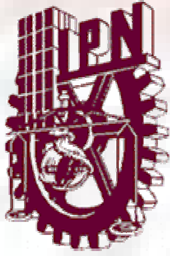

ANALYSIS OF THE MICROBIOTA OF THE GUT OF Dendroctonus spp.(Coleoptera:Curculionidae:Scolytinae)

ZAVALA-DIAZ DE LA SERNA, FRANCISCO JAVIERa, ZÚÑIGA-BERMUDEZ, GERARDOb, HERNÁNDEZ-RODRIGUEZ, CÉSAR HUGOa ESCUELA NACIONAL DE CIENCIAS BIOLOGICAS, INSTITUTO POLITECNICO NACIONAL, a Departamento de Microbiologia, b Departamento de Zoología. Prolongación de Carpio y Plan de Ayala s/n. Col. Casco de Sto.Tomás. Del. Miguel Hidalgo. Distrito Federal. México. 11340 email: javierzavala@hotmail.com

INTRODUCTION Bark beetles of the genus *Dendroctonus* (Scolytidae:Erichson) correspond to a group of insects associated to the trees of family Pinaceae, specially with the genus *Pinus*, *Picea*, *Pseudotsugae* and *Larix*. A total of 13 species of 19 described species have been reported in Mexico. They feed on phloem and vascular cambium from the crust of alive or fallen pines, causing great ecological and economic damage to the forestall sector (2). A fundamental part in the cycle of life of these insects is the association that they establish with fungi and other microorganisms. Insect structures like micangios show the importance of the symbiotic relationship. Also the insects have microbial endosimbiotic relationships into the alimentary canal. This microbiota, basically composed by bacteria, fungi and protistas, allows to insect obtain nutritional requirements, participate in the destoxification processes, and in the synthesis of insect pheromones, volatile compounds necessary to the communication among beetles.

MATERIAL AND METHODS: COLLECT: *Dendroctonus pseudotsugae* was collected in Coahuila (N 25°19' W 92°17'), and *D. mexicanus* (N 17°05' W 96°17'), *D. approximatus* (N 16°58' W 96°11') and *D. adjunctus* (N17°10' W 96°41') were collected in Oaxaca, Mexico. The species was determined by external morphological characters using the keys previously proposed (2). Finally, the chromosomal number was an useful diagnosis character. **EXTRACTION OF THE GUT**: the beetles was washed in a PBS solution (50 mm, pH 7,2) containing an antibiotic mixture. The insect was dissected to obtain the complete gut, and then it was separated into three parts (foregut, midgut and hindgut). Each part of the gut was placed in tubes containing sterile deionized water and its was stored to -20ºC. **EXTRACTION OF METHAGENOMIC DNA**: The tissues were mechanically disrupted and DNAs were extracted using DNAzol kit (Invitrogen) and proteinase K. **PCR**: A variable region of 16S rDNA was amplified from 10 ng of the extracted methagenomic DNA using a procedure previously proposed (1). A product of amplification of 233 pb was expected. Also we amplified a variable region of 28S rDNA for search of fungi **DGGE**: The denaturing gel ranged from 0 to 100 % of relative concentration of urea and formamide, the electrophoresis was ran during 16 h to 60 V. The gels were revealed with a silver stain (Biorad). The images was observed in a transiluminator of white light and they were digitalized. **16S rDNA LIBRARY AND RFLP**: ribosomal gene libraries were constructed from methagenomic DNA from different sections of the gut of *D. pseudotsugae*. RFLP analysis was performed using *Eco*RI, and *Hha*I restriction enzymes. The restriction products were electrophoresed on high resolution agarose. **ISOLATION**: We traslated a dilution 1:10 of the macerate of gut in Neutral medium and GAE medium. The Plates were incubated at 37°C for one week . Each colony was setting were put in liquid broth and to conserved in Glycerol at -70°C.

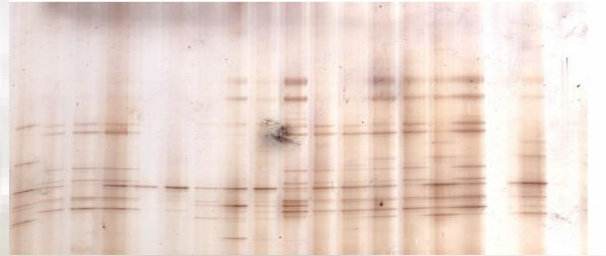

Figure 1. DGGE of the gut of *D. pseudotsugae*. The first six correpond to the foregut , the next five to midgut and the last five to the hindgut. the concentration of DGGE was 0 to 100% of dennaturing.

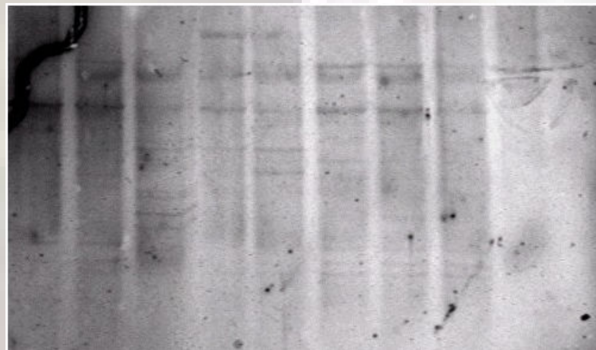

Figure 1. DGGE of the gut of *D. approximatus, D. adjuntus* and *D. mexicanus*. 1.-Foregut of *D. approximatus* 2.- Midgut of *D. approximatus* 3.- Hindgut of *D. approximatus* 4.- Foregut of *D. adjuntus* 5.- Midgut of *D. adjuntus* 6.- Hindgut of *D. adjuntus* 7.- Foregut of *D. mexicanus* 8.- Midgut of *D. mexicanus* 9.- Hindgut of *D. mexicanus.* The concentration of DGGE was 0 to 100% of dennaturing.

1 2 3 4 5 6 7 8 9

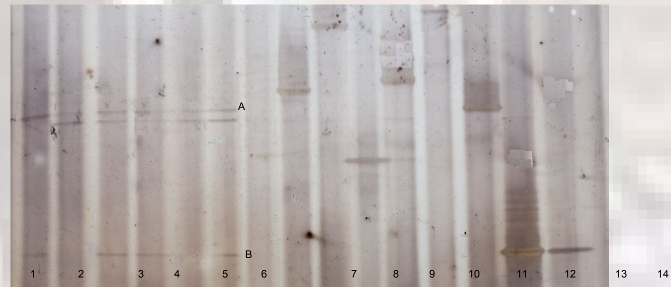

Figure 3. DGGE of the gut of *D. pseudotsugae* from region D1-D2 of 28S rDNA. 1-6 PCR from Metagenomic DNA 7-15 PCR from yeast isolated previously by culture.1 and 4 foregut, 2 and 5 midgut, 3 and 6 hindgut.7.- *Candida terebrans* 8.-*C. capsulata* 9.- *C. scolytidae* 10.- *C. arabinofermentans* 11.- *Candida* sp. 12.- *C. americana* 13.- *Candida* sp 14.-*C. oregonensis*. a and b were obtained from DGGE and sequenced. The analysis showed that these bands have relation with *C. americana* (A) and *C. oregonensis* (B). The concentration of DGGE was 0 to 100% of dennaturing.

RESULTS. In Fig. the display of DGGE can be observed. In general, the bacterial communities from the midgut and hindgut were similar, but foregut showed a different bacterial composition in the analyzed samples of Dendroctonus species. However, there are common organisms in all regions of the gut. Apparently, there are common bacteria in the gut of all species of bark beetles studied. Also Dendroctonus species-specific bacteria can be observed. A limited bacterial diversity was observed, only 8-14 bands of each sample were obtained. On the other hand, only 10 RFLP profiles from 250 clones were obtained from ribosomal libraries, supporting the idea that the gut of the insect maintain a limited bacterial community.

In Fig 3 the display of DGGE for fungi can be observed. Some yeasts were previously isolated (Rivera *et al*) and their DNA were extracted and amplified together with metagenomic DNA. Three bands were obtained and two of them were identified: *Candida oregonensis* and *Pichia americana*.

For culture we obtained six different morphology: coccus, sarcinae,staphylococci, long rod, short rod and actinomycetes like.(Fig 4.)

DISCUSSION. In this work, the low deduced bacterial diversity, compared with other insects as cockroach and termites, can be explained by the local environmental conditions and the conduct of the bark beetles. These pine insects are exposed to toxic volatile compounds and feed on phloem, a poor substrate that contains limited assimilable nutrients. A clear compartmentalization can be observed between midgut-hindgut and foregut. Identification of broad distributed and species-specific bacteria must be performed to clear the symbiosis role of the microbial partners in the life cycle of the bark beetles.

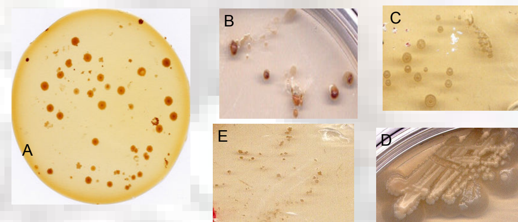

Figure 4 Bacterias isolated from hindgut of D. pseudotsugae♀.
A.Colony growth in GAE Medium plate.
B-E approaches from someone bacterias

REFERENCES

1. Muyzer, G., E. C. DeWaal, and A. G. Uitterlinden. 1993. Profiling of microbial population by denaturing gradient gel electrophoresis analysis of polymerase chain reaction amplified genes coding for 16S rRNA. Appl. Environ. Microbiol. 59:695-700.
2. Wood, S. L. 1982. The bark and ambrosia beetle of North and Central America (Coleoptera: Scolytidae). A taxonomic monograph. Great Basin Nat. Mem. 6.

ACKNOWLEDGES

FJZDS is a CONACYT and PIFI fellowship; GZB y CHHR are EDD y COFAA fellowships. This work was supported by CONAFOR and CONACYT grants.