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Antibiotic properties of Marine Natural Products (MNPs) derived from marine bacteria from New Caledonia against methicillin-susceptible *Staphylococcus aureus* (MSSA) and methicillin-resistant *S. aureus* (MRSA)

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INTRODUCTION

CONTEXT:

- 1 million death related to ARB in 2019¹.
- 10 million deaths related to ARB estimated for 2050 if no changes².
- Establishment of a list of bacteria against which new antibiotics are urgently needed by the WHO including several ARB from the ESKAPE group (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, *Enterobacter spp.*, *Escherichia coli*)³.
- Circulation of ARB in the Pacific Region including New Caledonia (Fr.)^{4,5}.
- New Caledonia recognized as marine biodiversity hotspot, source of innovative MNPs of pharmacological interest⁶.
- Bioprospecting campaigns in New Caledonia isolate certain marine bacteria with antibiotic properties^{7,8}.

OBJECTIVES & APPROACHES:

- To investigate the antibiotic potential of MNPs from New Caledonian marine bacteria and to understand their mode of action.
- To strengthen private partnership with the New Caledonian biotechnology start-up BIOTECAL.
- Implement microbiological tests and genomic approaches for bacterial identification.

MATERIALS & METHODS

Marine bacteria:

Marine bacteria collected in New Caledonian lagoons and isolated on Zobell[®] agar and liquid medium at 30°C or 37°C. Genus identification using 16S rRNA sequencing approach (Table 1).

Pathogenic bacteria:

MSSA (ATCC 25923) and MRSA (CIP 65.25) strains from ATCC and CIP collection. Cultured in Lysogeny Broth (LB) liquid and Mueller-Hinton Enriched (MHE) agar medium at 37°C.

Co-culture assays:

Marine bacteria co-cultured with pathogenic *S. aureus* (LB :Zobell, 1:2) for 1 week at 37°C. Collection of culture supernatant and bacterial pellet. Supernatant centrifuged to eliminate bacteria. Pellet lysed with DMSO 100%.

Contact test:

Deposit of an Zobell agar core of cultured marine bacteria on MHE plate in direct contact with seeded *S. aureus*. Incubation at 37°C for 96h. Daily macroscopic observation with measurement of inhibition halo. Growth of marine bacteria was detected around the Zobell core, and diameter measured.

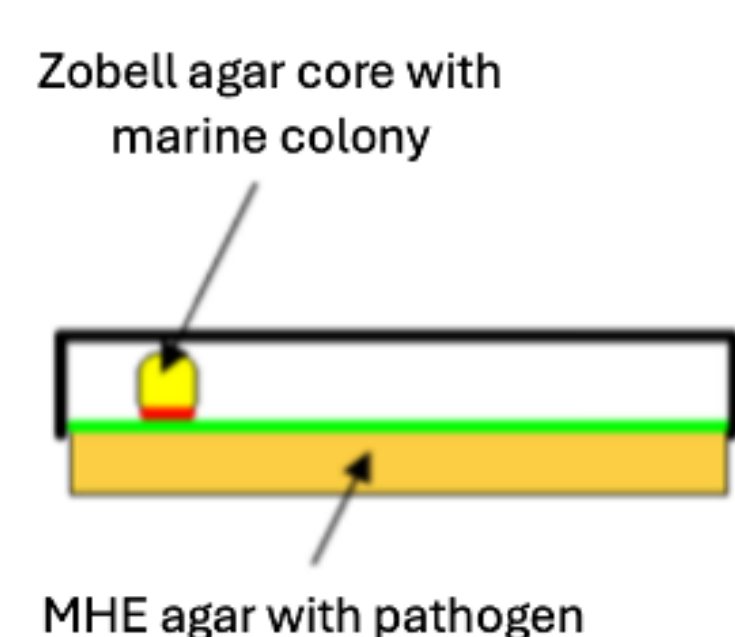


Figure 1: Contact test set-up (From BIOTECAL)

Well diffusion test:

LB agar seeded with *S. aureus* during the pouring of plate. Wells filled with supernatant from marine bacteria culture alone or co-cultured with *S. aureus*. Incubation at 37°C for 96h. Daily macroscopic observation with measurement of inhibition halo.

CONCLUSION & PERSPECTIVES

- 16 marine bacteria isolated from the lagoons of New Caledonia were screened and strains BT53, BT60 and BT115 belonging to the genus *Salinivibrio* showed an inhibitory effect on MSSA and MRSA during contact tests.
- Effect of the 3 *Salinivibrio* seems to be dependent of pre-culture incubation temperature.
- Evolution of inhibitory diameter during time suggests either an effect of marine bacteria on *S. aureus* mobility and/or an antibiotic effect.
- Co-culture of marine bacteria BT53, BT60 and BT115 with *S. aureus* showed inhibitory activity suggesting the need of pre-stimulation during culture possibly exerting regulatory effect on the production of metabolites of interests¹⁰.

Short term: Test other marine strains and resistant pathogens of the ESKAPE group; Produce extracts of marine bacteria by extractive chemistry.

Long term: Enhance the marine biodiversity of New Caledonia; Sustainable development; Response to the problem of antibiotic resistance.

References:

¹Murray et al., 2019, The Lancet; ²O'Neill et al., 2016, Analysis & policy observatory; ³Taccaronelli et al., 2017, The Lancet Infectious Diseases; ⁴Bourles et al., 2022, Journal of Global Antimicrobial Resistance; ⁵Colot et al., 2022, Infectious diseases Now; ⁶Motuhi et al., 2016, Marine drugs; ⁷Chalkiadakis et al., 2013, Journal of Applied Microbiology; ⁸Dufourcq et al., 2014, Letters in Applied Microbiology; ⁹Zobell et al., 1941, Journal of Marine Research; ¹⁰Maglangit et al., 2020, Marine drugs.

Acknowledgments:

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RESULTS

Table 1: Marine strains identified by 16S rRNA approach;

0: not yet tested, (+) activity, (-) no activity, the number of signs indicates the number of experiments carried out.

Bacterial CODE	Genus	Activity on MSSA & MRSA
BT 24	<i>Vibrio</i>	0
BT 36	<i>Photobacterium</i>	0
BT 50	<i>Bacillus</i>	(---)
BT 53	<i>Salinivibrio</i>	(+++)
BT 60	<i>Salinivibrio</i>	(+++)
BT 74	<i>Vibrio</i>	(-)
BT 88	<i>Bacillus</i>	(-)
BT 115	<i>Salinivibrio</i>	(+++)
BT 117	<i>Vibrio</i>	0
BT 122	<i>Photobacterium</i>	(--)
BT 135	<i>Vibrio</i>	0
BT 146	<i>Bacillus</i>	0
BT 161	<i>Pantoea</i>	(-)
BT211	not identified	(++)
BT 249	<i>Halomonas</i>	(-)
BT 280	<i>Pseudoalteromonas</i>	0
BT 284	<i>Pseudoalteromonas</i>	(-)



Figure 2: Contact test of BT50, BT53, BT60 and BT115 marine bacteria on MRSA. Observation after 96h idem figure 4;

Example of measured inhibition diameter (orange hatched), example of measured bacterial diameter (blue hatched), diameter of the 6mm Zobell agar core (black circle). T-: Zobell core without bacteria (6mm), T+: vancomycin BIORAD 67828 at 5 µg/disc.

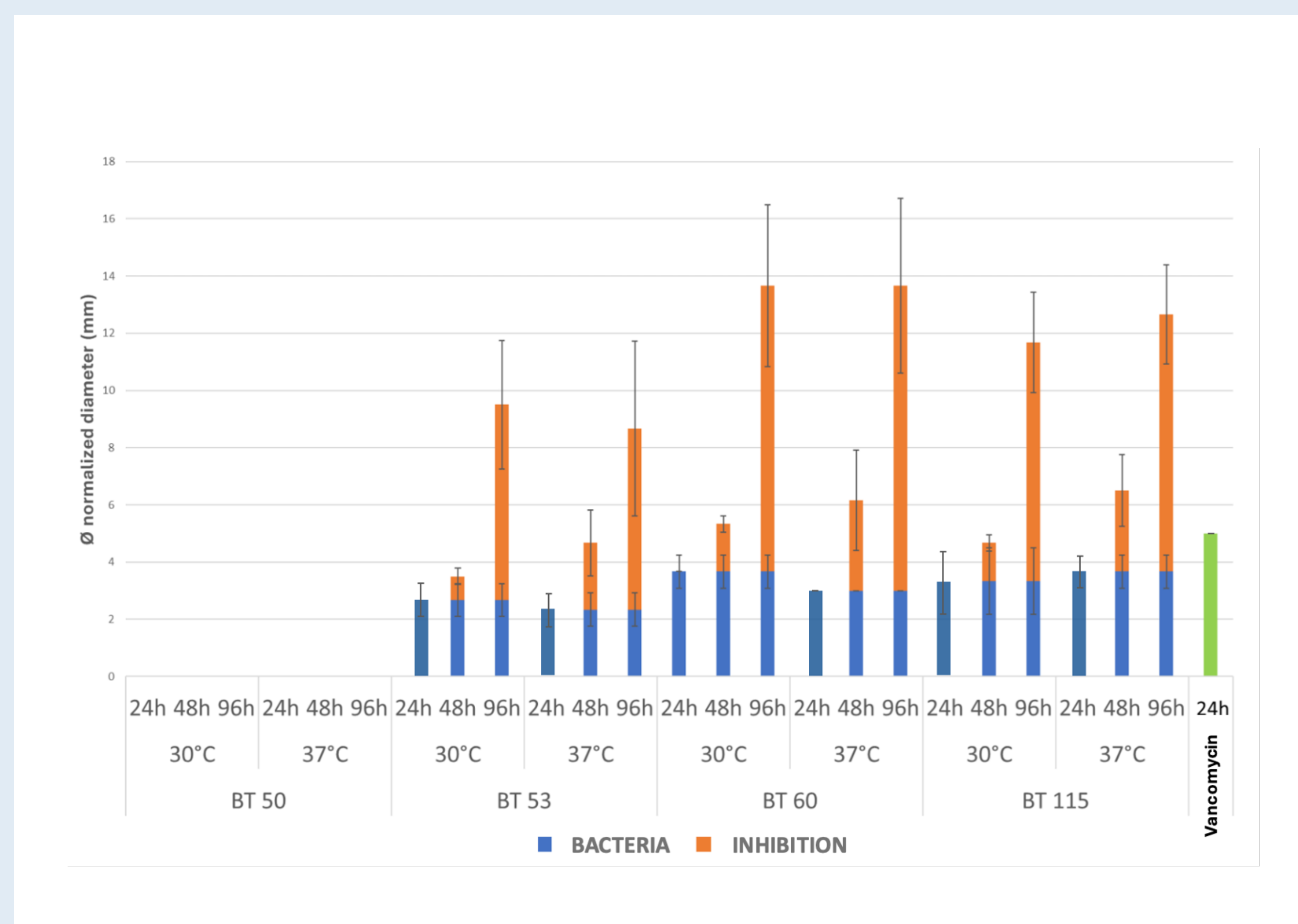


Figure 3: Evolution of inhibition diameter during time in contact test against MRSA at 30°C and 37°C, n=3 experiments, mean and sd;

Marine bacteria BT50, BT53, BT60 and BT115 were cultured at 30°C or 37°C prior to be tested against MRSA and diameter of the inhibition halo daily measured. Results show normalized halo where diameter of the Zobell core alone (6 mm) was subtracted. Growth of marine bacteria (blue) and inhibition diameter (orange) were measured. In green, the inhibition diameter of the reference antibiotic (vancomycin BIORAD 67828 at 5 µg/disc) without the disk.

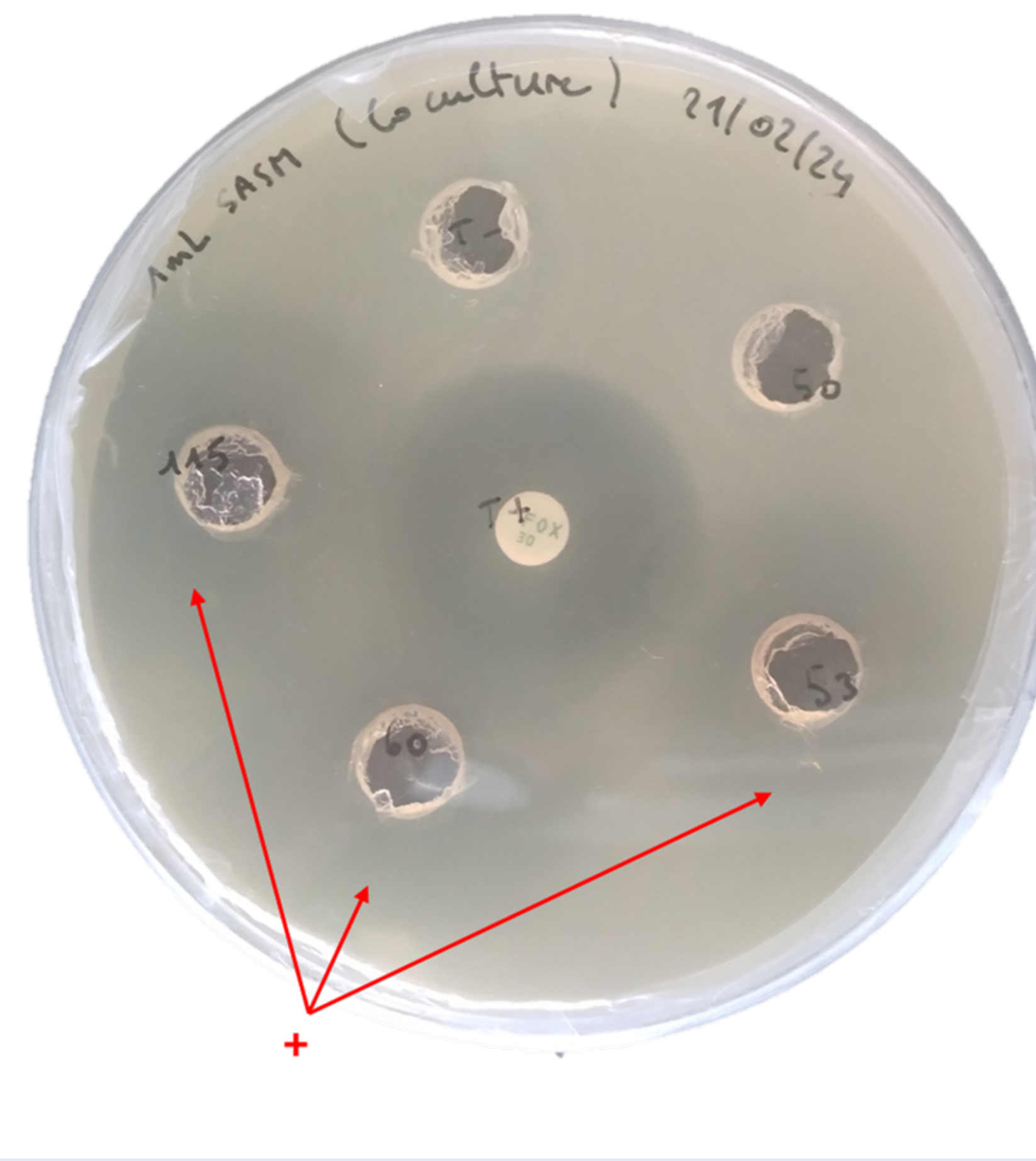


Figure 4: Well diffusion test of the coculture supernatant of BT50, BT53, BT60 and BT115 marine bacteria on MSSA; T-: (LB :Zobell, 1:2), T+ :cefotaxim BIORAD 66228 at 30 µg/disc, the red arrows connected to the sign show the areas where MSSA was inhibited by the co-culture supernatant of strains BT53, BT60 and BT115.

- Antibiotic assays with supernatant or bacterial pellet of marine bacteria cultured alone did not reproduce inhibitory effect observed in contact test.
- Thus, co-culture assay was set up to investigate if presence of *S. aureus* is necessary for inhibitory activity. While no effect of bacterial pellet was detected (not shown), results showed inhibition of MSSA by supernatant of co-cultured bacteria BT53, BT60 and BT115 but not BT50 (Figure 4), reproducing results observed in contact tests. These results suggest a potential stressful condition needed for antimicrobial activity.