomadura formosensis</i> NBRC14204 ¹	BCQQ000000000.1	23.10 (20.80-25.50)	77.91	80.01
<i>Actinomadura geliboluensis</i> A8036 ¹	VCKZ000000000.1	23.40 (21.10-25.80)	78.19	80.07
<i>Actinomadura hallensis</i> DSM45043 ¹	VFPO000000000.1	23.40 (21.10-25.80)	77.95	79.59
<i>Actinomadura harenae</i> NEAU-Ht49 ¹	RFFG000000000.1	23.00 (20.70-25.50)	77.64	79.04
<i>Actinomadura hibisca</i> NBRC15177 ¹	BCRO000000000.1	24.50 (22.20-27.00)	80.12	81.61
<i>Actinomadura kijaniata</i> NBRC14229	BCQR000000000.1	23.80 (21.50-27.40)	79.48	80.80
<i>Actinomadura latina</i> NBRC106108 ¹	BCQS000000000.1	23.00 (20.70-25.50)	78.10	79.92
<i>Actinomadura macra</i> NBRC14102 ¹	BCQT000000000.1	22.70 (20.50-25.20)	77.81	79.47
<i>Actinomadura madurae</i> DSM43067 ¹	FOVH000000000.1	23.00 (20.70-25.40)	78.15	79.91
<i>Actinomadura mexicana</i> DSM44485 ¹	FZNP000000000.1	23.10 (20.80-25.60)	78.38	79.94
<i>Actinomadura meyeræ</i> DSM44715 ¹	FZOR000000000.1	23.20 (20.90-25.70)	78.38	79.93
<i>Actinomadura montaniterræe</i> CYP1-1B ¹	WBMR000000000.1	23.70 (21.40-26.20)	78.84	80.31
<i>Actinomadura oligospora</i> ATCC43269 ¹	JADG000000000.1	22.80 (20.50-25.30)	77.83	79.06
<i>Actinomadura parvosata</i> subsp. Kistnae isolate1	OOHJ000000000.1	20.10 (17.90-22.50)	72.61	74.33
<i>Actinomadura pelletieri</i> DSM43383 ¹	RBWU000000000.1	22.40 (20.10-24.80)	76.93	78.98
<i>Actinomadura physcomitrii</i> LD22 ¹	WBMS000000000.2	23.40 (21.10-25.80)	78.61	80.41
<i>Actinomadura rayongensis</i> DSM102126 ¹	NZ_WUTW000000000	22.40 (20.10-24.90)	77.40	79.62
<i>Actinomadura rifamycinini</i> DSM43936 ¹	AULB000000000.1	22.80 (20.50-25.20)	77.70	79.31
<i>Actinomadura roseirufa</i> LMG30035 ¹	CAACVB000000000.1	23.90 (21.60-26.40)	78.08	79.97
<i>Actinomadura rubrobrunea</i> NBRC15275 ¹	BCQU000000000.1	23.50 (21.20-26.00)	78.03	79.92
<i>Actinomadura rudentiformis</i> HMC1 ¹	WBMT000000000.1	22.80 (20.60-25.30)	77.73	79.11
<i>Actinomadura syzygii</i> GKU157 ¹	VSFF000000000.1	22.60 (20.30-25.10)	77.55	79.28
<i>Actinomadura viridilutea</i> DSM44433 ¹	PVNI000000000.1	23.40 (21.10-25.90)	78.01	79.89

^e *in silico* DDH values were calculated using the GGDC web server available at <http://ggdc.dsmz.de/> (3).

^f ANI values were calculated using ANI calculator web server available at <http://jspecies.ribohost.com/jspeciesws> (4)

^g ANI values were calculated using EZBio cloudr web server available at <https://www.ezbiocloud.net/tools/ani> (5)

Table S6. Growth and phenotypic characteristics of strain RB29^T and RB68^T and closely related type strains of the genus *Actinomadura* after 12-14 days of incubation at 28 °C. Strains: 1. RB29^T; 2. *A. rayongensis* DSM 102126^T; 3. *A. atramentaria* DSM 43919^T; 4. RB68^T; 5. *A. hibisca* DSM 44148^T. All data was acquired in this study. Morphological feature: G growth, AM aerial mycelium, SM substrate mycelium, SP soluble pigment, Colour coding (N.) corresponding to Baumanns Farbatlas 1 in parentheses.

Medium	Morphol. feature	1	2	3	4	5
ISP1	G	Good	Good	Good	Good	Good
	AM	White	White	Sparse, white	None	None
	SM	Pale	Greyish-White	Light ochre	Light ochre (No. 10-00EA)	Red (No. 211-27PA)
	SP	Pure red (No.221-27NE)	None	Brown-pink (No.191-23IE)	None	None
ISP2	G	Good	Good	Good	Good	Good
	AM	White	White	Sparse, white	Sparse, white	None
	SM	Pale brownish	Brownish	White	Yellow (No. 69-08LA)	Red violet (No. 252-30LC)
	SP	Ruby red (No. 288-27NE)	None	Green-brown (No.89-08PI)	None	Black red (No. 229-27PI)
ISP3	G	Good	Good	Poor	Moderate	Good
	AM	White	White	None	Sparse, white	Sparse
	SM	Pale-pinkish	Greyish-white	Green-brown	White	Light pink
	SP	Antique pink (No. 241-30GC)	None	Greenish	None	Light pink (No. 206-27EA)
ISP4	G	Moderate	Moderate	Moderate	Moderate	Moderate
	AM	White	White	White	White	Sparse
	SM	Pale	Grey-brownish	White	White	Light pink
	SP	Pink (No 263-34 GA)	None	None	None	Light pink (No. 205-27CA)
ISP5	G	Good	Good	Moderate	Good	Moderate
	AM	None	Brownish	Poor, white	None	None
	SM	Grey (No. 5-1)	Brownish	Reseda green (No. 56-04LG)	Ochre	Light pink
	SP	Claret violet (No.229-27OI)	None	Green (No. 29-00NG)	Ochre (No. 68-08IA)	Light pink (No. 206-27EA)
ISP6	G	Good	Good	Poor	Good	Good
	AM	None	None	None	None	None
	SM	Grey, light purple touch	Green (No. 56-04LG)	Yellow-greenish	Light yellow (No. 10-00EA)	Red (No. 245-30PC)
	SP	Signal red (211-27PA)	Greenish	None	None	Light red

ISP7	G	Good	Moderate	Moderate	Good	Good
	AM	None	None	Poor, white,	None	Sparse
	SM	Grey (No5-I)	Light ochre	Green (No. 56-04LG)	Bright ochre (No. 101-13GC)	Red
	SP	Signal brown (No. 117-13PI)	Pink (No.191-23IE)	Dark brown (No. 148-17PN)	Bright ochre (No. 101-13GC)	Brown (No. 173-20PI)
Suter medium without tyrosine	G	Good	Moderate	Moderate	Moderate	Moderate
	AM	Poor, white	None	None	None	None
	SM	Pale brown (No. 115-13LI)	Brown-grey	Green (No. 56-04LG)	White-yellow (No. 67-08GA)	Light pink
	SP	Black red (No. 260-30PN)	None	Dark brown (No. 148-17PN)	None	None
Suter medium with tyrosine	G	Good	Moderate	Moderate	Moderate	Moderate
	AM	Poor, white	None	None	None	None
	SM	Pale brown (No. 115-13LI)	Grey-brown	Green (No. 56-04LG)	Light brown (No.108-13LE)	Light pink
	SP	Black red (No. 260-30PN)	Brown-pink	Dark brown (No. 148-17PN)	Brown (No. 117-13PI)	Brown (no. 173-20PI)

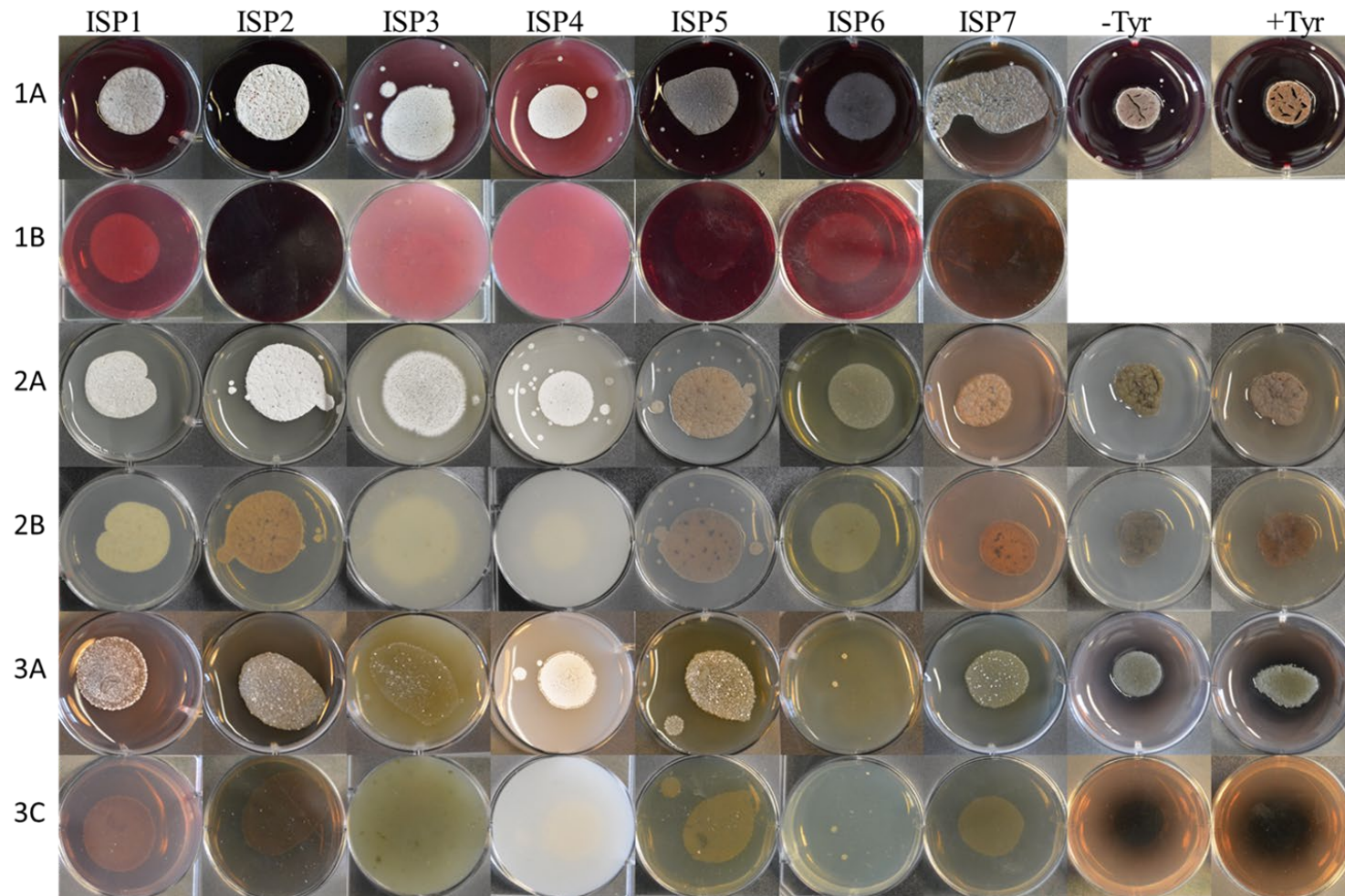


Figure S1. Morphology of strains grown for 9 days at 28 °C on different ISP-media and Suter-Medium (with: +Tyr [1 g/L] and without tyrosine: -Tyr).

Strains: RB29^T (1A: above, 1B: reverse); *Actinomadura rayongensis* DSM102126^T (2A: above, 2B: reverse); *Actinomadura artramentaria* DSM 44919^T (3A: above; 3B: reverse).

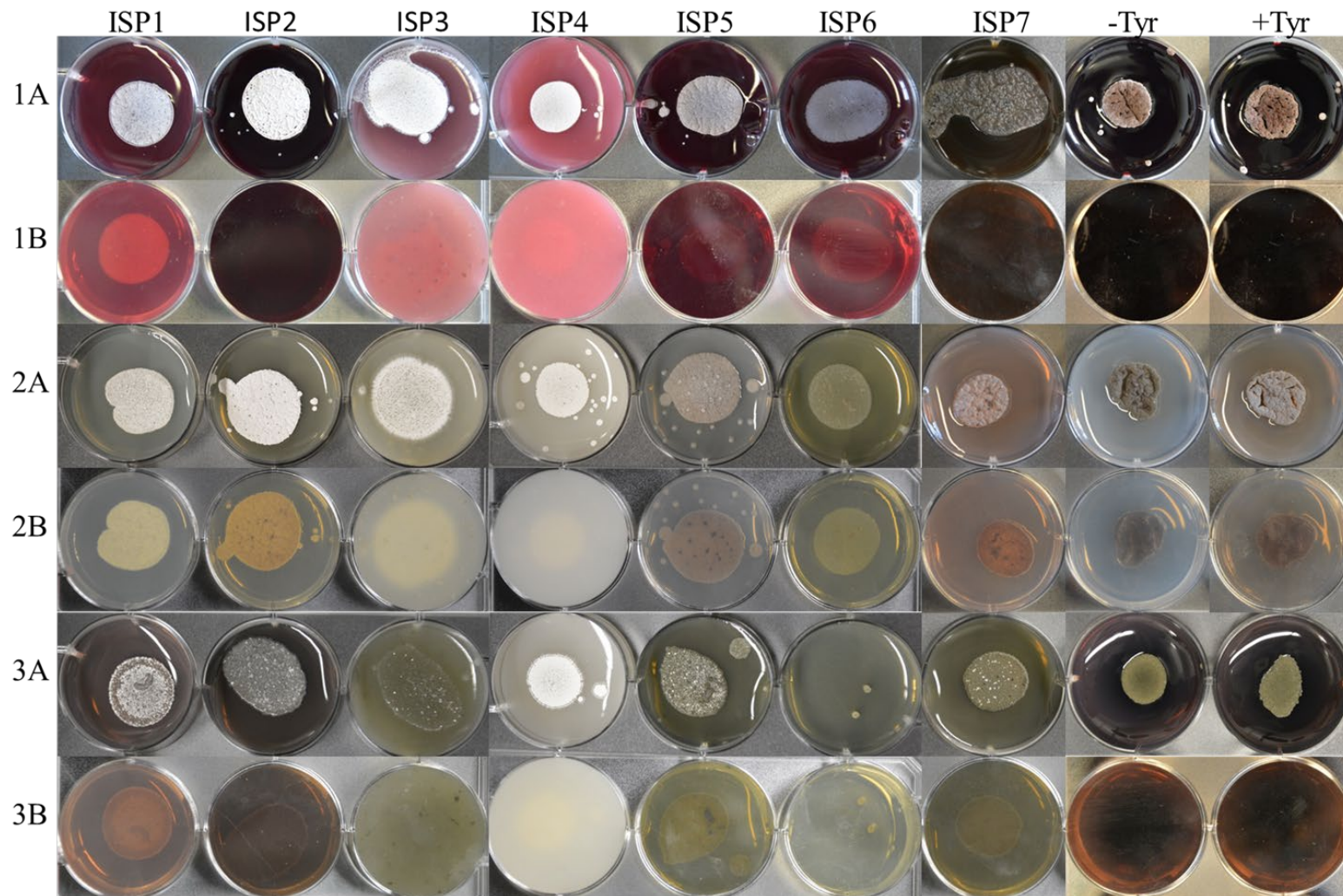


Figure S2. Morphology of strains grown for 14 days at 28 °C on different ISP-media and Suter-Medium (with: +Tyr [1 g/L] and without tyrosine: -Tyr).

Strains: RB29^T (1A: above, 1B: reverse); *Actinomadura rayongensis* DSM 102126^T (2A: above, 2B: reverse); *Actinomadura artramentaria* DSM 44919^T (3A: above; 3B: reverse).

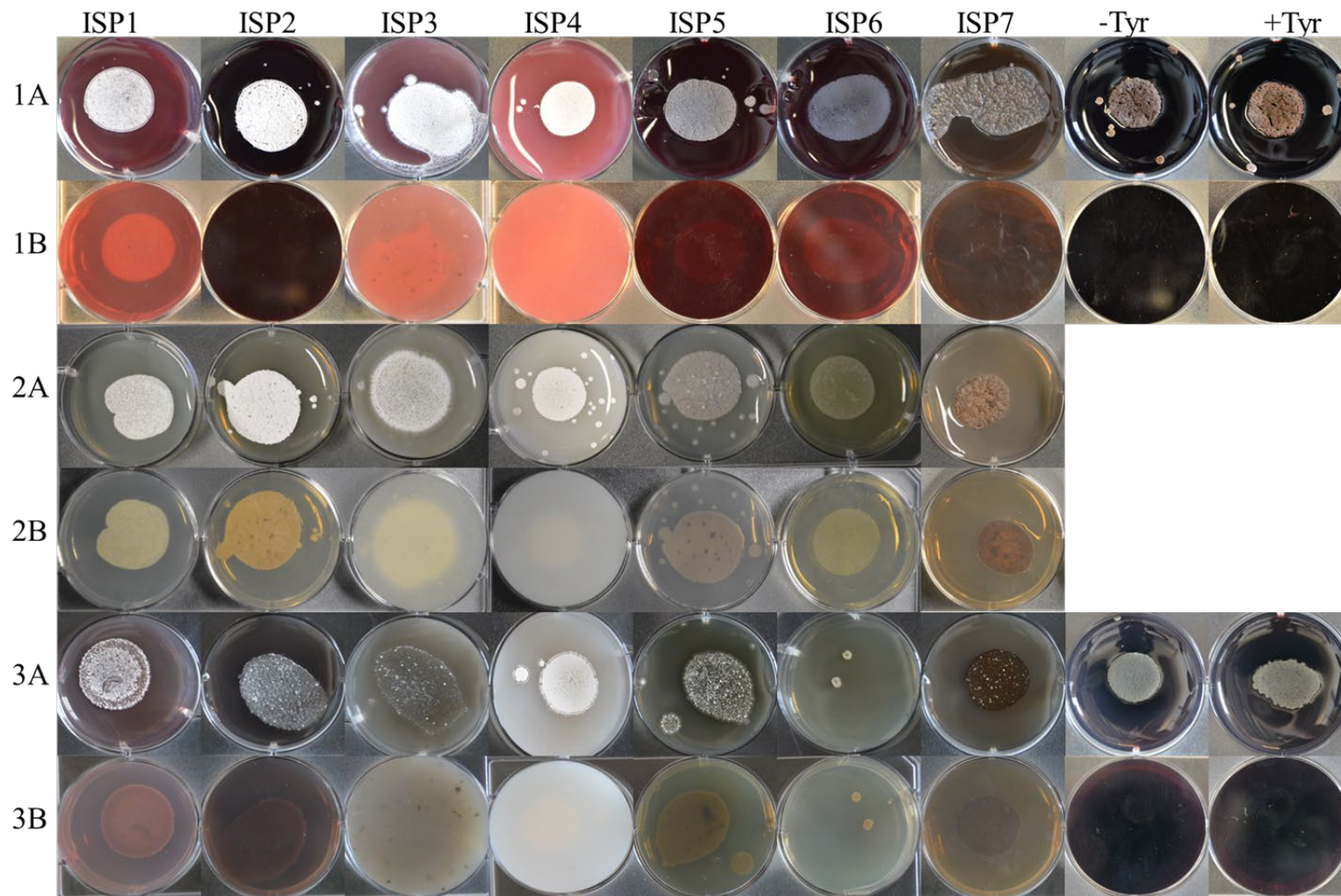


Figure S3. Morphology of strains grown for 18 days at 28 °C on different ISP-media and Suter-Medium (with: +Tyr [1 g/L] and without tyrosine: -Tyr).

Strains: RB29^T (1A: above, 1B: reverse); *Actinomadura rayongensis* DSM102126^T (2A: above, 2B: reverse); *Actinomadura aramentaria* DSM 43919^T (3A: above; 3B: reverse).

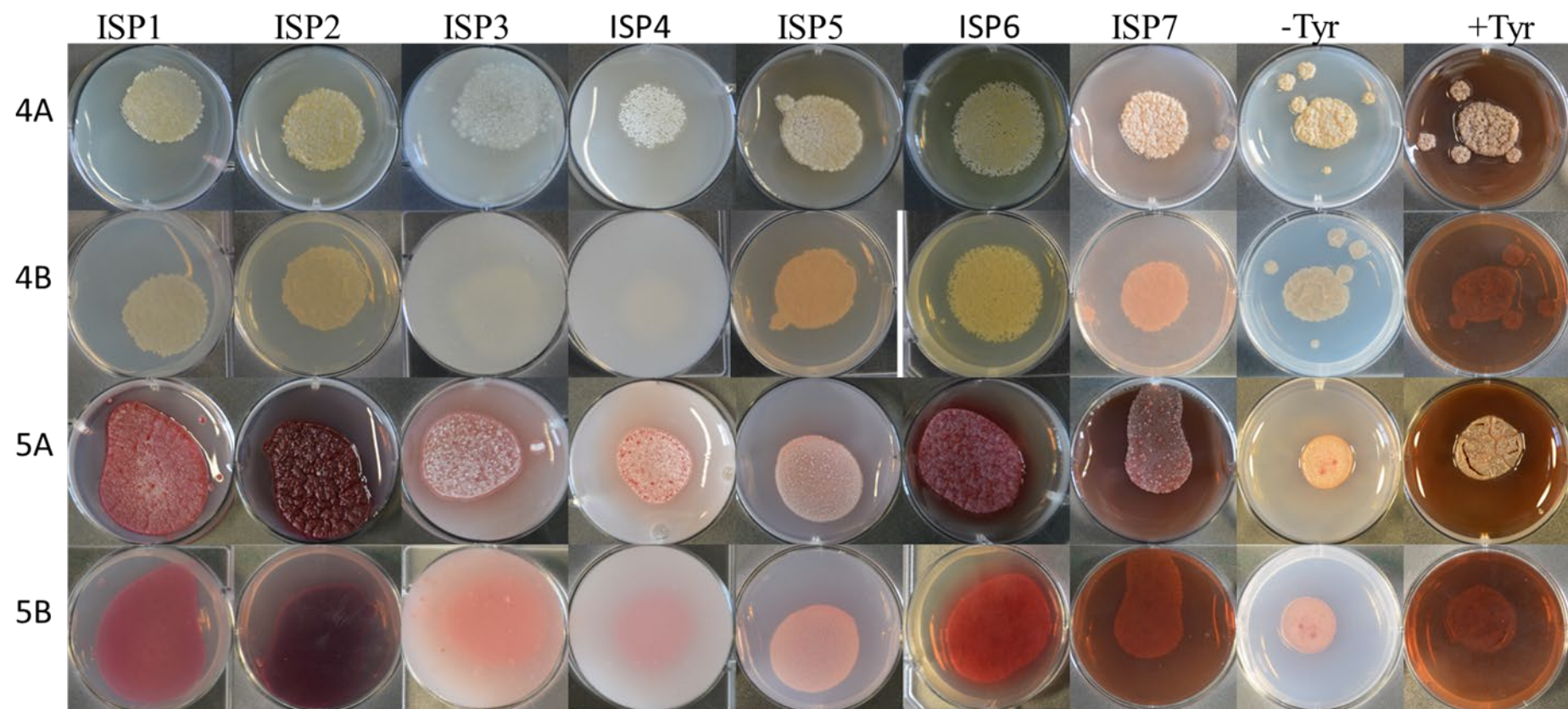


Figure S4. Morphology of strains grown for 9 days at 28 °C on different ISP-media and Suter-Medium (with: +Tyr [1 g/L] and without tyrosine: -Tyr).

Strains: RB68^T (4A: above, 4B: reverse); *Actinomadura hibisca* HK114 (=DSM 44148) (5A: above, 5B: reverse).

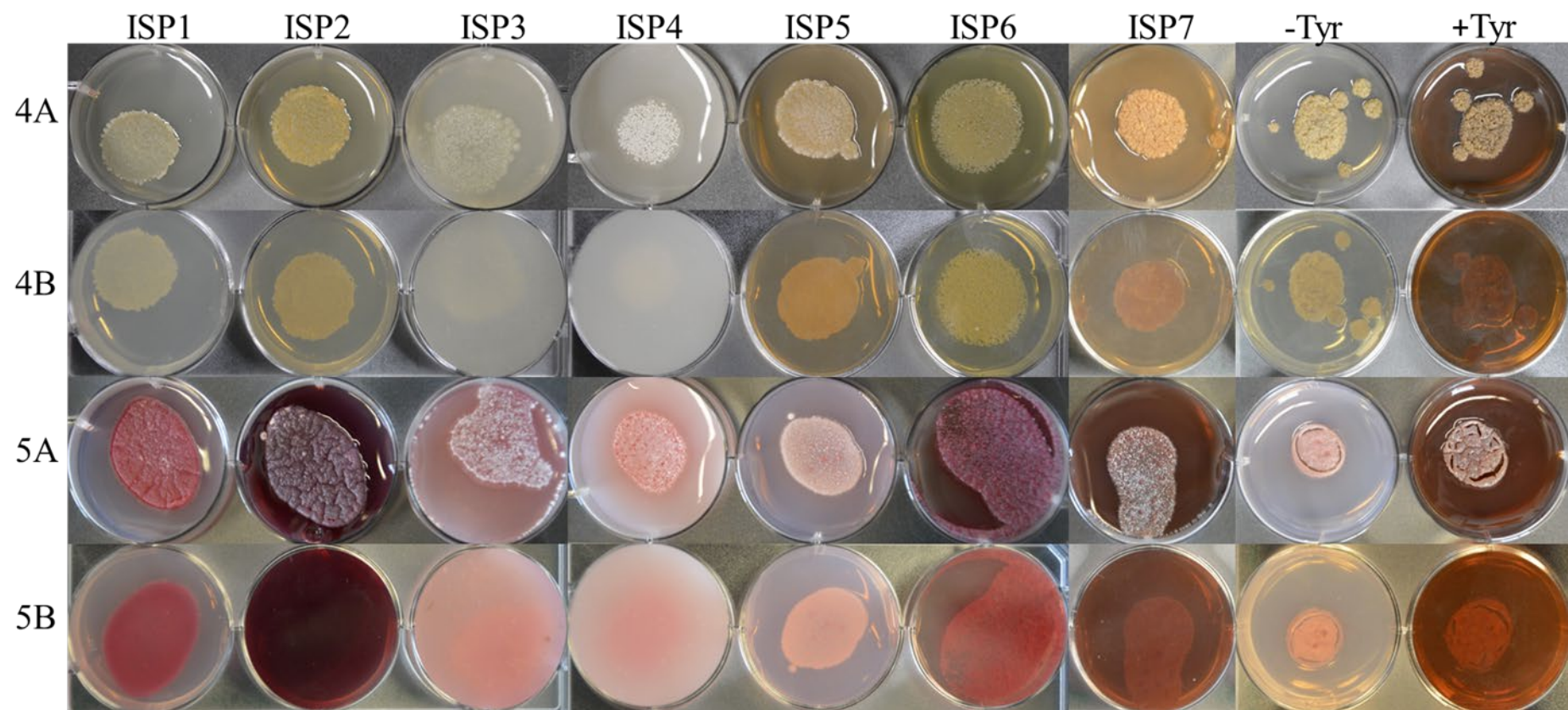


Figure S5. Morphology of strains grown for 14 days at 28 °C on different ISP-media and Suter-Medium (with: +Tyr [1 g/L] and without tyrosine: -Tyr).

Strains: RB68^T (4A: above, 4B: reverse); *Actinomadura hibisca* HK114 (=DSM 44148) (5A: above, 5B: reverse).

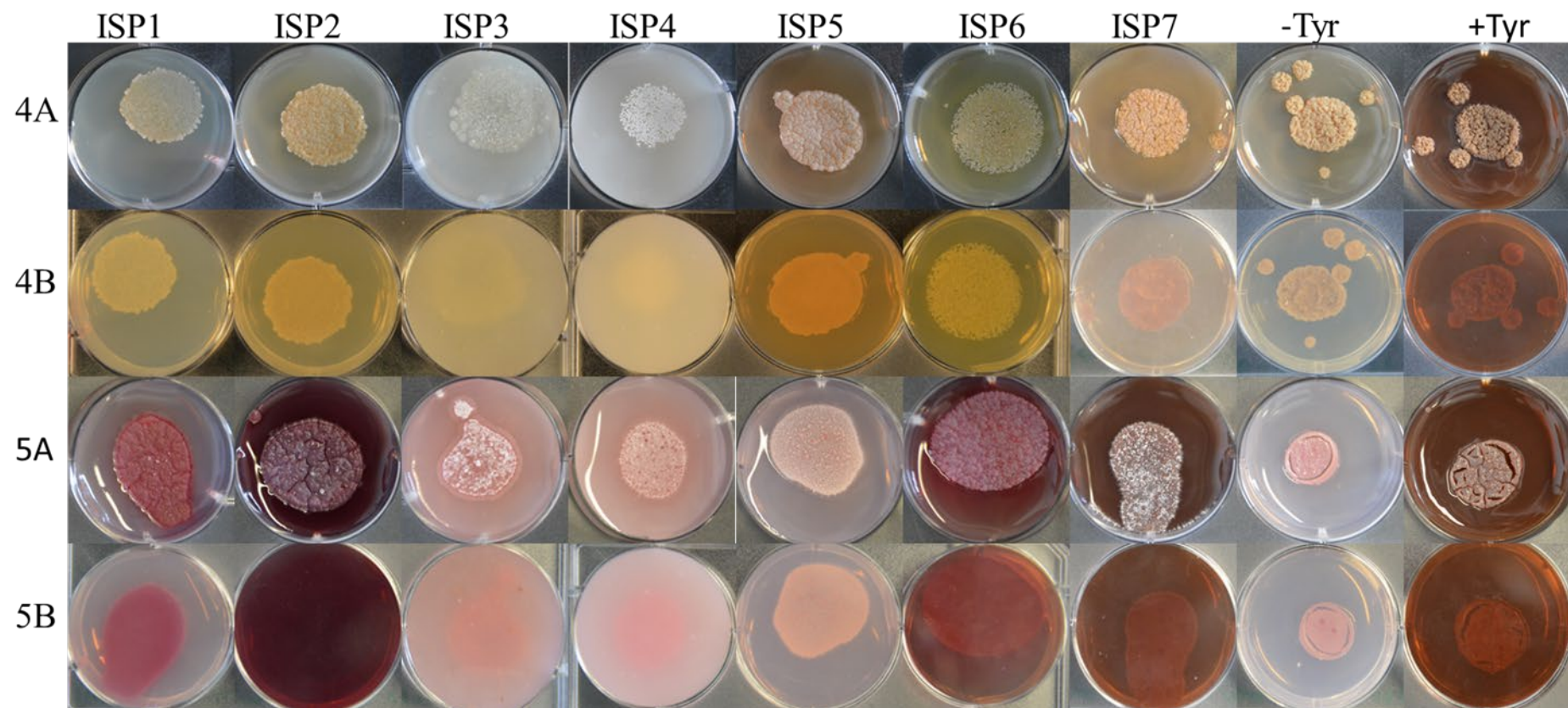


Figure S6. Morphology of strains grown for 18 days at 28 °C on different ISP-media and Suter-Medium (with: +Tyr [1 g/L] and without tyrosine: -Tyr).

Strains: RB68^T (4A: above, 4B: reverse); *Actinomadura hibisca* HK114 (=DSM 44148) (5A: above, 5B: reverse).

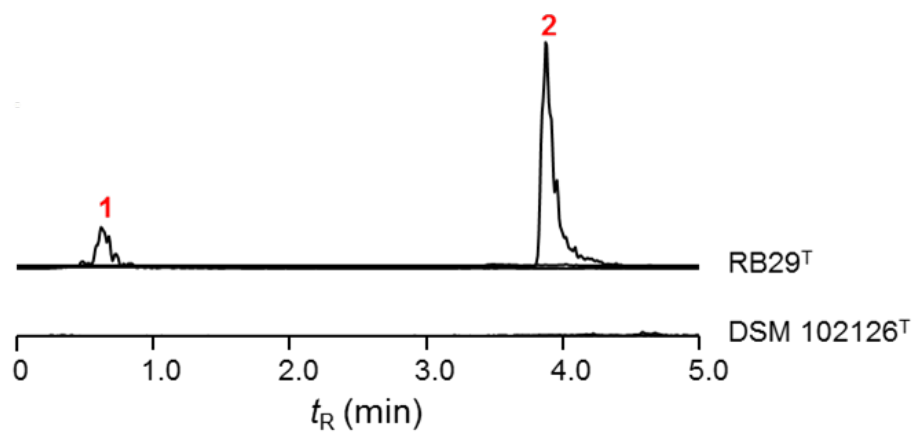


Figure S7. LC-MS chromatogram in selected ion mode (EIC mode) of solid phase (SP)-based metabolite extraction of culture extracts of RB29^T and DSM 102126^T. Both strains, RB29^T and DSM 102126^T, were grown in ISP2 for 10 days at 30 °C. Metabolite extraction was performed as stated in reference [1]. Formation of main rubterolones is shown as m/z peak 1 = 414.1 m/z and peak 2 = 496.1 m/z .

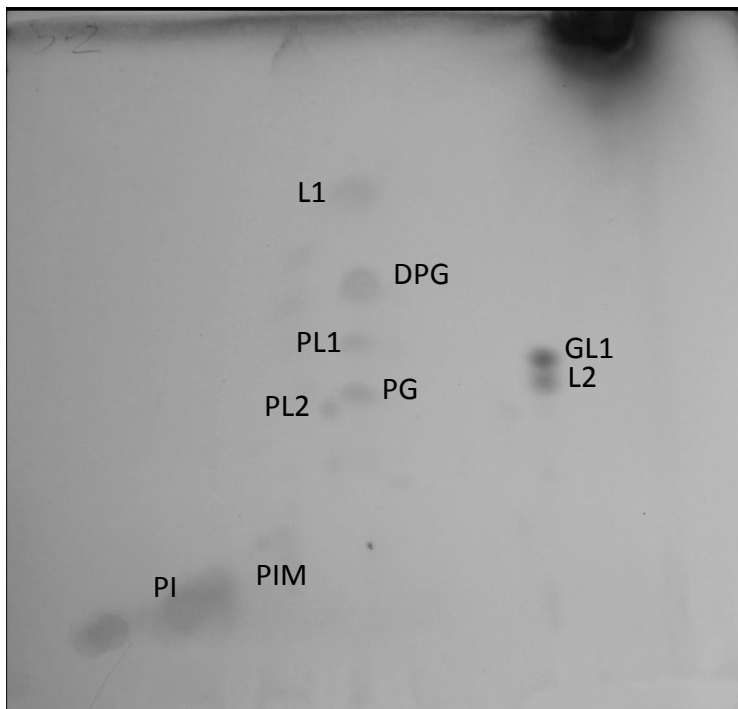


Figure S8. Polar lipid profile of strain RB29^T separated by two-dimensional thin layer chromatography and detection with molybdato-phosphoric acid. Abbreviations: DPG, diphosphatidylglycerol; PG, phosphatidylglycerol; PI, phosphatidylinositol; PIM, phosphatidylinositol-mannoside, PL1 and PL2, two unidentified phospholipids; L1 and L2, two unidentified lipids; GL1, glycolipid.

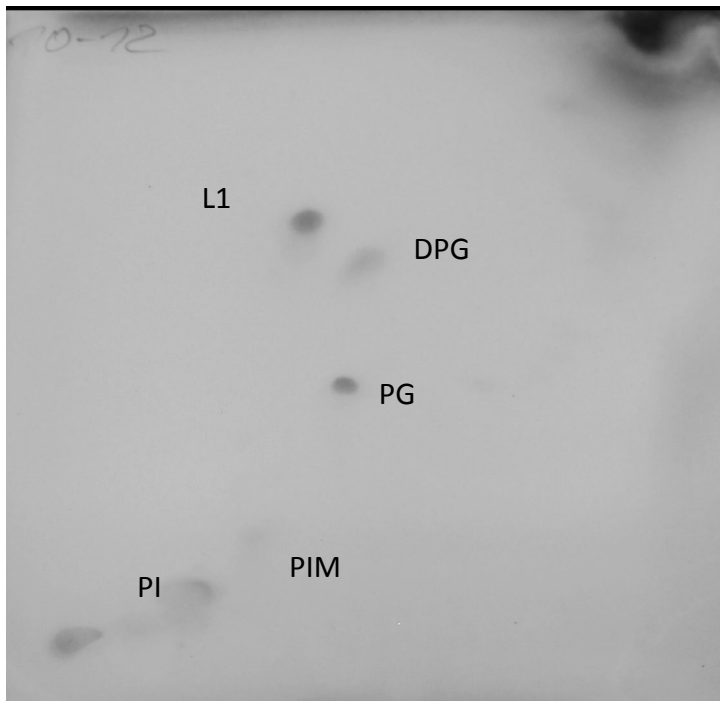


Figure S9. Polar lipid profile of strain RB68^T separated by two-dimensional TLC and detection with molybdato-phosphoric acid. Abbreviations: DPG, diphosphatidylglycerol; PG, phosphatidylglycerol, PI, phosphatidylinositol; PIM, phosphatidylinositol-mannoside, L1 unidentified lipid.

Table S7. Cellular fatty acids compositions (in %) of strains RB29^T and RB68^T and closely related type strains of the genus *Actinomadura*. Strains: 1. RB29^T; 2. *A. rayongensis* DSM 102126^T; 3. *A. atramentaria* DSM 43919^T; 4. RB68^T; 5. *A. hibisca* DSM 44148^T. Sum in feature 8 = C_{18:1} ω7cis and/or C_{18:1} ω6cis Amounts of fatty acids below 1.0% are marked with tr = traces (< 1%), - = fatty acid was not detected. All data was acquired in this study.

Fatty acids	1	2	3	4	5
Saturated fatty acids					
C _{14:0}	tr	2.0	2.1	5.0	2.2
C _{15:0}	2.0	2.2	4.6	tr	8.1
C _{16:0}	8.4	23.6	18.2	30.7	15.7
C _{16:0} 10-methyl	2.8	1.4	2.5	tr	tr
C _{16:0} 2-OH	2.8	6.0	6.8	-	-
C _{17:0}	1.7	2.0	6.4	tr	13.9
C _{17:0} 10-methyl	8.8	2.6	6.9	tr	4.5
C _{17:0} 2-OH	1.6	1.5	3.0	-	-
C _{18:0}	3.5	3.2	3.0	7.8	3.5
C _{18:0} 10-methyl	22.0	14.1	24.3	10.0	6.9
Unsaturated fatty acids					
C _{16:1} ω9cis	2.8	4.8	2.3	9.7	5.2
C _{17:1} ω9cis	2.3	2.5	2.0	tr	10.6
C _{18:1} ω9cis	7.0	22.2	5.4	19.7	14.5
Branched fatty acids					
<i>iso</i> -C _{16:0}	18.4	7.3	6.5	8.4	6.2
<i>iso</i> -C _{16:0} 2-OH	4.5	1.7	1.4	-	-
<i>iso</i> -C _{18:0}	4.8	1.0	1.1	1.6	1.2
C _{19:0} <i>cyclo</i> C ₁₁₋₁₂	2.6	-	-	-	-
Sum in Feature 8 ^C	-	-	-	-	3

References:

1. Guo H, Benndorf R, Lechnitz D, Klassen JL, Vollmers J, Gorls H, et al. Isolation, Biosynthesis and Chemical Modifications of Rubterolones A-F: Rare Tropolone Alkaloids from *Actinomadura* sp. 5-2. *Chemistry*. 2017;23(39):9338-45.
2. Meier-Kolthoff JP, Goker M, Sproer C, Klenk HP. When should a DDH experiment be mandatory in microbial taxonomy? *Arch Microbiol*. 2013b;195(6):413-8.
3. Meier-Kolthoff JP, Auch AF, Klenk HP, Goker M. Genome sequence-based species delimitation with confidence intervals and improved distance functions. *BMC Bioinformatics*. 2013a;14:60.
4. Richter DH, Rosselló-Móra, Oliver Glöckner F, Peplies J. JSpeciesWS: a web server for prokaryotic species circumscription based on pairwise genome comparison. *Genome Res* 2015;32:929-931.
5. Yoon SH, Ha SM, Lim J M, Kwon SJ, Chun J. A large-scale evaluation of algorithms to calculate average nucleotide identity. *Antonie van Leeuwenhoek* 2017;110:1281–1286.