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Supplementary Materials

Mechanism-Based Approach to New Antibiotic Producers Screening among Actinomycetes in the Course of the Citizen Science Project

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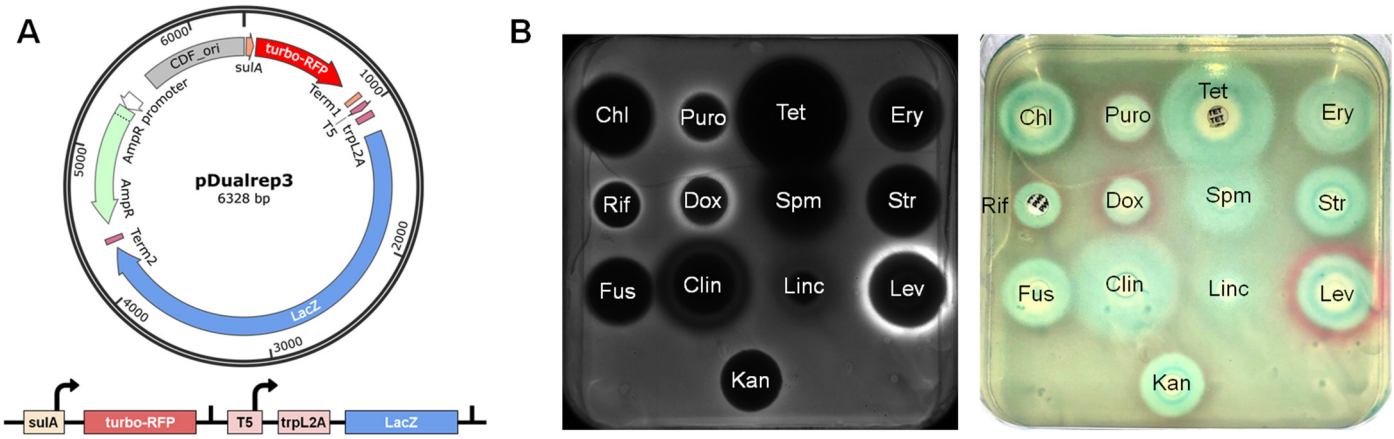
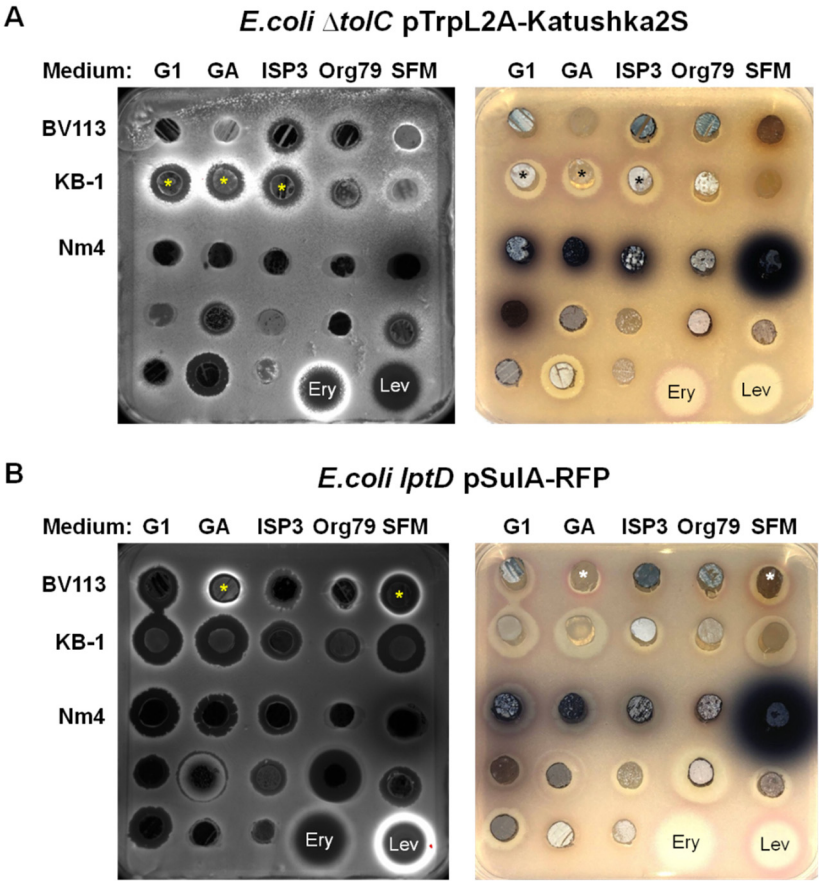
Contents

Figure S1. Double reporter system pDualrep3



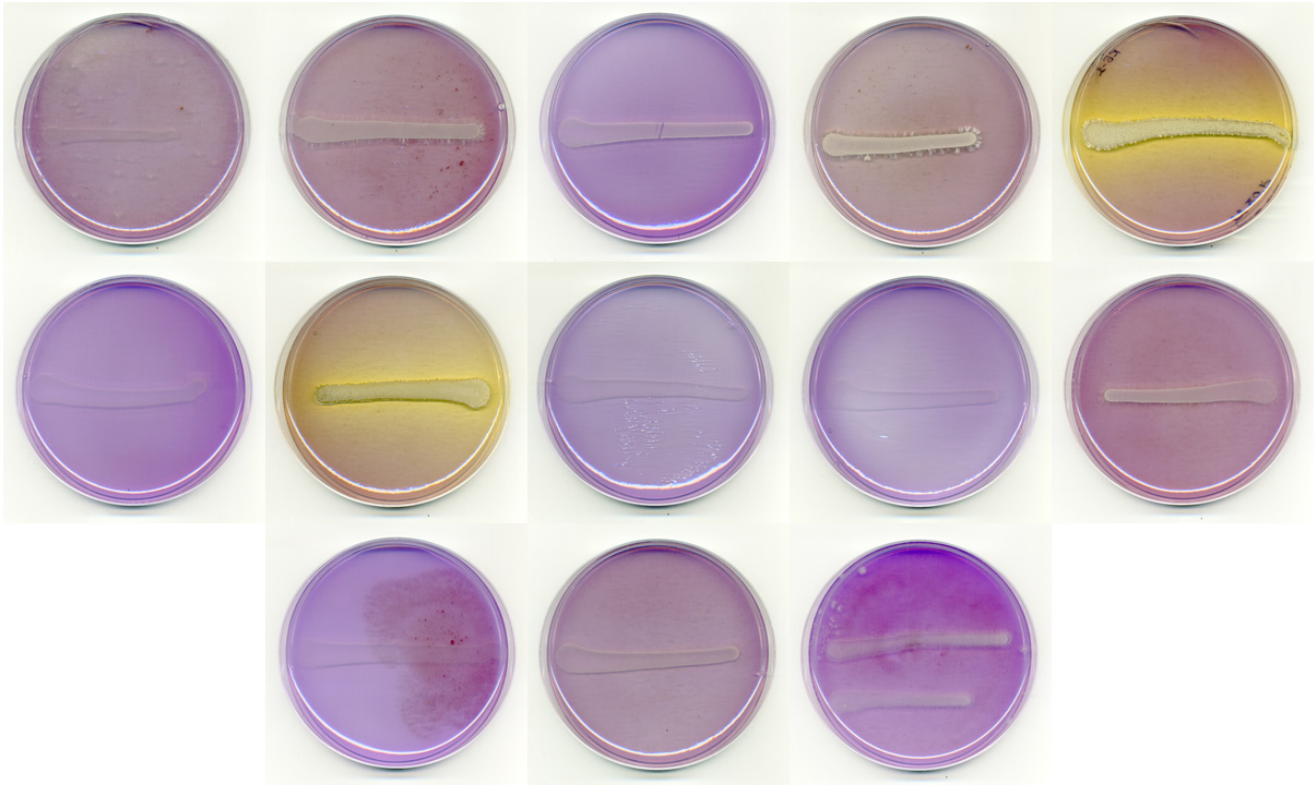
(**A**) pDualrep3 plasmid map and the reporter scheme. CDF_ori, CloDF13-derived CDF replicon; sulA, promoter of the *sulA* gene; turbo-RFP, red fluorescent protein gene; T5, bacteriophage T5 promoter; trpL2A, modified *trpL* leader open reading frame carrying W10A and W11A substitutions; LacZ, β-galactosidase gene. Transcription start sites are shown by arrows. Transcription terminators are shown by vertical dashes. (**B**) Comparison of fluorescence signals using an imaging system (left) and with the naked eye (right). The plate was scanned in the Cy3 (for TurboRFP) channel. *Turbo-rfp* expression is visible in red to the naked eye in the zone of antibiotic sublethal concentrations. *LacZ* expression is visible in blue to the naked eye in the zone of antibiotic sublethal concentrations. An agar plate was coated with the *E.coli ΔtolC* strain transformed with the pDualrep3 plasmid. A panel of antibiotics was used: chloramphenicol (Chl), puromycin (Puro), tetracycline (Tet), erythromycin (Ery), rifampicin (Rif), doxorubicin (Dox), spectinomycin (Spm), streptomycin (Str), fusidic acid (Fus), clindamycin (Clin), lincomycin (Linc), levofloxacin (Lev) and kanamycin (Kan).

Figure S2. Agar block diffusion assay of isolated actinomycetes (BV113, KB-1, Nm4)



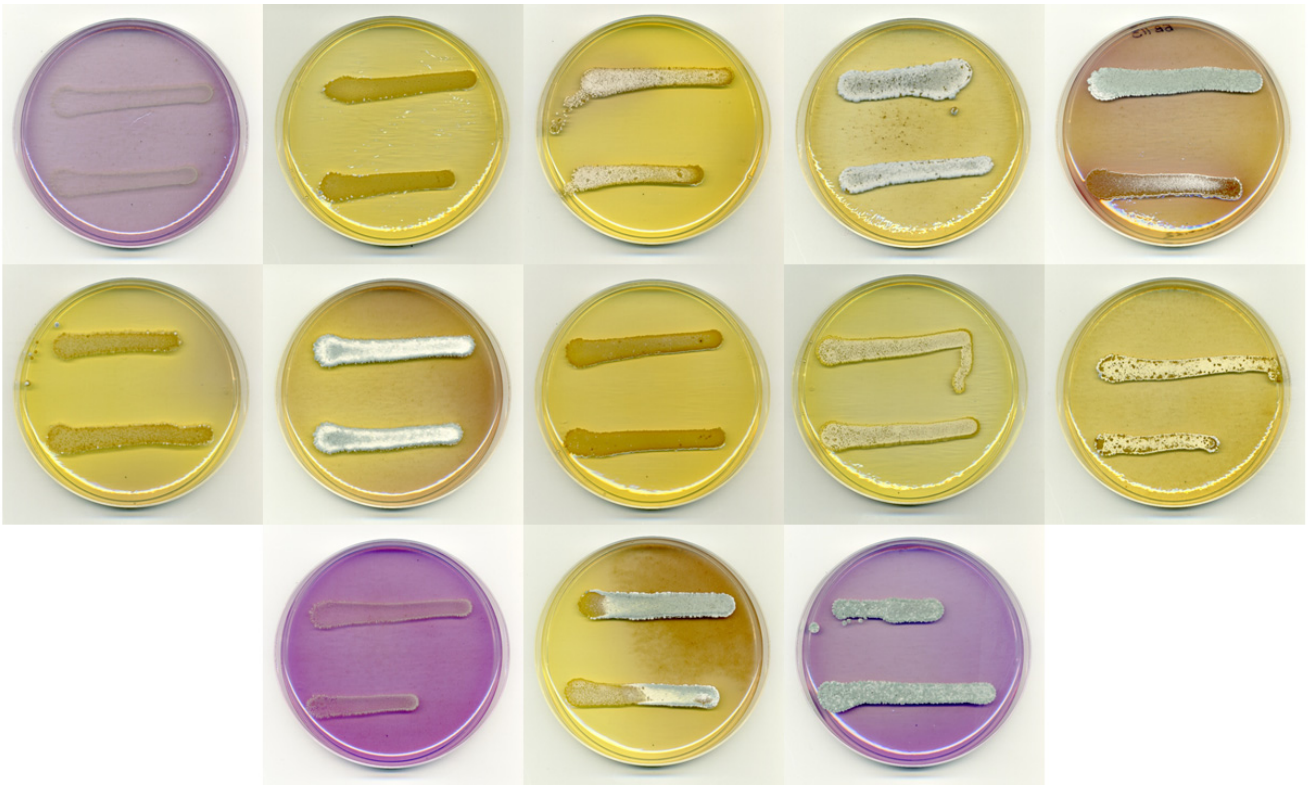
Different nutrient media (G1, GA, ISP3, Org79, SFM) were used to obtain blocks with mycelium and assess their antibacterial activity (for details, see Table S3). *Streptomyces* sp. KB-1 produces a compound that inhibits the growth of *E.coli ΔtolC* and *lptD* strains, revealing a strong pTrpL2A-Katushka2S reporter induction (marked with asterisks (*) in the picture **A**). *Streptomyces* sp. BV113 produces a compound that primarily inhibits the growth of *E.coli lptD* strain, revealing a strong pSulA-RFP reporter induction (marked with asterisks (*) in the picture **B**). Comparison of fluorescence signals using an imaging system (left) and with the naked eye (right). The upper plate (**A**) was scanned in the Cy5 (for Katushka2S) channel, the lower plate (**B**) was scanned in the Cy3 (TurboRFP) channel (left). Katushka2S and TurboRFP are visible to the naked eye in lilac and orange-red colors, respectively, in the zone of antibiotic sublethal concentrations (right). Agar plates were coated with either the *E.coli ΔtolC* strain transformed with the pTrpL2A-Katushka2S plasmid, or the *E.coli lptD* strain transformed with the pSulA-RFP plasmid. Antibiotics erythromycin (Ery) and levofloxacin (Lev) were used as controls.

Figure S3. Utilization of different sugars (1.0%, w/v) as sole carbon source by strain KB-1



Top row: basal agar medium (ISP9) without sugar, arabinose, fructose, galactose, glucose. Middle row: inositol, maltose, mannite, raffinose, rhamnose. Bottom row: sorbitol, sucrose, xylose.

Figure S4. Utilization of different sugars (1.0%, w/v) as sole carbon source by strain BV113



Top row: basal agar medium (ISP9) without sugar, arabinose, fructose, galactose, glucose. Middle row: inositol, maltose, mannite, raffinose, rhamnose. Bottom row: sorbitol, sucrose, xylose.

Figure S5. MS/MS spectrum of the pikromycin $[M+H]^+$ ion at *m/z* value 526.3370

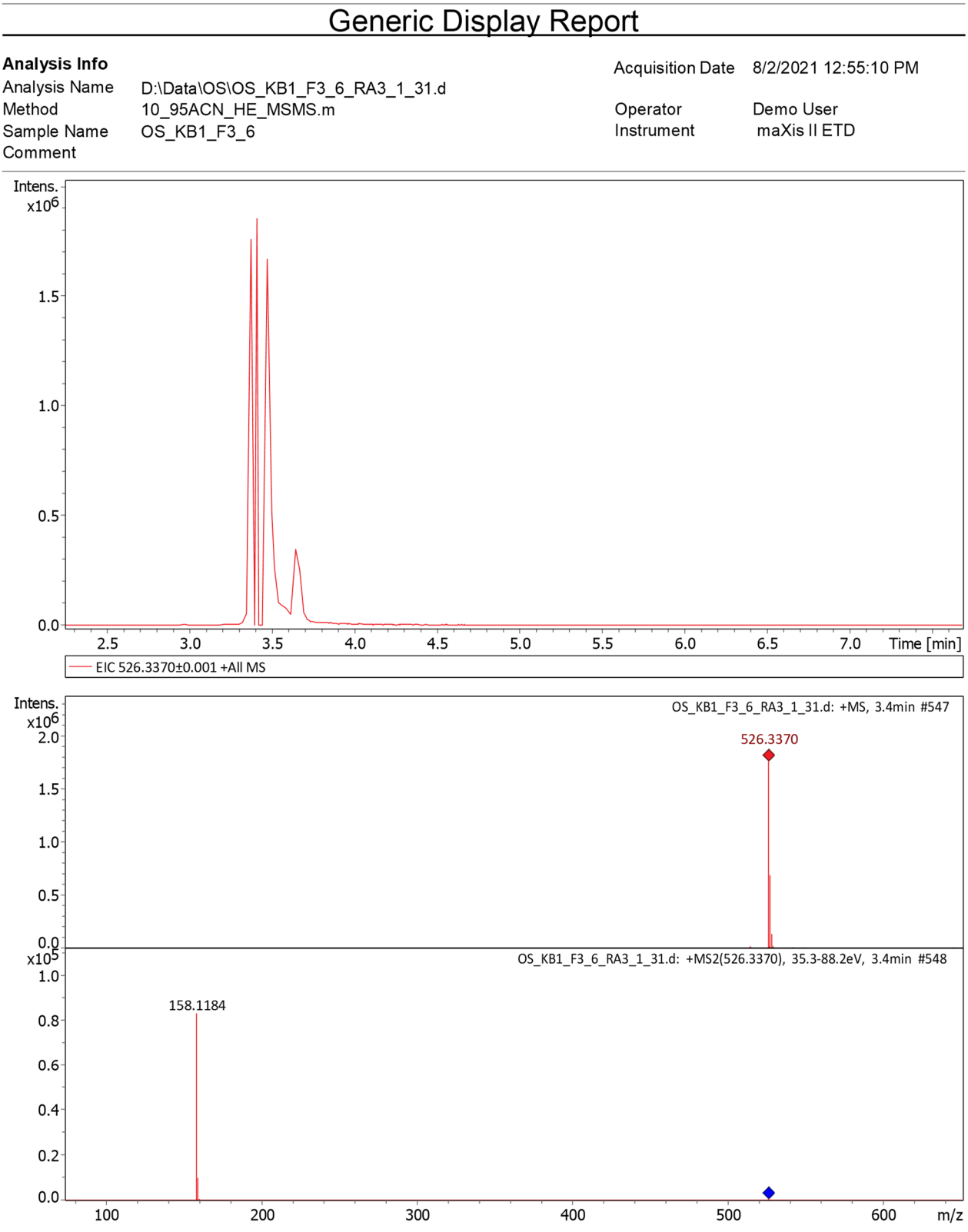
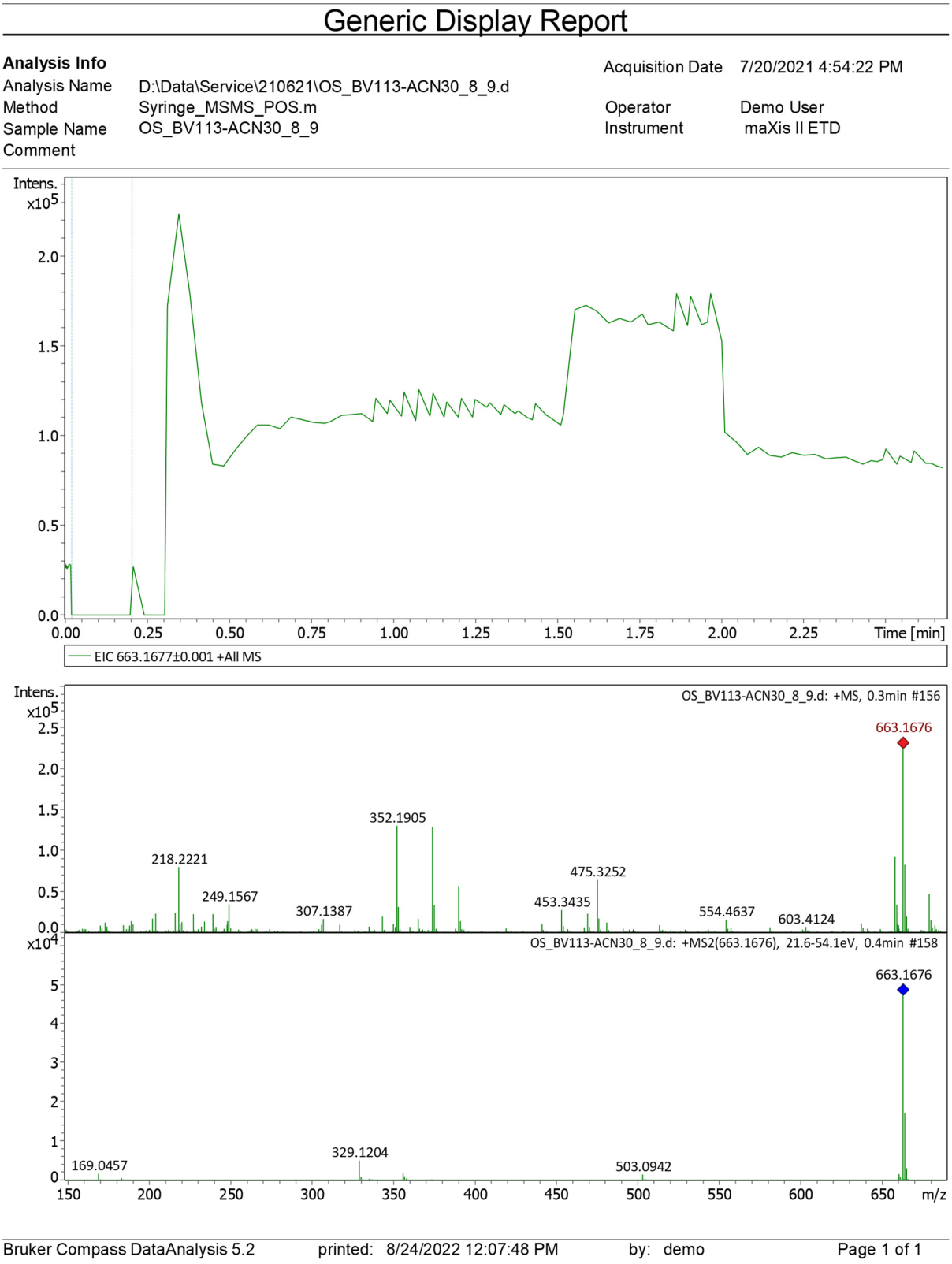


Figure S6. MS/MS spectrum of the chartreusin $[M+Na]^+$ ion at *m/z* value 663.1677



The data aquired with direct sample injection, no retention time included in this report.

Table S1. Physiological and biochemical characteristics of KB-1 isolate and closely related *Streptomyces* sp. strains

Properties	**KB-1**	***Streptomyces zaomyceticus* NRRL B-2038^T**	***Streptomyces exfoliatus* NBRC 13191^T**	***Streptomyces venezuelae* ATCC 10712^T**
Cultural characteristics				
Growth on ISP2/ISP3/ISP4	Good	Good	Good	Good
Color of aerial mycelium on ISP2/ISP3/ISP4	White/light gray/ light gray	White/gray/gray	White/rose/rose	Gray/white/gray
Colony Reverse on ISP2/ISP3/ISP4	Colorless	Brown/ivory/ivory	Ivory/yellow/gray brown	Brown/yellow/ivory
Diffusible pigment on ISP2/ISP3/ISP4	None	None	None	Brown/none/none
Melanoid pigment on ISP6	—	+	—	+
Morphological characteristics				
Spore surface	Smooth	Smooth	Smooth	Smooth
Spore chains morphology	Straight	Straight	Straight	Straight
Physiological characteristics				
Temperature-range and optimum (°C)	15-30 (25)	(30)	(30)	(30)
Acid from carbohydrates				
Arabinose	—	+	+	+
Fructose	—	+/-	+	+/-
Galactose	—	n/d	n/d	n/d
Glucose	+	+	+	+
Inositol	—	+/-	—	—
Maltose	+	n/d	n/d	n/d
Mannitol	—	+/-	—	—
Raffinose	—	—	+	—
Rhamnose	—	—	+	+/-
Sorbitol	—	n/d	n/d	n/d
Sucrose	—	+/-	+	—
Xylose	—	+/-	+	—
Degradation of				
Starch	+	n/d	n/d	n/d
Gelatin	+	+	n/d	+

Casein	+	n/d	n/d	n/d
Cellulose	++	—	n/d	—

+, positive; —, negative; n/d, not determined

The sources used for: *Streptomyces zaomyceticus* NRRL B-2038^T and *Streptomyces exfoliatus* NBRC 13191^T [1], *Streptomyces venezuelae* ATCC 10712^T (https://bacdive.dsmz.de (accessed on 11 April 2022)).

Table S2. Physiological and biochemical characteristics of BV113 isolate and closely related *Streptomyces* sp. strains

Properties	**BV113**	***Streptomyces osmaniensis* OU-63^T**	***Streptomyces galbus* JCM 4570^T**	***Streptomyces chartreusis* NBRC 12753^T**
Cultural characteristics				
Growth on ISP2/ISP3/ISP4	Good	Good	Good	Good
Color of aerial mycelium on ISP2/ISP3/ISP4	White/Pale green/ pale blue	Bluish gray	Silk gray/traffic gray/ light ivory	Pastel turquoise
Colony reverse on ISP2/ISP3/ISP4	None/yellow/none	Pale yellow	Orange brown/honey yellow/ochre brown	Colorless
Diffusible pigment on ISP2/ISP3/ISP4	None/yellow/none	None	Honey yellow/lemon yellow/honey yellow	None
Melanoid pigment on ISP6	+	+	—	+
Morphological characteristics				
Spore surface	Spiny	Spiny	Smooth	Spiny
Spore chains morphology	Spirales	Spirales	Spirales	Spirales
Physiological characteristics				
Temperature-range and optimum (°C)	20-40 (37)	15-40	(28)	(30)
pH-range and optimum	5-9 (7)	6-10	n/d	n/d
Acid from carbohydrates				
Arabinose	+	+	+	+
Fructose	+	+	+	+
Galactose	+	+	n/d	+
Glucose	+	+	+	+
Inositol	—	+	+	+
Maltose	—	+	n/d	n/d
Mannitol	+	+	n/d	+
Raffinose	+	+	+	+
Rhamnose	+	+	—	+
Sorbitol	—	+	n/d	n/d
Sucrose	+	+	+	+
Xylose	—	+	+	+
Degradation of				
Starch	+	+	n/d	+
Gelatin	+	+	+	+

Casein	+	—	n/d	+
Cellulose	+	+	+	+
Antibiotic resistance				
Erythromycin, 5 μg/disk	resistant	n/d	n/d	n/d
Vancomycin, 5 μg/disk	sensitive	n/d	n/d	n/d
Levofloxacin, 5 μg/disk	sensitive	n/d	n/d	n/d
Tetracycline, 12.5 μg/disk	sensitive	n/d	n/d	n/d
Streptomycin, 10 μg/disk	sensitive	sensitive	n/d	n/d

+, positive; —, negative; n/d, not determined

The sources used for: *Streptomyces osmaniensis* OU-63^T [2], *Streptomyces galbus* JCM 4570^T and *Streptomyces chartreusis* NBRC 12753^T (https://bacdive.dsmz.de (accessed on 11 April 2022)).

Table S3. Composition of some nutrient media used

Medium	Acronym	Ingredients (g/L)	References
Mineral agar Gauze 1	G1	Soluble starch - 20 KNO_3 - 1 $K_2HPO_4 \cdot 7H_2O$ - 0.5 NaCl - 0.5 $MgSO_4 \cdot 7H_2O$ - 0.5 $FeSO_4 \cdot 7H_2O$ - 0.01 pH adjusted to 7.2 Agar - 20	[3]
Organic medium 79	Org79	Glucose - 10 Peptone - 10 Casein hydrolysate - 2 Yeast extract - 2 NaCl - 6 pH adjusted to 7.0 Agar - 20	[4]
Glucose-asparagine agar	GA	Glucose - 10 Asparagine - 0.5 K_2HPO_4 - 0.5 Agar - 20	[5]
Soy flour mannitol agar	SFM	Mannitol - 20 Soy flour - 20 pH adjusted to 8.0 Agar - 20	[6]
Yeast extract-malt extract agar	ISP2	Bacto-yeast extract - 4 Bacto-malt extract - 10 Bacto-dextrose - 4 pH adjusted to 7.3 Bacto-agar - 20	[7]
Oatmeal agar	ISP3	Oatmeal - 20 $FeSO_4 \cdot 7H_2O$ - 0.001 $MnCl_2 \cdot 4H_2O$ - 0.001 $ZnSO_4 \cdot 7H_2O$ - 0.001 pH adjusted to 7.2 Agar - 18	[7]
Inorganic salts-starch agar	ISP4	Soluble starch - 10 K_2HPO_4 - 1 $MgSO_4 \cdot 7H_2O$ - 1 NaCl - 1 $(NH_4)_2SO_4$ - 2 $CaCO_3$ - 2 $FeSO_4 \cdot 7H_2O$ - 0.001 $MnCl_2 \cdot 4H_2O$ - 0.001	[7]

		$ZnSO_4 \cdot 7H_2O$ - 0.001 pH adjusted to 7.0 - 7.4 Agar - 20	
Peptone-yeast extract iron agar	ISP6	Bacto-peptone iron agar - 36 Bacto-yeast extract - 1 pH adjusted to 7.0 - 7.2	[7]
Basal agar medium	ISP9	Carbon source - 1% (w/v) $(NH_4)_2SO_4$ - 2.64 KH_2PO_4 - 2.38 $K_2HPO_4 \cdot 3H_2O$ - 5.65 $MgSO_4 \cdot 7H_2O$ - 1 $CuSO_4 \cdot 5H_2O$ - 0.0064 $FeSO_4 \cdot 7H_2O$ - 0.0011 $MnCl_2 \cdot 4H_2O$ - 0.0079 $ZnSO_4 \cdot 7H_2O$ - 0.0015 pH adjusted to 6.8 - 7.0 Agar - 15	[7]
Actinomyces Isolation Agar	M490	Sodium caseinate - 2 L-Asparagine - 0.1 Sodium propionate - 4 K_2HPO_4 - 0.5 $MgSO_4$ - 0.1 $FeSO_4$ - 0.001 pH adjusted to 8.1 ± 0.2 Agar - 15 Glycerol - 5 ml	[8] (HiMedia Laboratories)
Humic acid-vitamin agar	HV agar	Humic acid - 1 Na_2HPO_4 - 0.5 KCl - 1.71 $MgSO_4 \cdot 7H_2O$ - 0.05 $FeSO_4 \cdot 7H_2O$ - 0.01 $CaCO_3$ - 0.02 Thiamine-HCl - 0.0005 Riboflavin - 0.0005 Niacin - 0.0005 Pyridoxine-HCl - 0.0005 Inositol - 0.0005 Ca-pantothenate - 0.0005 *p*-aminobenzoic acid - 0.0005 Biotin - 0.00025 pH adjusted to 7.2 Agar - 18	[9]

Supplementary References

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