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RESEARCH ARTICLE

EXAMINATION OF ANTIMICROBIAL PEPTIDES FROM SERROGNATHUS TITANUS LARVAE.

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Abstract

This study was done to investigate the antimicrobial activity of stag beetle larvae extract induced by innate immune response by treating *Salmonella* strain. Agar diffusion assay showed no significant difference in antimicrobial activity compared to the control. However, when the activity was done by microtiter plate assay, the antimicrobial activity was increased compared to the control. The antimicrobial activity was measured according to the molecular weight to confirm that the activity was induced according to the molecular weight of the peptide. As a result, many antimicrobial substances against *S.* Gallinarum were found at below 3 kDa and above 30 kDa. Many antibacterial substances against S. Enteritidis were observed at below 3 kDa, 10-30 kDa and above 30 kDa. Therefore, it is suggested that stag beetle larvae induce an antimicrobial peptide, which is an innate immune response, against pathogenic microorganisms.

Introduction:-

Insect is known to be the largest and most abundant species, except for microbes on Earth, with more than a million reported species and more than one million species remaining unreported (Berenbaum and Eisner, 2008). Because insect is evolutionarily quite different from vertebrates, plants and microorganisms, scientists expect insect to evolve in the direction of creating compounds with unique structures that adapt to their particular environment (Ratcliffe et al., 2011). In addition, it has been deduced that if a physiologically active substance of an insect is well surveyed, a substance having a pharmacological activity that can be developed as a drug can be obtained (Dossey, 2010). Otherwise, insect has been widely used in many areas as folk remedies for treating or restoring diseases (Cherniack, 2010). However, since study on the search for new substances with pharmacological activity from insect has been limited in some Asian countries, more systematic studies are needed to discover bioactive compounds (Cherniack, 2010).

Stag beetles are collectively called beetles belonging to the family Coleoptera, Superfamily Scarabaeoidea, and family Lucanidae, and are popular as a pet and the most widely sold insect as a specimen. Stag beetles are distributed by almost the whole world. There are a variety of stag beetles in Korea including Lucanus, Dorcus, Serrognathus, Prosopocoilus, Prismognathus, Aegus, Platycerus, Figulus, and Nigidius.

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Currently, studies on stag beetle as a pet have progressed to a great extent, but little research has been done on the possibility as edible and medicinal. Therefore, we performed function analysis in this study for future use as food and medicine.

Materials and Methods:-

Experimental materials and Salmonella treatment

The samples of *Serrognathus titanus* larvae were purchased from Fabre company (Chungcheongnam, South Korea), which the stag beetle larvae (3 instar, female) were composed of 20 individuals. After the purchase, 10 individuals were grouped and acclimated for 1 week. CK111 (*S.* Typhimurium Δlon ΔcpxR), a *salmonella*-attenuated strain, was liquid-cultured, and 50 ml (1.5×10^8 CFU/ml) of the culture solution was mixed with 200 g of sawdust to rear the stag beetle larvae. In the control, PBS buffer solution was added instead of *Salmonella* culture solution. The second inoculation at two weeks after the first inoculation was performed with the same *Salmonella* strain, the samples were collected and analyzed at 24 hrs post the treatment.

Water extraction of stag beetle larvae

The collected larvae were homogenized by adding 9 times of distilled water to the weight of the larvae, and centrifuged at 3,000 x g for 10 min to collect the supernatant. The collected supernatant was filtered by Whatman No. 1 filter paper, and then filtered with a 0.2 μm syringe filter to completely remove the bacteria.

Antibacterial test

Minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) analysis were performed by DISC or punching method. *Salmonella* Typhimurium χ3339, *Salmonella* Enteritidis SN 107, and *Salmonella* Gallinarum HJL462 were used for antimicrobial activity analysis. These strains were pre-incubated by overnight at 37℃. Punching test was performed by adding appropriate amount of sample after punching with 7 mm cork borer. In addition, the microtiter plate assay was performed by adding appropriately diluted reaction solution to a microtiter plate and observing the reactivity during overnight incubation at 37℃.

Fractionation according to molecular weight

The filtered samples were separated by molecular weight using centricon (merckmillipore) at below 3 kDa, 3~10 kDa, 10~30 kDa, and above 30 kDa. The centricon loaded with sample was centrifuged at 3,500 x g for 20 min to collect the filtrate. The filter residue was collected by centrifugation at 1,000 x g for 2 min.

Purification by Sep-Pak R18 catridge

Samples separated by molecular weight were mixed with ice cold 0.1% trifluoroacetic acid (TFA) in equal amount. The mixed solution was centrifuged at 12,000 x g for 10 min at 4℃ to remove the precipitate. The collected supernatant was loaded into Sep-Pakⓡ plus C18 catridge (Waters). Two washes were done with a 10 bed volume of 0.05% TFA. Subsequently, the sample by each 20 ml of the solutions prepared by adding 10, 20, 30, 50, and 80% acetonitrile (ACN) in 0.05% TFA solution was sequentially eluted from a low concentration to a high concentration of ACN. The eluted solution was concentrated by decompressed condition, and concentrated by 10 folds to the used initial amount.

Results and Discussion:-

Changes in stag beetle by *Salmonella* vaccine treatment

The treatment of an attenuated *Salmonella* was done to identify whether production of AMPs was induced in the stag beetle larvae. As shown in Fig. 1, growth of the stag beetle larvae was significantly decreased when treated with live *Salmonella* strain.

The extract of the treated stag beetle larvae was analyzed for the antimicrobial activity enhancement by *Salmonella* treatment. As shown in Fig. 2, there were no significant differences in the agar plate by punching assay when compared to the control group according to *Salmonella* serotypes. However, as a result of microtiter plate assay, as shown in Fig. 3, the activity was enhanced in the group treated with live vaccine than the control group. Although a variety of antimicrobial peptides are known from invertebrates (Kim et al, 2017), no studies have been performed on AMPs from stag beetles yet. This study describes the first discoveries of AMPs derived from stag beetles.

Examination of antimicrobial activity by partial purification

In order to analyze the antimicrobial activity according to the molecular weight, the samples were fractionated by below 3, 3~10, 10~30, and above 30 kDa according to each molecular weight and then the activity was examined by microtiter plate assay. The activity against *S.* Gallinarum was detected at FT, WT, 20, 30, and 50% ACN at a fraction of below 3 kDa (Fig. 4). The activity was detected in FT, WT, and 20% ACN in the 3~10 kDa fraction. In the 10-30 kDa fraction, the activity was detected at 20, 30, and 50% ACN. The activity in above 30 kDa was detected in FT, WT, 10, 20, and 30% ACN.

On the other hand, the antimicrobial activity against *S.* Enteritidis was exhibited in Fig. 5. For *S.* Enteritidis, the activity was detected at FT, WT, 20, 30, and 50% ACN at a fraction of below 3 kDa. The activity was detected in FT, WT, and 20% ACN in the 3~10 kDa fraction. In the 10-30 kDa fraction, the activity was detected at 20, 30, 50, and 80% ACN. The activity in above 30 kDa was detected in FT, WT, 10, and 20% ACN.

Antimicrobial activities in various molecular weights are estimated to be a pattern similar to the study in the mealworm (our unpublished data). Since the antimicrobial activity against *S.* Gallinarum and *S.* Enteritidis was found by similar results according to the molecular weight, the antimicrobial activity is presumed to have essentially no difference in activity between the two serotypes. Therefore, it is assumed that further studies are needed for identification of characteristics in AMPs because of its various activities depending on the molecular weight and chemical properties.

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Fig 1:-Result of treatment with live-attenuated *S.* Typhimurium. Left panel; *Salmonella*-treated sample and right panel; PBS-treated control

Fig. 2. Antimicrobial activities of treatment with live-attenuated *S.* Typhimurium. (A) *Salmonella* Typhimurium χ3339, (B) *Salmonella* Enteritidis SN 107, and (C) *Salmonella* Gallinarum HJL462. Each susceptible bacterial strain was applied by 10^6 CFU/ml. The upper and lower laws in each panel mark control and vaccinated sample, respectively.

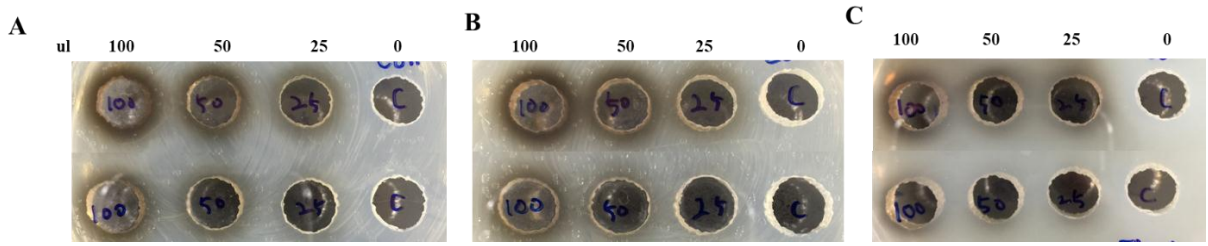


Fig 3:-Antimicrobial activities of treatment with live-attenuated *S.* Typhimurium. *Salmonella* Typhimurium χ3339 was added into solution by 10^6 CFU/ml, Left and right panels means control and vaccinated sample, respectively.

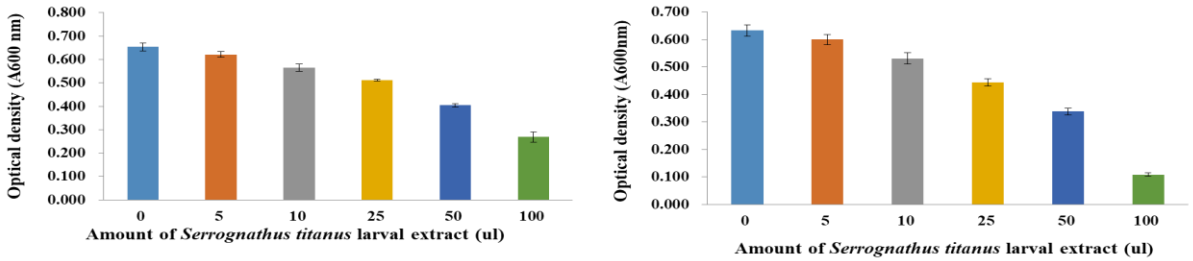


Fig 4:-Antimicrobial activities against *S.* Gallinarium depending on molecular weights for treated samples with live-attenuated *S.* Typhimurium. (A) below 3 kDa, (B) 3~10 kDa, (C) 10~30 kDa, and (D) above 30 kDa. *Salmonella* Gallinarum HJL462 was added into solution by 10^6 CFU/ml, 1. F.T; 2. W.T; 3. 10% ACN; 4. 20% ACN; 5. 30% ACN; 6. 50% ACN; 7. 80% ACN; Con, control.

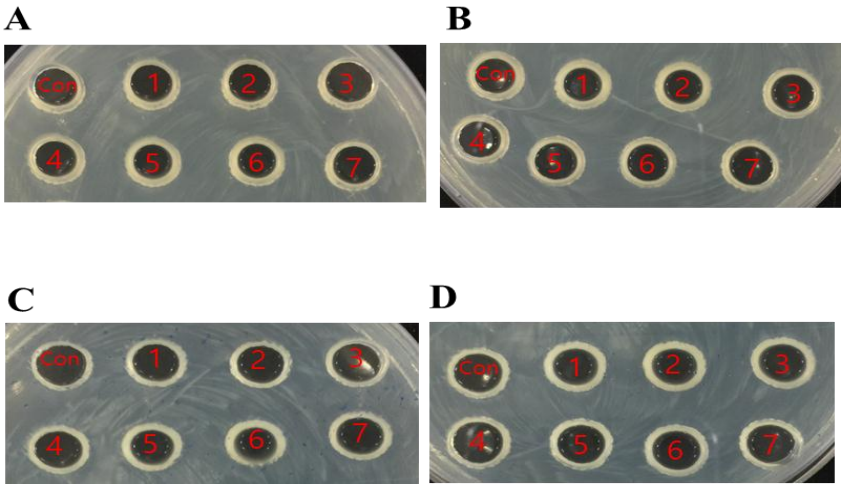
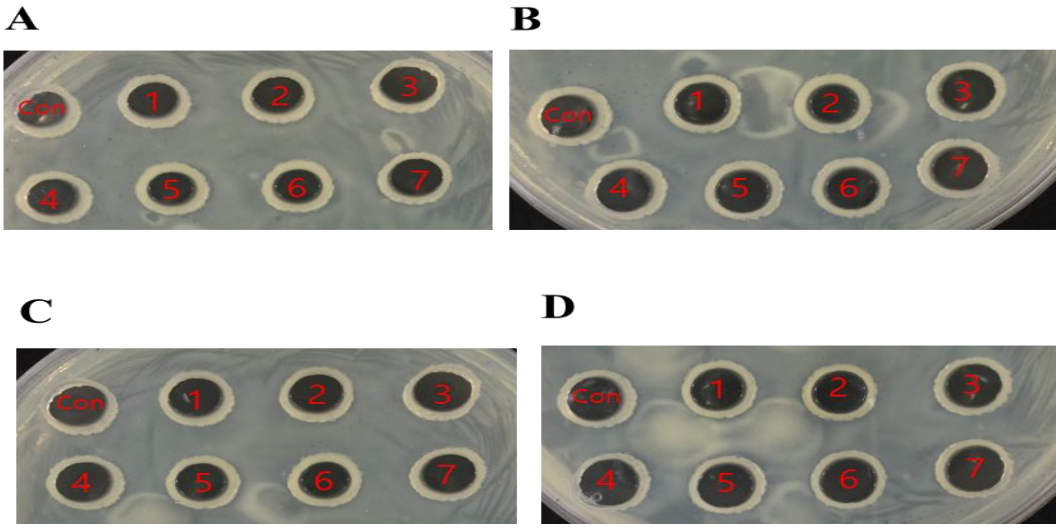


Fig 5:-Antimicrobial activities against *S.* Enteritidis depending on molecular weights for treated samples with live-attenuated *S.* Typhimurium. (A) below 3 kDa, (B) 3~10 kDa, (C) 10~30 kDa, and (D) above 30 kDa. Salmonella Enteritidis SN107 was added into solution by 10^6 CFU/ml, 1. F.T; 2. W.T; 3. 10% ACN; 4. 20% ACN; 5. 30% ACN; 6. 50% ACN; 7. 80% ACN; Con, control.



References:-

1. Berenbaum, M. R., and Eisner, T. 2008. Ecology. Bugs' bugs. Science 322, 52-53.

2. Dossey, A. T. 2010. Insects and their chemical weaponry: new potential for drug discovery. Nat Prod Rep 27, 1737-1757.
3. Cherniack, E. P. 2010. Bugs as drugs, Part 1: Insects: the "new" alternative medicine for the 21st century? Altern Med Rev 15, 124-135.
4. Kim, S. W., Bang, W. Y., Kim, T. W., Jeon, S. H., Noh, C. W., Lee, C. W., and Kim, I. S. 2017. Characteristics of antimicrobial peptides originated from invertebrates including insect. Int. J. Adv. Res. 5 (11), 284-289.
5. Ratcliffe, N. A., Mello, C. B., Garcia, E. S., Butt, T. M., and Azambuja, P. 2011. Insect natural products and processes: new treatments for human disease. Insect Biochem Mol Biol 41, 747-769.