

a wide variety of bacteria. Several bacterial species may survive unfavourable conditions or environmental changes after selecting mutations that improve their fitness in the new conditions. In order to understand present status of aqua-medicines use in fish disease management, under ICAR-Network project, survey was carried out at selected regions in Odisha, Andhra Pradesh, Jharkhand and Chhattisgarh. Detailed information on culture practices, farm inputs, measures taken by the farmer including application of drugs/ chemicals, formulations etc., were collected as per the format, from each farmer. The data were entered in data sheet, compiled and analysed. The paper presents the details of antimicrobial use in aquaculture and possible health hazard.

AH OR 08

Evaluation of antimicrobial potential of bioengineered peptides against fish pathogen

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Antibiotic resistance has been a great concern in recent years inviting attention of researchers from all over the world to discover/develop novel antimicrobial agents. Currently research is focussed on antimicrobial peptides (AMPs), as these molecules target multiple sites in the pathogen and kill them rapidly. So, the chances of developing resistance against AMPs by the microbes are very less. Naturally occurring AMPs are loaded with many advantageous features, however their application still face some challenges owing to their potential toxicity, susceptibility to proteases and extreme pH, lack of

sensitivity, incorrect folding in case of large AMP and associated high production costs. To overcome these problems, short peptides with antimicrobial activity can be engineered maintaining the typical features of AMPs but modified to increase stability and reduce toxicity. In the present study, two peptides of 7 and 14 amino acids length were designed using lysine (K), tryptophan (W) and isoleucine (I). The peptides were named as KK7 and KK14 (consists of two repeated sequence of KK7). Peptides were synthesized by solid phase peptide synthesis (SPPS) using standard 9-fluoroenylmethoxycarbonyl (Fmoc) chemistry. The synthesized peptides were purified by RP-HPLC. The peptides showed dominant helical structure in both secondary and three dimensional predictions by bioinformatics tools. Among these peptides, KK14 was found to inhibit the growth of ampicillin resistant fish bacteria, *Aeromonas hydrophilla*, which was not observed with same dosage of KK7. Lack of biological activity in KK7 may be due to decrease in hydrophobicity and net positive charge owing to its lesser number of residues. The peptide, KK14 showed promising antimicrobial activity against fish pathogen with low toxicity to the normal cells at its working concentration. Thus, KK14 has the potential to become a promising anti-infective agent and this designing strategy may be helpful in development of short, cost-effective peptide based anti-infectives.

AH OR 09

Studies on haemolytic activity of important fish and human pathogenic bacteria using fish blood

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Haemolysis is one of the striking features for the detection of the pathogenicity of the bacterial pathogens and has gained importance in the fish disease diagnostics. Haemolysis may be due to the presence of certain lytic enzymes which acts on the blood agar leading to lysis of red blood cells. In this study, fish blood was preferred instead of sheep, horse or rabbit to confirm and evaluate its performance as an alternate source as supplement for the preparation of blood agar for haemolysis test. Blood agar plates were prepared by supplementing 5% freshly collected fish blood (*Cyprinus rubrofasciatus*) to the basal medium and haemolytic activity against the fish and human pathogens was carried out. The reference strains used in this study were viz., *Edwardsiella tarda* (ATCC 15947), *Aeromonas hydrophila* (ATCC 35654), *Pseudomonas cloacae* (ATCC 13047), *Enterobacter aeruginosa* (ATCC 10145), *Escherichia coli* (ATCC 35218), *Staphylococcus aureus* (ATCC 43300), *Vibrio cholerae* (MTCC 3906) and *Salmonella paratyphi* (ATCC 15305). Overnight grown cultures (OD₆₀₀-0.5) were serially diluted and twenty-five microliter was spot inoculated onto freshly prepared fish blood (FB) agar plates in duplicates. Bacterial cultures showing haemolytic activity were observed after 48 h of incubation at 28°C and zone of haemolysis was measured. Results observed during this study were compared with haemolytic activity of same cultures on human blood (HB). Both human blood and fish blood showed similar β-haemolytic activity for all pathogens but *E. cloacae* did not show haemolysis on fish blood. The average diameter of haemolysis zone (mm) recorded in FB was 180, 280, 0, 220, 250, 140, 200, 220 and in HB is 130, 150, 140, 230, 170, 180, 150, 170 after 48 h

respectively for *E. tarda*, *A. hydrophila*, *E. cloacae*, *P. aeruginosa*, *E. coli*, *S. aureus*, *V. cholerae* and *S. paratyphi*. In conclusion, β-haemolytic activity observed better in FB plates when compared to HB plates and therefore, fish blood can be used as an alternative to sheep blood for haemolytic studies of fish pathogens.

AH OR 10

Abrogation of RLR downstream signalling by nervous necrosis virus (NNV) is mediated through Drp-1 dependent mitochondrial fission

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Mitochondria are highly dynamic sub-cellular organelles participating in signalling pathways involving innate antiviral mechanisms and cell death cascades. Member of the dynamin family of large GTPases, dynamin-related protein 1 (Drp1) dependent mitochondrial fission is an intricate process regulating both cellular and organ dynamics. Present study shows that NNV perturbs mitochondrial dynamics by promoting Drp-1 dependent mitochondrial fission, which attenuates MAVS mediated downstream signalling. NNV infected SISK cells revealed induction in Drp1 expression and manifested enhanced mitochondrial fragmentation at 24hpi and 48hpi. The mRNA transcript analysis revealed that MAVS expression was significantly up-regulated at 24hpi (p<0.05) and recovered to control level at 48h (p>0.05) *in vitro* post infection. The level of MAVS protein expression was up-regulated over a period of 24 hpi, but declined when NNV infection progressed at 48 and 72 hpi in SISK cells and was confirmed by western blot analysis.