SYNERGISTIC AND INHIBITORY EFFECTS OF SEPTICEMIA BACTERIA ON SILKWORM.

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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 inhibitory activity 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 compel to 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. Septicemic Disease causing silkworm bacteria (Serratiamarcescens, Serrataplymuthica 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, antiprototoxol, 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 productscan solve drug resistance epidemic. Selective nutritional supplements in culturing of septicemic bacteria augmentbacteriocinsproduction in an industrial scale.

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Methodology:
Well diffusion method
Serratia and Bacillus sp., inherently produces a wide variety of distinguished pharmacologically active compounds, their activity can be analyzed by 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 50µl, 100µl, 150µl, and 200µl 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. Bacillus cereus 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. plymuthica 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 obtained suggest 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 filtrate of Serratia strains like prodigiosin, proteases, chitinases, phosphatases and other inhibitory products which played role in antagonizing test bacteria. The pigment prodigiosin with pyrrolylpyromethane is a promising drug with reported anti-fungal, immunosuppressive and anti-proliferative activity (Khanafari et al., 2006). Bacteriocins benefits 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 degrees of concentration for bacteriocin that may be used as food preservatives, broad-spectrum therapeutics and in the treatment of infectious diseases.

References:-
1. Aunpad, R., Sripotong, N., Khamlak, K., Inchidjuy, S., Rattanasinganchan, P. & Pipatsatitpong, D. (2011). Isolation and characterization of bacteriocin with anti-listeria and anti-MRSA activity produced by food and soil isolated bacteria. African Journal of Microbiology Research, 5(29), 5297-5303.
2. Chang, C. C., Chen, W. C., Ho, T. F., Wu, H. S. & Wei, Y. H. (2011). Development of natural anti-tumor drugs by microorganisms. J Biosci Bioeng, 111(5), 501-511.
3. Dong, J., Ruan, J., Xu, N., Yang, Y. & Ai, X. (2016). In vitro synergistic effects of fisetin and norfloxacin against aquatic isolates of Serratia marcescens. Fems Microbiology Letters, 363(1), fnv220.
4. Foulds, J. D. & Shemin, D. (1969). Properties and characteristics of a bacteriocin from Serratia marcescens. J Bacteriol, 99(3), 655-660.
5. Genes, C., Baquero, E., Echeverri, F., Maya, J. D. & Triana, O. (2011). Mitochondrial dysfunction in Trypanosoma cruzi: the role of Serratia marcescens prodigiosin in the alternative treatment of Chagas disease. Parasit Vectors, 4, 66.
6. Goncalves, M. O., Coutinho-Filho, W. P., Pimenta, F. P., Pereira, G. A., Pereira, J. A., Mattos-Guaraldi, A. L. & Hirata, R., Jr. (2007). Periodontal disease as reservoir for multi-resistant and hydrolytic Enterobacter ial species. Letters in Applied Microbiology, 44(5), 488-494.
7. Hara, S., & Yamakawa, M. (1995). A novel antibacterial peptide family isolated from the silkworm, Bombyx mori. The Biochemical Journal, 310, 651–656.
8. Kadouri, D. E. & Shanks, R. M. (2013). Identification of a methicillin-resistant Staphylococcus aureus inhibitory compound isolated from Serratia marcescens. Res Microbiol, 164(8), 821-826.
9. Khanafari, A., Assadi, M. M. & Fakhr, F. A. (2006). Review of Prodigiosin, Pigmentation in *Serratia marcescens* 1Anita. *Journal of Biological Sciences*, 6(1), 1-13.
10. Rahul, S., Chandrashekhar, P., Hemant, B., Chandrakant, N., Laxmikant, S. & Satish, P. (2014). Nematicidal activity of microbial pigment from *Serratia marcescens*. *Natural Product Research*, 28(17), 1399-1404.
11. Sanchez, L. A., Hedstrom, M., Delgado, M. A. & Delgado, O. D. (2010). Production, purification and characterization of serraticin A, a novel cold-active antimicrobial produced by *Serratia proteamaculans* 136. *Journal of Applied Microbiology*, 109(3), 936-945.
12. Smaoui, S., Elleuch, L., Bejar, W., Karray-Rebai, I., Ayadi, I., Jaouadi, B., Mellouli, L. (2010). Inhibition of fungi and gram-negative bacteria by bacteriocin BacTN635 produced by *Lactobacillus plantarum* sp. TN635. *Appl Biochem Biotechnol*, 162(4), 1132-1146.
13. Wiegand, I., Hilpert, K. & Hancock, R. E. (2008). Agar and broth dilution methods to determine the minimal inhibitory concentration (MIC) of antimicrobial substances. *Nat Protoc*, 3(2), 163-175.