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Research Paper

SURVIVOR AS AN ALTERNATIVE TO ANTIBIOTICS TO CONTROL PATHOGENIC BACTERIA *IN VITRO*

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ABSTRACT:- The aim of the present study was to use Survivor (Microl Remedies product) as biocontrol measure against pathogenic bacteria sp. in shrimp culture. The test pathogenic bacteria used are *Bacillus cereus*, *Staphylococcus aureus* and *Flavobacterium columnare*. Three pathogenic solutions and replica plated on specific media and survivor at 0.05%, 0.1%, 0.2% and 0.3% was added to the pathogenic solution and plated on same and observed the viability of pathogenic bacteria. The *in vitro* study showed that the Survivor at dosages of 0.2% and 0.3% significantly ($p < 0.001$) reduced the viability of pathogenic bacteria and it could be used as probiotic against pathogenic bacteria sp. in Aqua culture industry.

Keywords:- Pathogenic bacteria, Probiotic, Minimal Inhibition Concentration

I. INTRODUCTION

Aquaculture is currently the fastest growing food production sector in the world, but diseases especially bacterial infections remain primary constraints to its continued expansion (Abd *et al*., 2009 and El-Haroun *et al*., 2006). Micro-organisms have been implicated in this problem and its control in aquaculture is a challenge (Ringo and Birkbeck 1999). Thus heavy reliance on vaccines and antibiotics to combat these diseases is inevitable and their use for decades.

Lactic acid bacteria have been used as probiotic against shrimp pathogens (Gatesoupe 1999; Skjermo and Vadstein 1999) and it was used as an alternative to antibiotics in disease control strategy (Fuller and Turvey 1971, Parker 1974, Roach S and Tannock 1980, Fuller 1978, Smoragiewicz *et al*., 1993). Bacillus constitutes a large part of the microflora of the gills, skin and intestinal tracts of shrimps (Sharmila *et al*., 1996), which has been used as probiotic against fish and shellfish pathogenic bacteria (Gatesoupe 1999, Rengipipat *et al*., 2000). Survivor is a 1-Monoglycerides from C3 to C12, with glycerol, as antibacterial and anti-mould substance. 1-monoglycerides have long been known for their antibacterial and antiviral effects in the human pharmaceutical and animal husbandry industries. As a sustainable substitution for the preventive use of antibiotics, these molecules are being evaluated in the fight against early mortality syndrome in shrimp. The 1-monoglycerides prevent the bacteria that cause EMS: 1-monoglycerides disturb in fact specific structures within the membranes of bacteria (G. Bergsson *et al*., 2001), and destabilize the fat-envelope of viruses (H. Thormar *et. al*. 1987). Thereby 1-monoglycerides inhibit multiplication of these pathogens. The molecules are effective during the entire gastrointestinal tract and are also taken up by the blood stream.

The purpose of this study was to test pathogenic bacteria with 1-monoglycerides and to evaluate importance of monoglycerides for controlling the pathogenic bacteria i*n* vitro.

II. MATERIAL AND METHODS

Bacterial strains: Three pathogenic bacterial strains were used for testing i.e., *Bacillus cereus, Staphylococcus aureus* and *Flavobacterium columnare.* These cultures were obtained from culture bank of Microl Remedies.
Survivor product: Three pathogenic bacteria were tested with Survivor (Microl Remedies product) manufactured by SILO, Italy. Survivor at 0.05%, 0.1%, 0.2% and 0.3% were tested against pathogenic bacteria.

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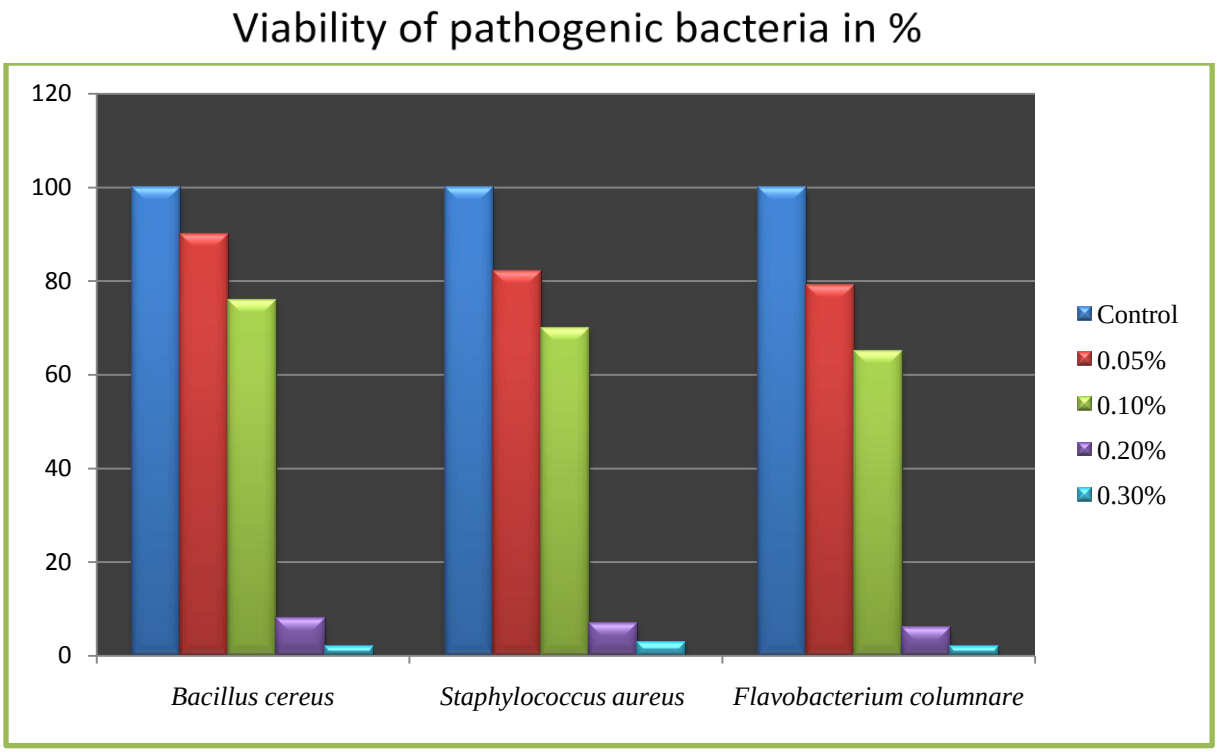
III. PROTOCOL

Pathogenic bacteria were grown in SCD (Soybean-Casein Digest Agar) medium for 24 Hrs and checked the microscopy for growth. Each bacterial broth was adjusted to 1 x 10^8 cfu per ml through Haemocytometer and added Survivor dosages i.e., 0.05%, 0.1%, 0.2% and 0.3% and mixed well. After 30 mins, 0.1 ml of sample was taken and analyzed in selective agar media before and after treatment with Survivor and documented the viability of pathogenic bacteria in percentage. Repeated the each dosage twice separately against every pathogenic bacteria.

IV. RESULTS

The survivor was tested for its antagonistic activity *in vitro* condition against pathogenic bacteria. The viability of testes pathogens after treatment with survivor results were recorded after 20 Hrs of incubation. The viability percentages of Survivor against test pathogen were calculated (Fig: 01). When pathogens treated with Survivor at 0.05% and 0.1% did not cause a significant decrease in the viability, where as the Survivor at 0.2 % and 0.3% significantly reduces viability of the pathogenic bacteria compared to the non treated control. *In vitro* studies concluded that Survivor was tested against pathogenic bacteria at different dosages and 0.2% and 0.3% showed the highest inhibition activity.

Fig 01: Study of the Survivor against Pathogenic bacteria viability after 30 Mins of treatment on SCD medium



The viability % was significantly ($p<0.001$) reduced when treated with the Survivor @ 0.2% and 0.3%

V. DISCUSSIONS

In vitro investigation clearly showed that the survivor showed the good inhibition capability against pathogenic bacteria. There are several *Bacillus sp*. (*B. acidophilus, B. subtilis, B. sulphureus, B. aerogens, B. radiatus, B. licheniformis*, etc) have been used commercially as a potential probiotic bacteria. According to survey made by Anikumari *et al*., (2003), the commercial probiotics were used in most of the shrimp farms and found that Vibrio proliferation was controlled by the use of probiotics. Authors have reported that a commercially prepared bacterial mixture of Bacillus sp. mixed into the rearing water increased survival and production. In 1972, Kabara et *al*., (1972) showed that monoglycerides of fatty acids are more effective in terms of Minimal Inhibition Concentration (MIC) values against different pathogens compared to the original organic acid. Batovska *et al*., (2009) concluded that 1-monoglycerides are more active than the fatty acids. The commercial level in vitro experiments will be an area for future research along with elucidation of the mechanism of inhibition between Survivor and pathogen.

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