

Supplementary Material

Statistical Medium Optimization for the Production of Anti-Methicillin-Resistant *Staphylococcus aureus* Metabolites from a Coal-Mining-Soil-Derived *Streptomyces rochei* CMB47

Contents

Table S1. Composite design matrix and results of anti-MRSA compounds production.

Table S2. Cultural characteristics of the isolate CMB47 on international *Streptomyces* project (ISP) and some media.

Table S3. Physiological properties of strain CMB47.

Figure S1. HPLC-DAD/ESI-MS analysis of the bioactive fraction 11 B from ethyl acetate crude extract of *S. rochei* CMB47 strain: (a) extracted ion chromatograms showing the three signals at $t_R=6.9$ min, $t_R=8.2$ min, $t_R=10.4$ min, corresponding to (b) the MS spectra in positive ion mode with $[M+H]^+$ ions at m/z 207, 221 and 237 respectively.

Figure S2. HPLC-DAD/ESI-MS analysis of the bioactive fraction 11B from ethyl acetate crude extract of *S. rochei* CMB47 strain: (a) the same UV spectrum for each of the three compounds corresponding to the three peaks at $t_R=6.9$ min, $t_R=8.2$ min, $t_R=10.4$ min (b) UV chromatogram acquired at 296 nm.

Table S1. Composite design matrix and results of anti-MRSA compounds production.

Run N°	Natural values				Coded values						
	Starch (g/L)	NaNO ₃ (g/L)	Incubation time (days)	pH	X ₀	X ₁	X ₂	X ₃	X ₄	Diameter of inhibition zones (mm) Y	\hat{y}
1	6	2	5	5	1	-1	-1	-1	-1	15.83±0.76	17.62
2	14	2	5	5	1	+1	-1	-1	-1	16.33±0.76	18.60
3	6	4	5	5	1	-1	+1	-1	-1	14.16±1.89	16.80
4	14	4	5	5	1	+1	+1	-1	-1	18.33±0.28	17.78
5	6	2	9	5	1	-1	-1	+1	-1	17.33±0.28	17.48
6	14	2	9	5	1	+1	-1	+1	-1	17.67±1.04	18.45
7	6	4	9	5	1	-1	+1	+1	-1	15.67±0.57	16.66
8	14	4	9	5	1	+1	+1	+1	-1	15±1	17.64
9	6	2	5	9	1	-1	-1	-1	+1	17.67±0.57	18.34
10	14	2	5	9	1	+1	-1	-1	+1	17±0	17.37
11	6	4	5	9	1	-1	+1	-1	+1	16.67±0.28	17.52
12	14	4	5	9	1	+1	+1	-1	+1	14.33±0.28	16.55
13	6	2	9	9	1	-1	-1	+1	+1	18.33±0.57	19.57
14	14	2	9	9	1	+1	-1	+1	+1	19.5±0	18.59
15	6	4	9	9	1	-1	+1	+1	+1	18±0	18.75
16	14	4	9	9	1	+1	+1	+1	+1	16.33±0.28	17.77
17	10	3	7	7	1	0	0	0	0	18±0	17.92
18	10	3	7	7	1	0	0	0	0	17±0	17.92
19	10	3	7	7	1	0	0	0	0	18±0	17.92
20	10	3	7	7	1	0	0	0	0	18±0	17.92
21	10	3	7	7	1	0	0	0	0	18±0	17.92
22	10	3	7	7	1	0	0	0	0	18.5±0	17.92
23	2	3	7	7	1	-2	0	0	0	15.83±0.28	17.92
24	18	3	7	7	1	+2	0	0	0	17.67±0.57	17.92
25	10	1	7	7	1	0	-2	0	0	15.5±0	18.74
26	10	5	7	7	1	0	+2	0	0	16.16±0.57	17.10
27	10	3	3	7	1	0	0	-2	0	16±0	17.37
28	10	3	11	7	1	0	0	+2	0	15.5±0	18.46
29	10	3	7	3	1	0	0	0	-2	18±0	17.20
30	10	3	7	11	1	0	0	0	+2	16.83±0.57	18.06

Table S2. Cultural characteristics of the isolate CMB47 on international *Streptomyces* project (ISP) and some media.

Culture media	Growth	Substrate mycelium	Aerial mycelium	Diffusible pigments	Appearance
ISP1 (Tryptone yeast extract agar)	Weak	Pale white	Pale white	No	Small elevated, Rough
ISP2 (Yeast extract-Malt extract agar)	Moderate	Dark violet	Dark violet with gray	Brown to violet	Rough with irregular edges
ISP3 (Oatmeal agar)	Abundant	Dark violet	Light pink	No	Elevated, powdered, round, smooth edges
ISP4 (Inorganic salt starch agar)	Abundant	Violet	Violet	Light violet	Rough with irregular edges
ISP5 (Glycerol asparagine agar)	Abundant	Dark violet	Light pink to dark gray	Yellow	Flat with smooth edges
ISP6 (Peptone yeast extract iron agar)	Weak	Light violet	Light violet to light white	No	Slightly elevated with irregular edges
ISP7 (Tyrosine agar)	Moderate	Light violet	Light violet	No	Flat, elevated at center, powdered with irregular edges
SCA (Starch Casein Agar)	Moderate	Dark violet	Violet to gray	Yellow	Elevated at centre, round, irregular edges
Gausse agar	Moderate	Dark violet	Dark violet	Light violet	Elevated at centre, round, irregular edges
Czapeck agar	Abundant	Dark violet	Violet	Yellow	Elevated, Rough, round, irregular edges

Table S3. Physiological properties of strain CMB47.

Characteristics	Results
Carbon source utilization	
Glucose	+
Starch	+
Cellulose	+
fructose	+
galactose	+
mannitol	+
mannose	+
sucrose	+
xylose	+
Growth temperature range (°C)	
4	-
28	+
37	+
40	+
50	-
Growth pH range	
3	+
5	+
7	+
9	+
11	+
NaCl tolerance range (w/v, %)	
0	+
0.2	+
0.4	+
1	+
2	+/-
4	+/-
6	-
8	-
Metal ions tolerance	
Fe	+/-
Cr	+/-
Cu	+/-
Zn	-
Cd	-
Mixture	-
Extracellular enzymes production	
Amylase	+
Cellulase	+
Protease	+
Lipase	+
L-tyrosinase	+
Alkaline phosphatase	+

Esterase lipase (C8)	+
Leucine arylamidase	+
Valine arylamidase	+
Lipase (C14)	-
trypsin	-
α -chymotrypsin	-
<i>N</i> -acetyl- β -lucosamidase	-
β -glucuronidase	-
α -mannosidase	-
α -fucosidase	-

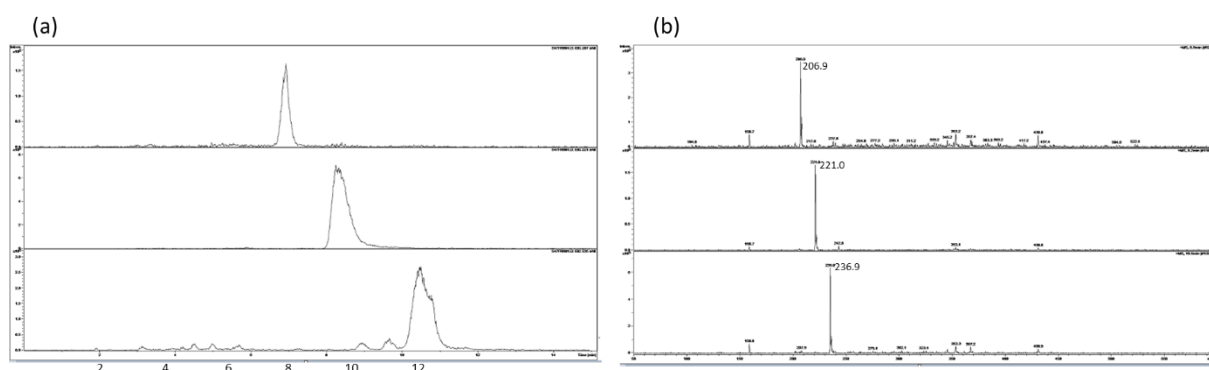


Figure S1. HPLC-DAD/ESI-MS analysis of the bioactive fraction 11 B from ethyl acetate crude extract of *S. rochei* CMB47 strain: (a) extracted ion chromatograms showing the three signals at $t_R=6.9$ min, $t_R=8.2$ min, $t_R=10.4$ min, corresponding to (b) the MS spectra in positive ion mode with $[M+H]^+$ ions at m/z 207, 221 and 237 respectively.

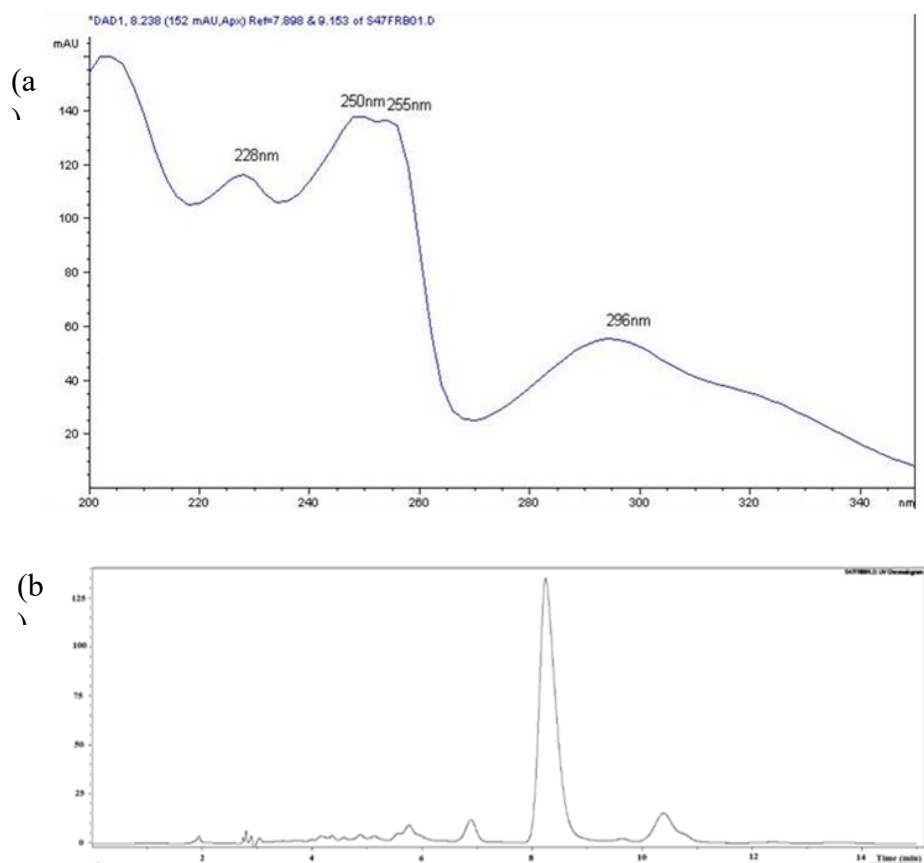


Figure S2. HPLC-DAD/ESI-MS analysis of the bioactive fraction 11B from ethyl acetate crude extract of *S.rochei* CMB47 strain: (a) the same UV spectrum for each of the three compounds corresponding to the three peaks at $t_R=6.9$ min, $t_R=8.2$ min, $t_R=10.4$ min (b) UV chromatogram acquired at 296 nm.