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RESEARCH ARTICLE

SYNERGISTIC AND INHIBITORY EFFECTS OF SEPTICEMIA BACTERIA ON SILKWORM.

Ashok kumar H. K, Ramakrishna S, Rizwan Sharief and Rakesh Hand Girishkakade A. S.
Department of Zoology, Bangalore University, Bangalore-560 056, India.

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Abstract

Microbes spend energy to produce secondary metabolites (defensins) in order to colonize various environments. Production of defensins by septicemia causing bacteria of silkworm and its of synergistic or inhibitoryactivity on selected bacteria were studied. Well diffusion method was employed to define the defensins activity in microenvironment. Different aliquots of supernatant containing defensins from*Serratia* sp., and *Bacillus* sp., were examined on test cultures of *Escherichia coli* and *Staphylococcus aureus*. Results revealed inhibition of *E. coli* and *S. aureus* by supernatants of *Serratia* species in different degree based on different concentrations. Therapeutic applications of defensins may potentially serve as an alternate to classical drug therapy for infections of humans by their specific and narrow spectrum of activity.

Introduction:-

The increasing concern of multi drug resistance (Goncalves et al., 2007) in bacterial species compelsto combat sustainable resistance by a novel method. In absolving drug resistance and treatment of sensitive strain causing diseases using bacteriocinshence becomes expedient.

Bacteria produces an extraordinary array of compounds *viz.*, antibiotics, lytic agents, protein exotoxins, and bacteriocins for their defense. SepticemicDisease causing silkworm bacteria (*Serratiamarcescens*, *Serratiaplymuthica* and *Bacillus cereus*) with secondary metabolites producing ability are considered to be of paramount importance (Dong et al., 2012; Aunpad et al., 2011). *Serratia*species are known to colonize wide range of ecological niches by producing a spectrum of secondary metabolites as extracellular products including chitinases, proteases, lipases, nucleases, bacteriocins, carbapenemase, SiderophoreSerratigen and polysaccharide Serratimannan (anti-tumor) that synergisticallyare anti-nematodes anti-bacterial, antifungal, antiprotozoal, anti-malarial, immunosuppressive and anti-cancerous (Chang et al., 2011; Genes et al., 2011;Rahul et al., 2014). These are organically soluble, stable to heat, cold-active and surfactants (Smaoui et al., 2010; Sanchez et al., 2010).

The production of bacteriocins from different bacterial species needs to be further investigated through Immunization of silkworm (Hara &Yamakawa, 1995) or by genetic engineering techniques. The production of these remarkable productscansolve drug resistance epidemic. Selective nutritional supplements in culturing of septicemic bacteria augmentbacteriocinsproduction in an industrial scale.

Corresponding Author:-Ashok kumarh.k.
Address:-Department of Zoology, Bangalore University, Bangalore-560 056, India.

Methodology:-

Well diffusion method

Serratia and Bacillus sp., inherently produces a wide variety of distinguished pharmacologically active compounds, their activity can be analyzedby well diffusion method. Supernatants of silkworm septicemia causing bacteria (*Serratia* sp. and *Bacillus* sp.) were cultured in optimal nutrient broth at 30°C for 48h. Supernatants of *Serratia* sp., and *Bacillus* sp., were obtained by centrifugation at 6000 rpm. Gram-negative and Gram positive test bacteria *Escherichia coli* and *Staphylococcus aureus*were cultured by pour plate method on Nutrient Agar medium to obtain lawns. Wells were made on bacterial lawns using cork borer to add supernatants in aliquots of 50 µl, 100 µl, 150 µl, and 200 µl and incubated at 37°C for 24hrs. (Wiegand et al., 2008).

Results:-

Isolates producing secondary metabolites were employed on test bacteria to be assessed by well diffusion method

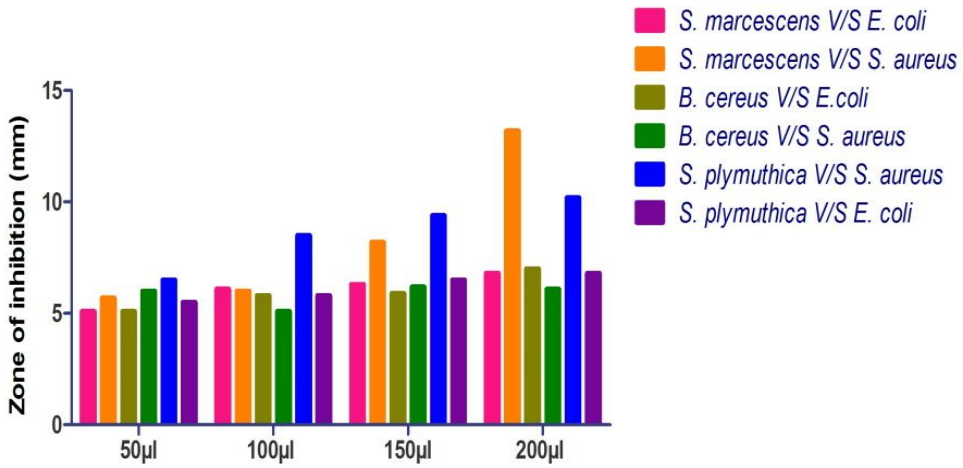
The standardization and application of varied concentrations were identified by the Well diffusion method. The secondary metabolite produced in septicemic*S. marcescens*, *S. plymuthica* and *B. cereus*were recognized and varied inhibition concentration was evaluated using above method. The antagonistic effects of septicemia causing bacteria species were assessed by dispensing 50µl, 100µl, 150µl, and 200µl into wells.

B. cereus filtrate on examination against test bacteria *S. aureus*and *E. coli* lawns, the diameter of inhibition zone formed including well was found to be low in comparison to other examined bacteria. The *S. marcescens* inhibition zones increased in steady state with an increase in aliquots of supernatants on lawns of S. *aureus*, however on *E. coli* inhibition zones were comparatively low. *S. plymuthica*on lawns of *S. aureus* showed high inhibition zone while with *E. coli* exhibited low inhibition zones even with increase in concentrations of filtrate aliquots. *Bacilluscereus* showed meager inhibition diameters for both *S. aureus* and *E. coli*. The summary of well diffusion results are summarized in Table1.

Table1:-Inhibition of septicemia bacteria by well diffusion method

Antagonism of bacteria	**supernatant in µl and zone of inhibition in mm**			
	50µl	**100µl**	**150µl**	**200µl**
B. cereus* V/S *S. aureus	6.0 ± 0.5	5.1 ± 0.5	6.2 ± 0.5	6.1 ± 0.5
B. cereus* V/S *E.coli	5.1 ± 0.5	5.8 ± 0.5	5.9 ± 0.5	7.0 ± 0.5
S. marcescens*V/S *S. aureus	5.7 ± 1.0	6.0 ± 1.0	8.2 ± 1.0	13.2 ± 1.0
S. marcescens*V/S *E. coli	5.1 ± 1.0	6.1 ± 1.0	6.3 ± 1.0	6.8 ± 1.0
S. plymuthica*V/S *S. aureus	6.5 ± 1.0	8.5 ± 1.0	9.4± 1.0	10.2 ± 1.0
S. plymuthica*V/S *E. coli	5.5 ± 0.5	5.8 ± 0.5	6.5 ± 0.5	6.8 ± 0.5

mm = mili meter, µl= microleter,
Values are means (±SD), n=3 for each condition



Graph 1:-Inhibition of septicemia bacteria by well diffusion method.

Discussion:-

Serratia sp., and *Bacillus* sp., exhibits antagonistic activity against*Bacillus paeni*, *Bacillus lysine*,*E. coli* and *S. aureus*were reported as insect pathogens (Foulds&Shemin, 1969; Aunpad et al., 2011; Kadouri& Shanks, 2013). *S. marcescens*was found to inhibit the growth of *S. aureus* isolates and other Gram-positive bacterial species.

The results obtainedsuggest that *B. cereus* had little effect on test strains. Culture filtrates of *S. marcescens* and *S. plymuthica* showed a wide inhibition zone on test bacteria, further with increase in concentration of supernatant, showed progressive-wider zone of inhibition. There were many factors in filterate of *Serratia* strains like prodigiosin, proteases, chitinases, phosphatases and other inhibitory products which played role in antagonizing test bacteria. The pigment prodigiosin with pyrrolylpyrromethane is a promising drug with reported anti-fungal, immunosuppressive and anti-proliferative activity (Khanafari et al., 2006). Bacteriocinsbenefits in the field of pharmaceuticals as affordable, biodegradable and broad spectrum antagonistic antibiotics. The culture filtrate concentration procedure would help industrial breeders in production of antibiotics of various degreesof concentration for bacteriocinsthat may be used as food preservatives, broad-spectrum therapeutics and in the treatment of infectious diseases.

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