Title Polyphasic characterization of antibiotic resistant and virulent enterococci isolated from animal feed and stored-product insects

Author Lakshmikantha Channaiah, Subramanyam Bhadriraju,Ludek Zurek, Keith Behnke and Scott Beyer

Citation Thesis, Doctor of Philosophy (Grain Science and Industry), Kansas State University. 125 pages. 2009.

Keywords Animal feed, Antibiotic resistance, Enterococci, Stored-product insects; Virulence

Abstract

Feed samples and live stored-product insects from feed mills and swine farms were collected and cultured for *Enterococcus* spp. The mean concentration of enterococci in insect and feed were 2.7 ± 0.5 × 10^1 cfu/insect and 6.3 ± 0.7× 10^3 cfu/g respectively. A total of 362 isolates of enterococci collected from 89 feed samples and 228 stored-product insects were identified to the species level using PCR. These isolates were represented by *Enterococcus casseliflavus* (53.0%), *E. gallinarum* (20.4%), *E. faecium* (16.2%), *E. hirae* (5.2%), and *E. faecalis* (5.0%). Enterococci were phenotypically resistant to tetracycline (48.0%), erythromycin (14.3%), streptomycin (16.8%), kanamycin (12.1%), ciprofloxacin (11.0%), ampicillin (3.3%), and chloramphenicol (1.1%). All isolates were susceptible to vancomycin and gentamicin. Tetracycline resistance was encoded by *tetM* (50.0%), *tetO* (15.1%), *tetK* (0.5%), *tetS* (0.2%) and other unknown tetracycline determinants. Enterococci carried virulence genes including gelatinase (*gelE* ; 21.5%), an enterococcus surface protein (*esp* ; 1.9%), and cytolysin (*cylA* ; 2.2%). An aggregation substance (*asa1*) gene was detected in 61.0% of *E. faecalis* isolates. Fifty perncet of *E. faecalis* isolates were phenotipically tested positive for aggregation substances. Enterococci with *cylA* genes were hemolytic (52.0%) and with *gelE* genes were gelatinolytic (18.5%). The *ermB* gene, encoding erythromycin resistance was detected in 8.8% of the total isolates. The Tn*916/1545* family of conjugative transposons was detected in 10.7% of the isolates.

Laboratory experiments showed that adults of the red flour beetle, *Tribolium castaneum* (Herbst), fed on poultry and cattle feeds inoculated with *E. faecalis* OG1RF:pCF10, were able to successfully acquire enterococci and contaminate sterile poultry and cattle feeds. To assess the potential of horizontal gene transfer, conjugation assays were carried out with *E. faecalis* using a donor (wild strains) and recipient (*E. faecalis* OG1SSP) in ratio of 1:10. Only one isolate (1 out of 18 *E. faecalis*) could transfer *tetM* to a recipient using broth mating. However, filter mating assay, followed by PCR confirmation

revealed that 89.0% (16 out of 18 *E. faecalis)* of isolates could transfer *tetM* to *E. faecalis* . Transfer ratios of transconjugant per recipients ranged from 2.6×10^{-4} to 1×10^{-9} .

In summary, feed (52.0%) and stored-product insects (41.6%) collected from feed mills and swine farms carried antibiotic-resistant and potentially virulent enterococci. Our study showed that *T. castaneum* , a pest commonly associated with feed, served as a potential vector for enterococci in the feed environment. Conjugation assays followed by PCR confirmed presence of the *tetM* gene on a mobile genetic element(s) such as Tn *916* and may be horizontally transferred to other *Enterococcus* species and to other bacteria of clinical significance.