Unlocking rhizospheric bacteria secondary metabolism: genome analysis for the discovery of novel antimicrobial compounds

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The emergence of antimicrobial resistance in pathogenic agents has raised awareness among society and nowadays is a recognized threat to public health. This problem is aggravated due to the misuse of current antibiotics and the lack of novel antimicrobial compounds. Soil microorganisms are a potential source of new antibiotics and, thanks to the study of their genomes, we can guide the search for undescribed antimicrobial compounds (1, 2).

MATERIALS AND METHODS

We have isolated two bacterial strains from a rhizospheric soil, belonging to the genera *Brevibacillus* and *Streptomyces*, which were revealed as antimicrobial agents, inhibiting the growth of bacteria and fungi with different profiles of antimicrobial resistance. These bacterial strains were grown in Müller-Hinton Agar (28°C) for one week; then, they were subjected to an antibiosis assay against several microorganisms, including G+, G-, yeasts and filamentous fungi).

In order to study in deep their encoded metabolic potential to produce antimicrobial compounds, we sequenced the genome of these strains using the Illumina MiSeq platform. The gene calling and genome annotation were done through the RAST tool (v2.0) (3). antiSMASH (v5.1) (4) was used to annotate in depth those genes related to the secondary metabolism of both strains.

Table 1: BGCs related to the secondary metabolism of *Brevibacillus* sp. and *Streptomyces* sp.

BGCs	*Brevibacillus* sp.	*Streptomyces* sp.
NRPS	6	7
Terpenes	1	9
Bacteriocines	2	2
TransAT-PKS-like	2	-
TransAT-PKS	2	-
LAP	1	1
Siderophores	1	2
Linaridines	1	4
Tiopeptides	-	1
NRPS-like	-	1
T1PKS	-	2
CDPS	-	5
Indoles	-	1
Butirolactones	-	1
Lantiopeptides	-	2
Melanines	-	2
hgIE-KS	-	2
PKS II	-	1
Lasopetides	-	1

RESULTS AND DISCUSSION

Inhibition tests showed that both strains were able to inhibit different microbes (Figure 1).

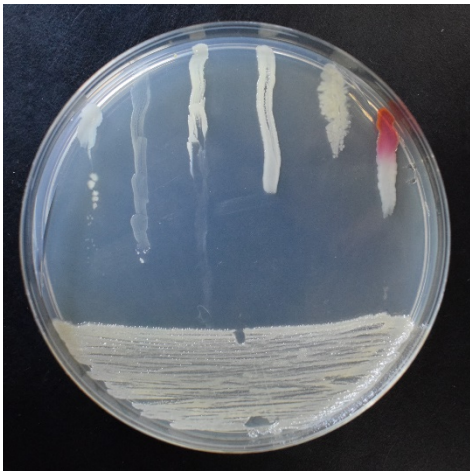

Fig. 1. In vitro *Brevibacillus* sp. antibiosis assays

Microorganisms from left to right *Bacillus subtilis, Staphylococcus* sp, *Arthrobacter phenantrenivorans, Klebsiella oxytoca* ,*Escherichia coli* and *Serratia marcescens*

The *Streptomyces* strain inhibits G+ *(Arthrobacter phenantrenivorans* and *Bacillus subtilis),* G- *(Pseudomonas bohemica),* yeasts (*Candida humilis* and *Saccharomyces cerevisiae*) and filamentous fungi (*Aspergillus* sp.).

Brevibacillus strain inhibits G+ (*Enterococcus faecium, Streptococcus pyogenes, Staphylococcus* sp., *A. phenantrenivorans* and *B. subtilis*) and G- (*Serratia marcescens, Acinetobacter baumannii, Klebsiella oxytoca* and *Escherichia coli*)

Genome analyses showed diverse antimicrobial potential encoded within these 2 genomes. Table1 shows the different biosynthetic gene clusters (BGCs).

In sum, 61 BGCs related with the secondary metabolism were annotated, of which 16 correspond to the *Brevibacillus* strain and 45 to the *Streptomyces* strain. The most abundant BGCs were non-ribosomal peptide synthetase (NRPS), terpenes and siderophores. Interestingly, some of these BGCs showed no similarity to any of the already described ones involved in the production of antimicrobial compounds.

CONCLUSIONS

The selected strains were able to inhibit several pathogens *in vitro*; thus, these strains are good candidates for further antimicrobials characterization. Futhermore, the genetic machinery encoded in both genomes might provide us the basis for the discovery of novel antibiotics against multidrug resistance pathogens.

REFERENCES. 1. Ziemert et al., 2016.RSC. 33, 988-1005; 2. Adamek et al., 2019. RSC. 36, 1295-1312; 3. Aziz et al., 2008. BMC Gen. 9, 75.;4. Blin et al., 2019. Nucleic Acids Res. 47, 81-87