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Bioprospecting for antimicrobial peptides from insects: *In vitro* antimicrobial activity of acidified methanol extract of dung beetles

S. Gundappa*, J. Jayappa, and K. Chandrashekara

Department of Entomology, UAS, GKVK, Bangalore - 560 065, Karnataka, India

ABSTRACT

***In vitro* antimicrobial activity of acidified methanol extracts of individuals of sixteen dung beetle species 'challenged' (with overnight grown culture of *Escherichia coli*) was determined by Kirby-Baer disk diffusion bioassay. Extracts from 'unchallenged' individuals were also screened for antimicrobial activity along with Tetracycline as positive control in bioassays. Considerable variation in antimicrobial activity was observed between 'unchallenged' and 'challenged' extracts and between the species. The highest mean zone of inhibition was observed in extracts of *Copris repertus* (283.84 ± 202.06 mm^2) and (267.86 ± 237.48 mm^2) at 10 and 20 h post-treatment, respectively. Among the test microorganisms the highest mean zones of inhibition of 179.79 ± 120.08 mm^2 and 188.34 ± 125.85 mm^2 were recorded against *Staphylococcus aureus* at 10 and 20 h post-treatment, respectively. The mean zone of inhibition of the extracts against *E. coli* was 166.51 ± 166.43 mm^2 and 128.49 ± 119.86 mm^2 at 10 and 20 h post-treatment, respectively. The antimicrobial activity against *E. coli* was particularly important since the strain of *E. coli* used in the study was resistant to all antibiotics, including Methicillin. The mean zone of inhibition of the extracts against *Candida albicans* was 26.56 ± 43.13 mm^2 and 30.44 ± 44.46 mm^2 at 10 and 20 h post-treatment, respectively.**

Key words : Antimicrobial peptides, acidified methanol extract, dung beetle.

The cationic antimicrobial peptides (AMP) have been identified as major components of the humoral immune response of insects. The first such AMP was isolated from the moth *Hyalophora cecropia* by Steiner *et al.* (1981) and named Cecropin. Since then more than 300 AMPs (including those identified by *in silico* analysis) have been identified from insects alone, out of about 700 AMPs of animal origin (Otvos, 2000; Brahmachary *et al.* 2004). The AMPs from insects are generally small with most peptides having 30-40 amino acids (Hetru *et al.* 1998; Marshall, 2003), and up to 100-150 amino acids in rare cases (Jayappa, 2008). The AMPs have been reported both, as being constitutively present and produced on induction by pathogenic or any invading microbe (Hetru *et al.* 1998; Hancock and Diamond, 2000). The AMPs are synthesized in fat bodies and a few cell types in the haemolymph and released in the haemolymph at quantities ranging from 1 to 10 microlitres (Hetru *et al.* 1998; Lamberty *et al.* 2001). Alternatively, they may also be present constitutively in haemocytes and released upon challenge by pathogenic microbes (Lamberty *et al.* 2001). These peptides are heat stable, resistant to action of proteases and exhibit a broad spectrum of activity against bacteria, fungi and even virus and tumour cells (Hetru *et al.* 1998; Bulet and Stocklin, 2005). The high stability of the AMPs has made them attractive for therapeutic use in human, animal health and the genes coding for antifungal AMPs have already been cloned to develop disease resistant transgenic crops (Tripathi *et al.*, 2004; Vilcinskas and Gross, 2005).

A compilation of AMPs of insect origin when analyzed with respect to the distribution across different taxa revealed that only six AMPs were from four species of coleopteran insects (Jayappa, 2008). AMPs from *Zophobas atratus* (Bulet *et al.* 1991), *Tenebrio molitor* (Moon *et al.* 1994) (Cucujoidea), *Holotrichia diamphalia* (Lee *et al.* 1994), and *Allomyrina dichotoma* (Miyanoshita *et al.* 1996) (Scarabaeoidea) were reported more than fifteen years ago suggesting a major gap in bioprospecting Coleoptera, the largest taxa in Animalia (Jayappa, 2008). Four out of these six antibacterial peptides

*Corresponding author's E-mail: gundu6100@gmail.com