Culture medium modelisation for optimization of anti methicillin resistant *Staphylococcus aureus* metabolites by a coal mining soil derived *Streptomyces rochei* CMB47 strain

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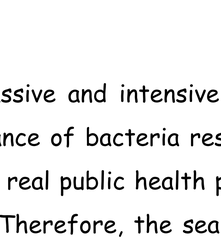
Introduction

- The massive and intensive use of antibiotics, in both humans and animals, has led to the accelerated appearance of bacteria resistant to these molecules. The emergence of MRSA strains on a global scale poses a real public health problem, because they are responsible for a wide variety of infections (WHO, 2022). Therefore, the search for new active molecules with a new mechanism of action or structurally distinct from those currently used is urgently needed.
- Actinobacteria has so far been the major source of new bioactive natural products. A challenge in the screening of these microorganisms lies in the search for growth conditions favorable for the production of bioactive secondary metabolites and the dereplication of known molecules.
- The peculiar ecosystem of Saharan soils is abundant in rare actinobacteria that have proven to be major producers of new antimicrobial molecules.

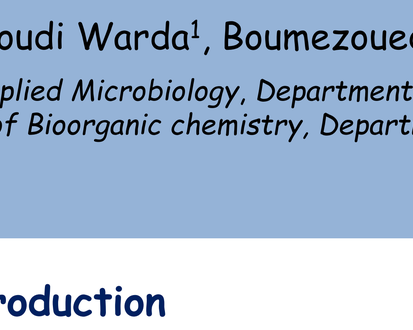
Results

1.Isolation of actinobacteria strains and antibacterial screening

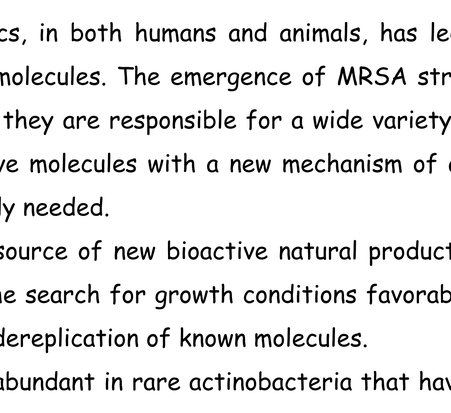
Isolation of 119 actinobacteria strains from a coal mine sample, collected from Bechar region, Algeria.



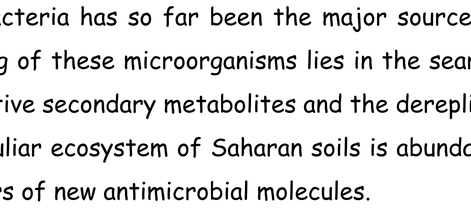
Screening of the antibacterial potential against *E. coli* ST131, *S. aureus* ATCC 25923, MRSA ATCC 43300, *Candida albicans* ATCC 10231 and *P. aeruginosa* ATCC 27853



Percentage of active isolates out of 119 tested strains



Venn diagram representation of antimicrobial traits of 119 actinobacteria isolates showing four profiles representing the four tested pathogenic germs

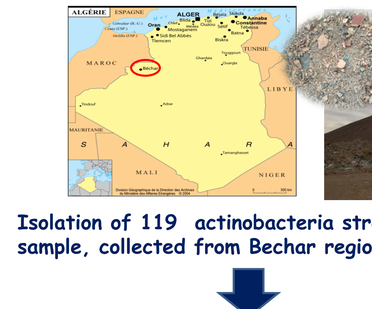


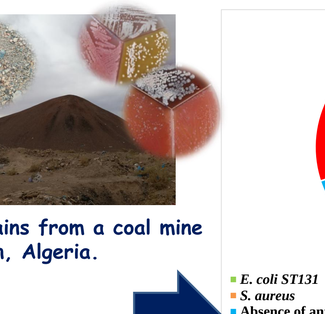
Selection of CMB47 strain

Principal component analysis (PCA) of screening for antimicrobial activity of 119 actinobacteria strains.

2.Screening the best culture medium and kinetic study on the production of anti-MRSA metabolites

Among four culture media used, the highest antibacterial activity (20.16 mm inhibition diameter) was observed in Czapeck medium against MRSA, containing per liter:
10g of soluble starch, 3g of $NaNO_3$, 1g of K_2HPO_4, 0.5g of $MgSO_4{\cdot}7H_2O$, 0.5g of KCl, 10mg of $FeSO_4.7H_2O$.





MIC evaluation of ethyl acetate and methanol crude extracts are estimated at 0. 439 and 1.95 µg/mL

Selection of Czapeck medium for further optimization study using statistical approaches

Polyphasic and molecular characterization of the selected active CMB47 Strain

Streptomyces rochei with strain A-1T 99.47% sequence homology

(**a**), Anti-MRSA activity variation of CMB47 based on culture media composition,
(**b**), anti-MRSA compounds production kinetics on Czapeck medium of CMB47 strain

3.Optimization of parameters for the production of anti-MRSA metabolites using experimental Central Composite Design (CCD) analysis

Factors	Levels				
	$-\alpha$ (–2)	–1	0	+1	$+\alpha$ (+2)
Starch (g/L)	2	6	8	14	18
$NaNO_3$ (g/L)	1	2	3	4	5
Incubation time (days)	3	5	7	9	11
pH	3	5	7	9	11

Coded and actual values of the variables for experimental CCD.

Minitab 16.0 (Minitab Inc., Pensylvania, USA) software.

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

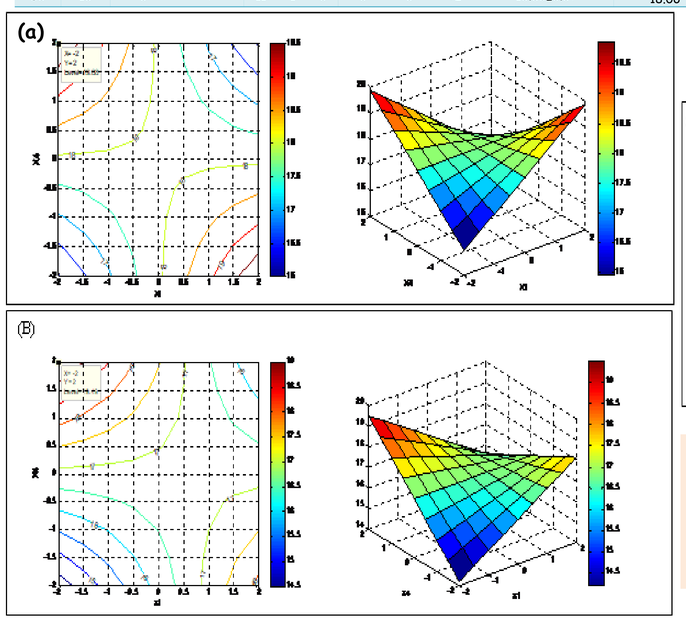
The model equation for anti-MRSA activity after discarding the insignificant coefficients is as follows:

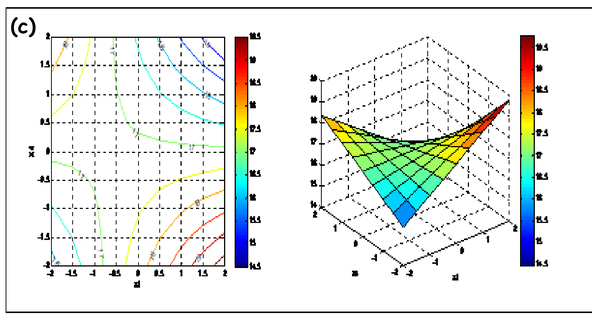
Positive effect of incubation time

$$\hat{y} = 17.92 - 0.41.X_2 + 0.27.X_3 - 0.49.X_1.X_4 + 0.34.X_3.X_4 - 0.47.X_2^2 - 0.49.X_3^2$$

Negative effect of $NaNO_3$

starch (X_4) and pH (X_1) affect the response with other factors in interaction ($X_1.X_4$), ($X_3.X_4$)



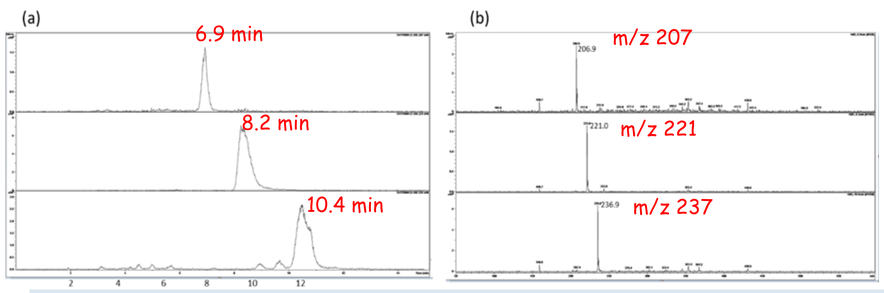


Contour plots and response surface plots at different conditions.
(a): X_2= 0, X_3= 0,
(b): X_2= +1, X_3= +1,
(c): X_2= -1, X_3= - 1.

The anti-MRSA activity ranged from 19.5 mm to 20 mm under different parameters The best 20 mm value was selected, corresponding to : **2 g/L of starch concentration** (X1 = –2), **3 g/L of NaNO3 concentration** (X2 = 0), **7 days of incubation time** (X3 = 0) and a media **pH of 11** (X4 = +2).

The measured anti-MRSA activity of 18.5 mm, close to the predicted 20 mm value, indicates that the developed model has a high degree of accuracy

4.Secondary metabolite profile of the bioactive fraction from CMB47 ethyl acetate extract by DAD-HPLC/ESI-MS analysis



The analysis of the bioactive **fraction 11 B** provided:
(a) an extracted ion chromatograms showing signals at the indicated retention time corresponding to (b) the MS spectra in positive ion mode with $[M+H]^+$ ions at *m/z* 207, 221 and 237.

The molecular structures of the three metabolites present in the bioactive fraction are related because differ by 14 units, attributable to a different number of CH2 unit, or to the presence of one/two OMe replacing OH groups or NMe replacing NH units.

Conclusions

- The novel strain, *Streptomyces rochei* CMB47 showed significant activity against MRSA.
- The CCD analysis applied with the aim of maximizing anti-MRSA activity allowed to determine optimal fermentation process conditions reaching 20 mm in inhibition zone diameter.
- DAD-HPLC/ESIMS analysis of the bioactive fraction provided preliminary structural indications on the metabolites responsible for MRSA inhibition. To the best of our knowledge, no similar data were found for antibiotic molecules isolated from *S. rochei* , hence the interest in further chemical investigation.

Reference

Djinni I.et al. *Fermentation* 2023, 9, 381.