Expression of a phenol hydrolase promoter of *Burkholderia unamae* y *Burkholderia nodosa*.

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Introduction. Many species within the genus *Burkholderia* possess significant biotechnological potential in bioremediation and biological control. The *Burkholderia* strains are investigated for their ability to degrade major xenobiotic pollutants, and to update information on their taxonomy, metabolic capacity and genomes[1].
In this project we obtained *Burkholderia* conjugants expressing transcriptional fusions of the promoter of the phenol hydrolase operon (FH) of *B. nodosa* Br3437^T and *B. unamae* MTl-641^T with the *GUS* reporter gene.

Methods. PCR amplification of the FH promoters was observed in *B. nodosa* Br3437^T and *B. unamae* MTl-641^T, this region was cloned into the vector pFAJ1700[2], which was introduced into the donor strain *E. coli* DH5α, and then transferred by conjugation to the wild type strains *B. nodosa* Br3437^T and *B. unamae* MTl-641^T, using *E. coli* pRK2013 as helper. The conjugants obtained were confirmed by PCR and GUS assays.

Results. PCR amplification revealed a fragment of 1372 bp from *B. unamae* and a fragment of 1312 pb from *B. nodosa,* whose sequence corresponded to the promoter of the phenol hidrolase operon. Fig 1.

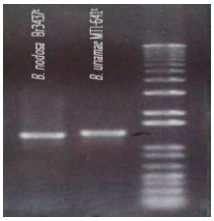

Fig.1 Products of PCR. 1) *B. nodosa* Br3437^T 2) *B. unamae* MTl-641^T 3) Ladder 1Kb

In the process of conjugation we selected one clone from each species, *B. unamae*/pFAJ1700gus and *B.* nodosa/pFAJ1700gus, which were shown by PCR to contain the fusion FH(p)::GUS, whose size corresponds to the amplified fragment of 3224 bp. Fig. 2

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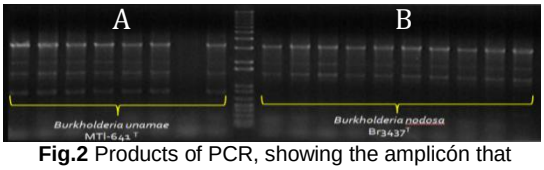

Fig.2 Products of PCR, showing the amplicón that confirms the transcriptional fusion FH(p)::Gus. A) *B. unamae* MTl-641^T. B) *B. nodosa* Br3437^T

The GUS assay was positive (indigo blue staining) when the conjugants were in contact with phenol. This result was not observed when succinic acid was used as carbon source. Additional conjugants expressing *GUS* from a constitutive promoter (S7)[2] were used as positive control.

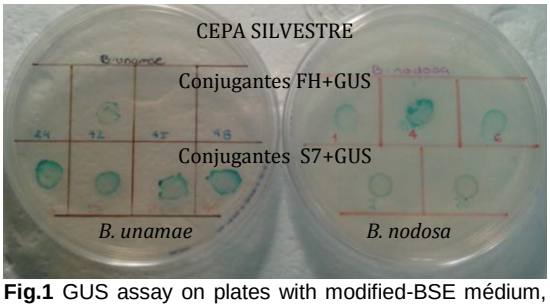

Fig.1 GUS assay on plates with modified-BSE médium, containing phenol 4.23 mM.

Conclusions.
The transcriptional fusion of the FH promoter with the GUS gene allows to analyze FH operon expression in *B. unamae* and *B. nodosa*.

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