MULTIPLE ANTIBIOTIC RESISTANCE FOUND BY METAGENOMICS TECHNIQUES IN WASTE WATER FROM LERMA DE VILLADA (*EDOMEX*)

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Introduction. Antibiotic resistance is a serious and growing phenomenon in contemporary medicine, especially in Mexico, where consumption has not been controlled for a long time (Amavile C. 2010). Functional metagenomics allow to identify enzymatic activities and physiological mechanisms of interest, in samples of unculturable microorganisms (Schmieder R. and Edwards R.2010). The aim of this work was to determine the antibiotic resistance present in waste water by metagenomics techniques.

Methods. A waste water sample was isolated in Lerma (Edomex). DNA was extracted with the BIO101 FastDNA Spin kit. (Qbiogene). Fragments ranging between 1000 to 4000 pb in size were selected and inserted into the pBluescript SKII(+) plasmid (Strategene). A metagenomic library was constructed. Resistance to four antibiotics, spectinomycin, tetracycline, kanamycin and chloramphenicol was tested.

Results. Approximately 200,000 recombinant clones were obtained with different insert size (Fig 1.)

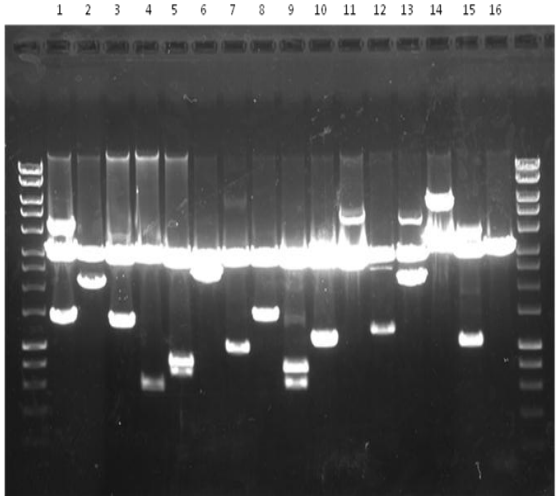

Fig.1 Fig.1 Restriction digestion analysis of 15 random clones with *Xho*I and *Xba* in a 1% agarose gel (Lanes 1-15).. Lane 16 negative control, pBluescript without insert. MM: 100bp Ladder.

Clones from the metagenomic library were screened in LB with the corresponding antibiotic. A total of 2 colonies were resistant to spectinomycin, 146 to kanamycin, 8 to chloramphenicol and 10 to tetracycline (Fig. 2) To exclude chromosomal mutations, plasmid DNA from these colonies were retransformed into *E. coli* and screened again in the presence of the corresponding antibiotic. A total of 6 antibiotic resistant clones were obtained.

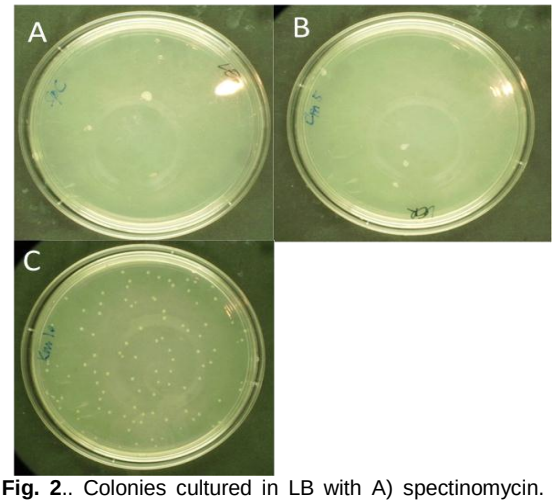

Fig. 2.. Colonies cultured in LB with A) spectinomycin. B). chroramphenicol and C) kanamycin.

Conclusions. Metagenomic techniques allowed the isolation of 6 antibiotic resistance clones. This methodology could be a rapid methodology to identify resistant strains in a great variety of environments.

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References.

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2. Robert Schmieder and Robert Edwards. 2010. Insights into Antibiotic Resistance Through Metagenomic Approaches *Future Microbiol.* 7(1):73-89.