

Supplementary Materials

Mechanism-based approach to new antibiotic producers screening among actinomycetes in the course of civil science

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I. Supplementary Figures

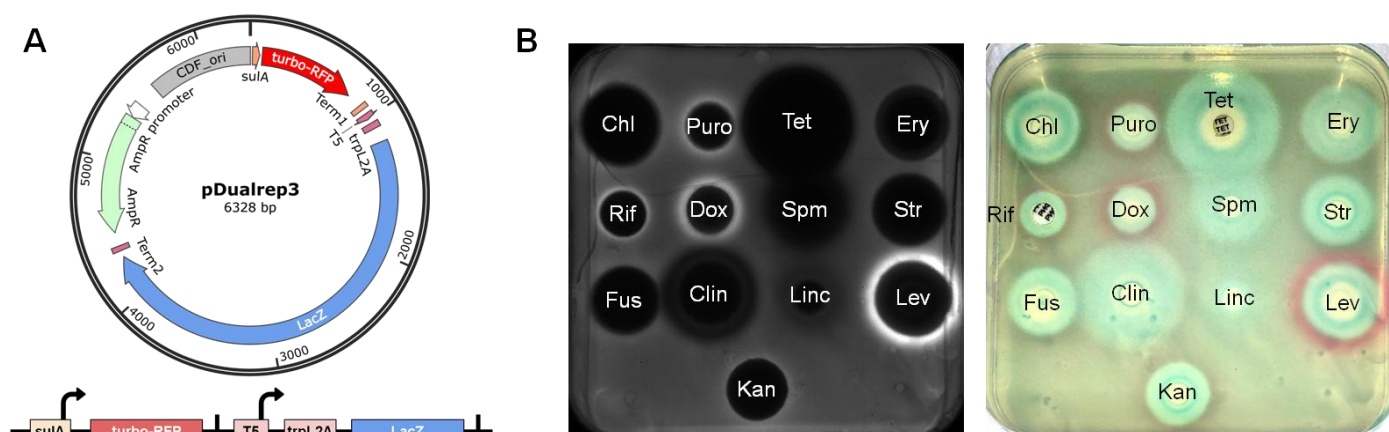


Figure S1. Double reporter system pDualrep3. **(A)** pDualrep3 plasmid map and the reporter scheme. CDF_ori, CloDF13-derived CDF replicon; *sulA*, promoter of the *sulA* gene; *turbo-RFP*, red fluorescent protein gene; T5, bacteriophage T5 promoter; *trpL2A*, modified *trpL* leader open reading frame 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 induction detection using an imaging system (left) and with the naked eye (right). The plate was scanned in the TurboRFP (Cy3) 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. Agar plate coated with the *E.coli* $\Delta tolC$ strain transformed with the pDualrep3 plasmid and spotted with the panel of antibiotics: 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).

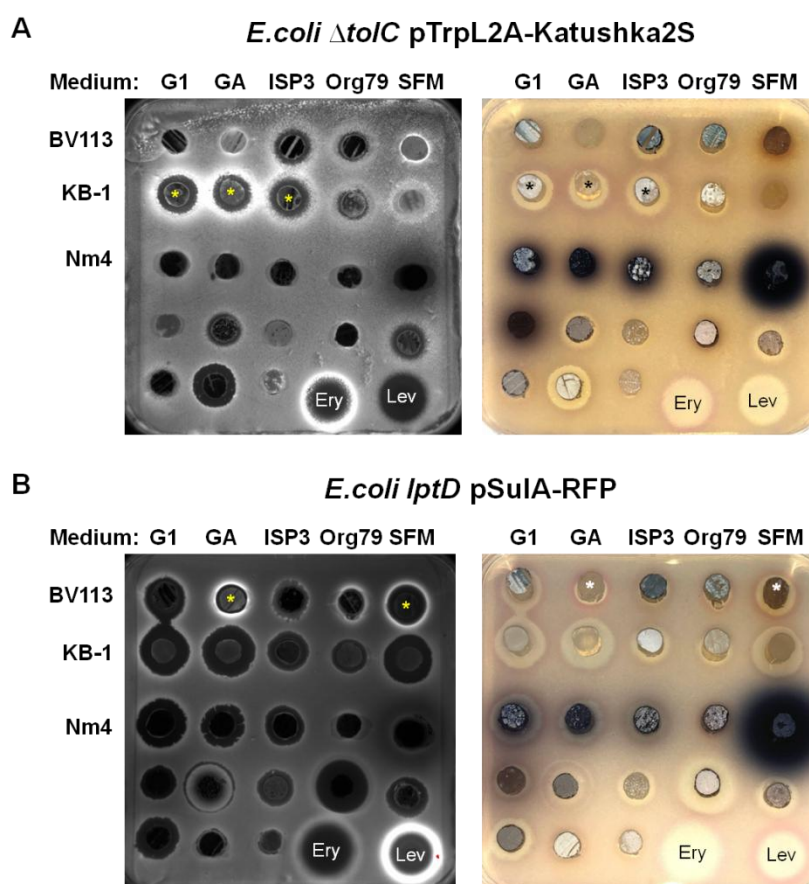


Figure S2. Agar blocks with isolated actinomycetes (BV113, KB-1, Nm4) were tested on the reporter strains. Different nutrient media (G1, GA, ISP3, Org79, SFM) were used to obtain blocks and assess their antibacterial activity (for details, see Table S3). *Streptomyces* sp. KB-1 produces a compound that inhibits the growth of *E.coli* $\Delta tolC$ and *lptD* strains revealing 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 strong pSulA-RFP reporter induction (marked with asterisks in the picture B). Comparison of fluorescence induction detection using an imaging system (left) and with the naked eye (right). The upper plate was scanned in the Katushka2S (Cy5) channel, the lower plate was scanned in the TurboRFP (Cy3) 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* $\Delta tolC$ 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.

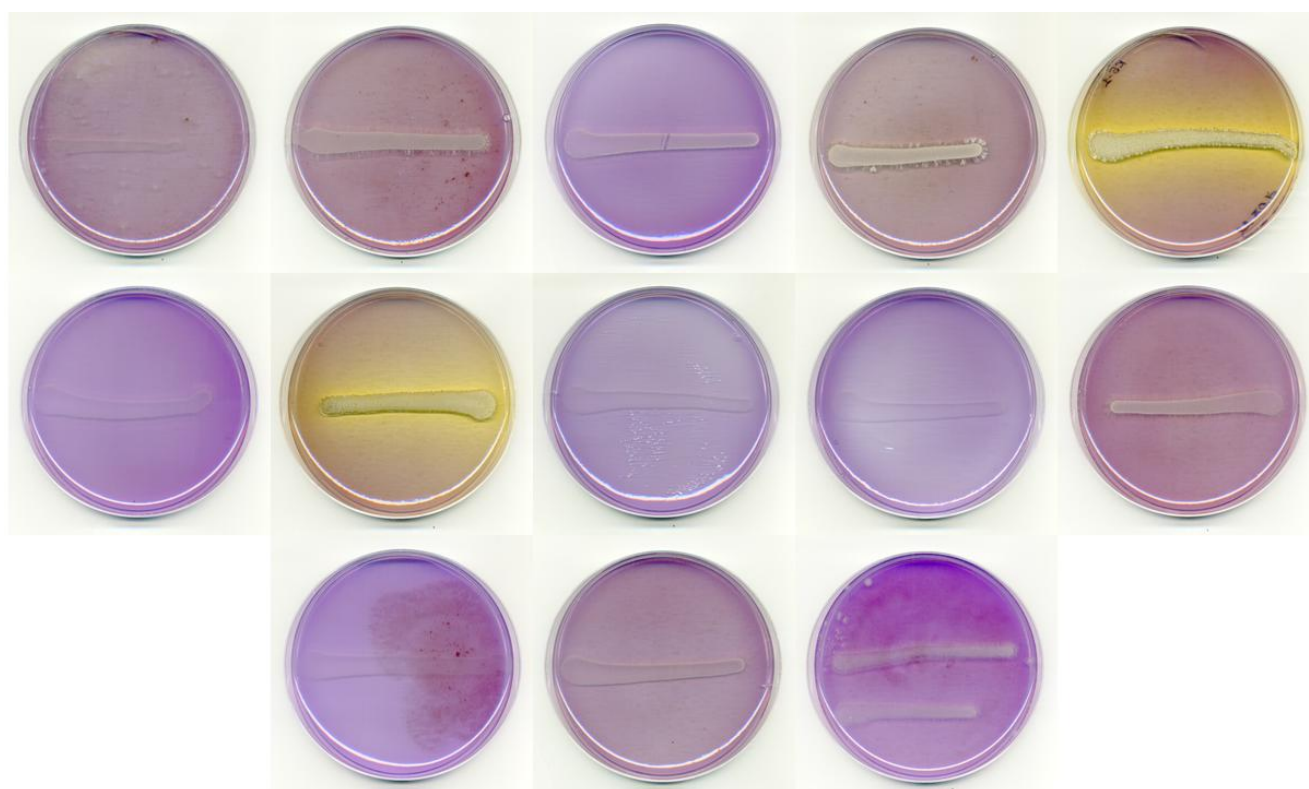


Figure S3. Utilization of different sugars (1.0%, w/v) as sole carbon source by strain KB-1. Top row: basal agar medium (ISP9) without sugar, arabinose, fructose, galactose, glucose. Middle row: inositol, maltose, mannite, raffinose, rhamnose. Bottom row: sorbitol, sucrose, xylose.



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.

II. Supplementary Tables

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

Properties	KB-1*	<i>Streptomyces zaomyceticus</i> NRRL B-2038 ^T	<i>Streptomyces exfoliatus</i> NBRC 13191 ^T	<i>Streptomyces venezuelae</i> 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	—
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+, positive; —, negative; n/d, not determined

* The data were obtained experimentally

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>).

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

Properties	BV113*	<i>Streptomyces osmaniensis</i> OU-63 ^T	<i>Streptomyces galbus</i> JCM 4570 ^T	<i>Streptomyces chartreusis</i> 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 data were obtained experimentally

The sources used for: *Streptomyces osmaniensis* OU-63^T [2], *Streptomyces galbus* JCM 4570^T and *Streptomyces chartreusis* NBRC 12753^T (<https://bacdiv.dsmz.de>).

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 ₂ HPO ₄ · 7H ₂ O - 0.5 NaCl - 0.5 MgSO ₄ · 7H ₂ O - 0.5 FeSO ₄ · 7H ₂ O - 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 ₂ HPO 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 · 7H ₂ O - 0.001 MnCl ₂ · 4H ₂ O - 0.001 ZnSO ₄ · 7H ₂ O - 0.001 pH adjusted to 7.2 Agar - 18	[7]
Inorganic salts-starch agar	ISP4	Soluble starch - 10 K ₂ HPO ₄ - 1 MgSO ₄ · 7H ₂ O - 1 NaCl - 1 (NH ₄) ₂ SO ₄ - 2 CaCO ₃ - 2 FeSO ₄ · 7H ₂ O - 0.001 MnCl ₂ · 4H ₂ O - 0.001 ZnSO ₄ · 7H ₂ O - 0.001 pH adjusted to 7.0 - 7.4 Agar - 20	[7]

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) ₂ SO ₄ - 2.64 KH ₂ PO ₄ - 2.38 K ₂ HPO ₄ · 3H ₂ O - 5.65 MgSO ₄ · 7H ₂ O - 1 CuSO ₄ · 5H ₂ O - 0.0064 FeSO ₄ · 7H ₂ O - 0.0011 MnCl ₂ · 4H ₂ O - 0.0079 ZnSO ₄ · 7H ₂ O - 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 ₂ HPO 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 ₂ HPO ₄ - 0.5 KCl - 1.71 MgSO ₄ · 7H ₂ O - 0.05 FeSO ₄ · 7H ₂ O - 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 <i>p</i> -aminobenzoic acid - 0.0005 Biotin - 0.00025 pH adjusted to 7.2 Agar - 18	[9]

III. Supplementary References

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