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Studies on β-Hemolysin producing Aermonas hydrophilla MTCC 646 source Antibacterial activity

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Abstract

Aeromonas hydrophila MTCC646 was purchased from Institute of microbial Technology (IMTECH) Chandigarh, and it was investigated for the hemolytic, Proteolytic and antibacterial activities. The ability of the Aeromonas hydrophila MTCC646 to exhibit the above activities was confirmed by the results of blood agar plates, Muller Hinton Agar (MHA) plates seeded with the bacterial pathogens.

Keywords: Aeromonas hydrophila MTCC646, antibacterial activity, hemolysin.

Introduction

Aeromonas hydrophila MTCC 646 is a gram-negative, nonsporeforming, rod shaped bacteria in the family of Aeromonadaceae. *Aeromonas hydrophila* can survive in aerobic and anaerobic environments. It causes infections in marine, fresh water and ornamental fish[1,2]. Sea foods have been contaminated (37%) by *Aeromonas* from the west coastal region of southern India[3].

Aeromonas hydrophila was isolated from extra intestinal specimens from the coastal region of Southern Karnataka and it can cause serious infections among immune compromised as well as immune competent individuals[4]. *Aeromonas hydrophila* CRI14 produces siderophores. Although A.*hydrophila* CRI14 produced siderophores, production speed was not in accordance with its growth in fish sera. Simultaneously A. *hydrophila* protease compensates its adequacy of siderophore for iron[5].

Haemolysin, protease and extracellular polysaccharides (EPS) from pathogenic *Aeromonas hydrophila An4* shows antibacterial activity against some bacterial pathogens of marine ecosystem. Haemolysins are one the important bacterial virulence factors [6]. Extracellular polysaccharides also play a major role in virulence of pathogenic bacteria and it mediates the interaction between pathogenic bacteria and their environment through adhesion to the host[7].

Nile tilapia is a popular fresh water aquaculture species in the Philippines. The study focused on the bacteriological examination of Nile tilapia (*Oreochromis niloticus*) during disease outbreaks in various aquaculture farms shows ampicillin resistant *Aeromonas hydrophila.* Consistent isolation of A.*hydrophila* from various tissues of diseased Nile tilapia during disease outbreaks shows a recurring septicemia[8].

Aeromonas hydrophila has been isolated from wounds of five species of brackish water fish including species such as *Platosus anguillaries*, *Lates calcarifer*, *Epinephelus megachie*, *Labeo rohita*, and *Serotherodon nilotica*[9]. Aerolysin binds to specific glycoprotein receptors on the surface of eukaryotic cells before inserting into the lipid bilayer and forming holes. Such aerolysin and haemolysin genes were detected in A. *hydrophila* strains isolated from infected Koi carp (*Cyprinus carpio*)[10].

The expression of extracellular proteinases and haemolysins under altered atmospheres were studied well and optimal conditions for the expression were determined[11]. More widespread recognition of the prevalence and pathogenic significance of this species have stimulated a number of investigations of the ability of A. *hydrophila* to grown under modern commercial refrigerated storage conditions[12, 13].

Mass mortality in *Misgurnus anguillicaudatus* (Korean cyprinid loach) was caused by *Aeromonas hydrophila* . Additionally it was proved that isolated *Aeromonas hydrophila* containing the *tet*E gene (i.e., Tetracycline resistant gene) exists in Korean aquaculture system and has virulence[14]. In Kolkata the multi-drug resistant *Aeromonas hydrophila* was isolated from surface waters. The *Aeromonas hydrophila* has been examined for their enteropathogenic potential in the sealed-adult -Mouse model using live bacterial cells. Exposure of Mouse intestine to the isolates of *Aeromonas hydrophila* caused epithelial damage of villi associated with architectural distortion of polymorphonuclear cells extending through the mucus[15].

The activity of some β-lactum antibiotics upon 20 strains of *Aeromonas hydrophila* and some properties of their β-lactamases has been studied. The results show that resistance of

Aeromonas hydrophila to β-lactum antibiotics. The high degree of resistance to benzyl penicillin and lower resistance to ampicillin and cephaloridine was observed[16,17]. Aeromonas *hydrophila* was present in faces of buffaloes[18]. *Aeromonas* hydrophila was found to produce haemolytic and proteolytic exotoxin lethal to *Tilapia nilotica*, as well as heat stable unknown virulent factors that were responsible for 20% mortality. The lethality of ECP was decreased by heating and completely inactivated by boiling at 100^0C for 10 mins[19]. *Aeromonas hydrophila* was isolated from (Tamilnadu) stool samples of children with acute diarrhea shows multiple antibiotic resistance character.

Some strians of *Aeromonas hydrophila* were isolated from infected skin from common fresh water fish in Bangladesh and tested for enterotoxin production, hemolysin production and proved that strains have resistant to ampicillin[20]. *Aeromonas hydrophila* is also reported as to produce pathogenesis in Gold fish (*Carrassius auratus*) and Koi (*Cyprinus carpiokoi*)[21].

The formation of extracellular haemolysin by *Aeromonas hydrophila* in relation to protease and staphylolytic enzyme was well studied[22]. Isolation rates of *Aeromonas hydrophila* from stool samples of symptomatic and asymptomatic individuals were examined for several common enteric media. Sheep blood agar with 10µg of ampicillin per ml, preceded by overnight enrichment in alkaline peptone water, yielded 2.6 times the number of isolates as the other media examined for isolation of *Aeromonas hydrophila* from humans[23]. The cytopathogenic response to the α-hemolysin was reversible, whereas cells treated with small amounts of the β-hemolysin for only 1mimute invariably died within a few hours. Thus, the two hemolysins from *Aeromonas hydrophila,* despite dissimilarities in their interactions with cultured cells[24].

Material and Methods

Source of organism: *Aermonas hydrophila* MTCC 646 was purchased from Institute of Microbial Technology (IMTECH), Chandigarh.

Detection of Proteolytic activity: *Aeromonas hydrophila* MTCC646 spot was inoculated on casein agar plate and stab inoculated in 10% gelatin agar in test tube. The proteolytic activities were observed in the form of zone clearance and liquefaction after 48 hours of incubation.

Detection of Hemolytic activity: Hemolytic activity was isolated by streaking the *Aeromonas hydrophila* MTCC 646 on blood agar base plate supplemented with 7% human blood. The Blood should be aseptically transferred to the media. After sterilizing the media, the blood is added, in order to avoid denaturation.

Determination of Antimicrobial Activity: *Aeromonas hydrophila* MTCC 646 was cultured in 100ml Nutrient Broth (NB) medium at room temperature for 5 days in a rotary shaker. After five days the culture medium was centrifuged at 8000 rpm for 8 minutes and filtrate was screened for antimicrobial activity by agar- diffusion technique on Muller Hinton Agar media (MHA) that was previously seeded with test pathogens. The plates were incubated for 12hrs. Formation of any zone of inhibition was recorded[25]. The test pathogens as follows; *Klebsiella pneumonia*, *Bacillus subtilis*, *Streptococcus aureus*, *Salmonella typhi*, *staphylococcus aureus*, *Pseudomonas Species*

Results and Discussion

In the proteolytic activity *Aeromonas hydrophila* MTCC 646 was spot inoculated on casein agar media and stab was inoculated in 10% gelatin agar in test tube, does not from clearance of zone and liquefaction. This shows that *Aeromonas hydrophila* MTCC 646 does not produce protease extracellularly. Hemolytic proteins are commonly isolated from pathogenic bacteria and *β*-hemolysin is one of the important bacterial virulence factors. Hemolysin and its related proteins containing cystathionine *β* synthase (CBS) domains are bacterial toxins that function by assembling identical subunits into a membrane-spanning pore[26]. It has been suggested that proteolytic enzymes of fish pathogen, *Aeromonas hydrophila,* play an important role in causing massive tissue damage in the host which may facilitate establishment of infection. In Hemolytic activity *Aeromonas hydrophila* strain MTCC 646 interestingly demonstrated a very clear β-hemolysin in the form of clearance zone along the streak on blood agar plate within 24 hours of incubation at room temperature (figure-1). We have demonstrated the hemolytic activity of the *Aeromonas hydrophila* MTCC 646 by using blood agar supplemented with 7% human blood. In antimicrobial activity shows that, filtrate obtained from NB inoculated with *Aeromonas hydrophila* MTCC 646 has antimicrobial activity against some test organisms such as *Klebsiella pneumonia* , *Bacillus subtilis Streptococcus aureus*, *Salmonella typhi*, *staphylococcus aureus*, *Pseudomonas Species.* Attention has been given to the ptoduction of haemolysin by *Aeromonas hydrophila*, and it regarded as indication of pathogenic potential, though non-haemolytic aermonads have also been considered as human pathogen [27]. We have investigated that the *Aeromonas hydrophila* MTCC 646 for proteolytic activity. The results show that the strain *Aeromonas hydrophila* MTCC 646 does not exhibit proteolytic activity. In previous study[6] shows that *Aeromonas hydrophila* An4 have antibacterial activity against marine bacterial species.

Conclusion

The present study concluded that, the *Aeromonas hydrophila* MTCC 646 shows antibacterial activity against some bacterial pathogens. It suggested that further studies are required to determine the antimicrobial activity of *Aeromonas hydrophila* MTCC 646.

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