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CHARACTERIZATION OF BACTERIOCIN PRODUCED BY *Bradyrhizobium japonicum* ISOLATED FROM MAIZE FIELD

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ABSTRACT

Bacteriocin producing *Bradyrhizobium japonicum* strain was isolated from maize field. Bacteriocin production by *Rhizobium* sp was carried out in modified TY medium and extracted using 0.22µm membrane filter. The bacteriocin has purified by ammonium sulphate precipitate and SDS-PAGE. Biochemically it was pure protein moiety and the molecular weight was 28kDa. It also showed broad range of antibacterial activity against some major pathogens. The study revealed the possibility of using bacteriocin as a biocontrol agents.

KEYWORDS: Bacteriocin, *Bradyrhizobium japonium*, SDS-PAGE, antagonistic activity, Well diffusion method.

1. INTRODUCTION

Bacteriocins are proteinaceous toxins secreted by Gram-positive and Gram-negative bacteria. They have a narrow inhibitory spectrum against bacteria that are closely related to the producing bacterium . Bacteriocins were first identified almost 100 years ago as a heat labile product present in cultures of *Escherichia coli V* and were toxic to *E.coli* S. These were given the name of colicin to identify the producing species.[1] Since then, bacteriocins have been found in all major lineages of bacteria and, more recently, have been described as universally produced by some members of the Archaea.[2,3]

Bacteriocins are usually ribosomally synthesized.[4,5,6] These polypeptides have attracted much attention due to their potential use as antibacterial agents for the treatment of infections, as well as preservation of food and animal feed. The bacteriocin family includes a diverse number of proteins in terms of size, microbial target, mode of action, release and immunity mechanisms and can be divided into two main groups: those produced by Gram-negative and those produced Gram-positive bacteria.[7]

The symbiosis between legumes and N_2-fixing bacteria (rhizobia) is of huge agronomic benefit, allowing many crops to be grown without nitrogenous fertilizers. It is a sophisticated example of coupled development between bacteria and higher plants.[8] The ability of soil bacteria to produce bacteriocins, defined as specific, non-self propagating inhibitory agents causing antagonism between closely related strains, and bacteriocinogenic activity has been described in almost all rhizobial species.[9] *Rhizobium* strains have been shown to produce bacteriocins that have been characterized as small, medium or large based on their assumed sizes and diffusion characteristics.[10,11]

2. MATERIALS AND METHODS

Bacteriocin production

Bacteriocin production by *BradyRhizobium japonicum* from rhizophere soil of maize plants was carried out in modified TY medium. *Rhizobium* spp was inoculated (10ml) into 100ml of the production medium and incubated in orbital shaker for 150rpm (37^0C) for 3 days. Cells were harvested by centrifugation at 15000 rpm for 15 mins at 4^0C and cell free supernatant was sterilized with 0.22µm filter membrane under sterile conditions and stored at 20^0C for further studies.[12]

Bacteriocin Protein Purification

Cell free supernatant (CFS) was used to carry out protein extractions, Twenty percent cholorform was added to the CFS in a separatory funnel. The aqueous phase formed was separated and used for precipitating out the proteins. Protein precipitation was carried out at 4^0C by the addition of analytical grade ammonium sulphate. The aqueous phase was saturated with cold ammonium sulphate 80% (w/v) saturations and gradually stirred with a glass stirrer for 10-15 min. The aqueous phase was kept overnight at 4^0C. The precipitate was collected by centrifugation at 11,000 rpm for 20 min. The solid pellet dissolved in distilled water and dialyzed against distilled water at room temperature for 24 hours . The suspension obtained

was designated as proteinaceous fraction or crude bacteriocin fraction and subjected to SDS PAGE to determine the molecular weight of the protein.[13]

Determination of molecular weight in SDS-PAGE

The molecular weight of the bacteriocin was determined by 10% sodium dodecyl sulfate polyacrylamide gel electrophoresis. After electrophoresis, the gel was stained with comassie Brilliant Blue R-250.[14]

Antimicrobial Screening by Well Diffusion Technique

Antimicrobial activity of bacteriocin against all pathogenic microorganisms was determined by well diffusion method under aerobic conditions. Agar plates were inoculated with 200µl of *E.coli, Klebsiella pneumoniae, Staphylococcus aureus* and *Bacillus subtilis* after growing them in a nutrient broth and diluting appropriately. The inhibitory activity against all pathogenic microorganisms was tested on Muller-Hinton agar. Wells (10mm) were cut in Muller-Hinton agar plate and 25 to 75 µl cell free culture supernatant (crude Bacteriocin) of the *Rhizobium* was added into each well. After incubation at 37^0C for 24h, the diameter (mm) of the inhibition zone around the well was measured.[15]

3. RESULTS AND DISCUSSION

The bacteriocin producing strain was isolated from the rhizosphere soil of maize plants and the strain was identified as *Bradyrhizobium japonicum* based on its physiological and biochemical characteristics. Production of bacteriocin are shown in fig 1. The purification of bacteriocin protein was carried out by saturating with 80% cold ammonium sulphate and dialyzed to obtain bacteriocin fraction are shown in fig 2.

Figure 1: Bacteriocin production

Figure 2: Purified bacteriocin

Molecular weight of the bacteriocin was determinized by SDS-PAGE gel electrophoresis (Fig 3). Single protein band was observed when stained with comassie blue and it clearly indicated the purity of the protein. The molecular weight of the purified bacteriocin was calculated to be about 28KDa.

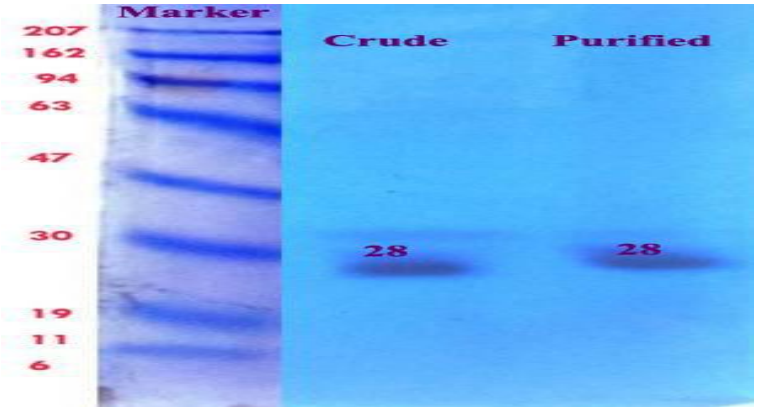

Figure 3: SDS – PAGE of bacteriocin

The susceptibilities of various Gram-positive and Gram-negative bacteria to growth inhibition by the supernatant of *BradyRhizobium japonicum* are presented in Table 1. It shows inhibitory activity against *E.coli, Klebsiella pneumeoniae, Staphylococus aureus and Bacillus subtilis*. Among these, maximum activity was observed against *Staphylococcus aureus* and *Bacillus subtilis* and minimum activity observed against *E.coli* [Fig.4,5,6 & 7].

Table 1: Antibacterial activity of bacteriocin against pathogens

Name of the pathogen	Zone of inhibition			Standard (methicillin)
	25 µl	50 µl	75 µl	
E.coli	11 mm	13 mm	19 mm	Nil
Klebsiella pneumoniae	12 mm	14 mm	16 mm	Nil
Staphylococcus aureus	12 mm	16 mm	18 mm	Nil
Bacillus subtilis	12 mm	16 mm	18 mm	Nil

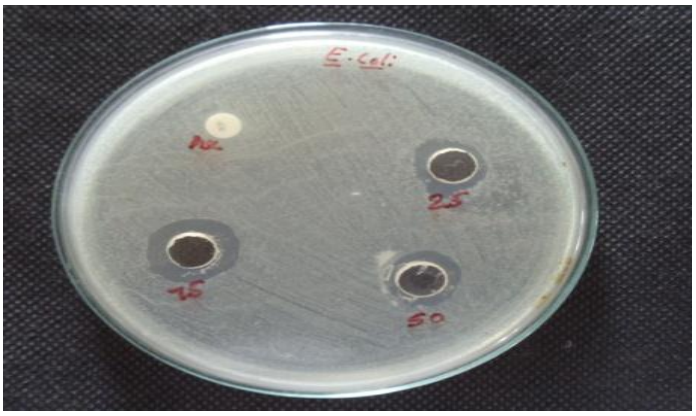

Figure 4: Bacteriocin Vs *E.coli*

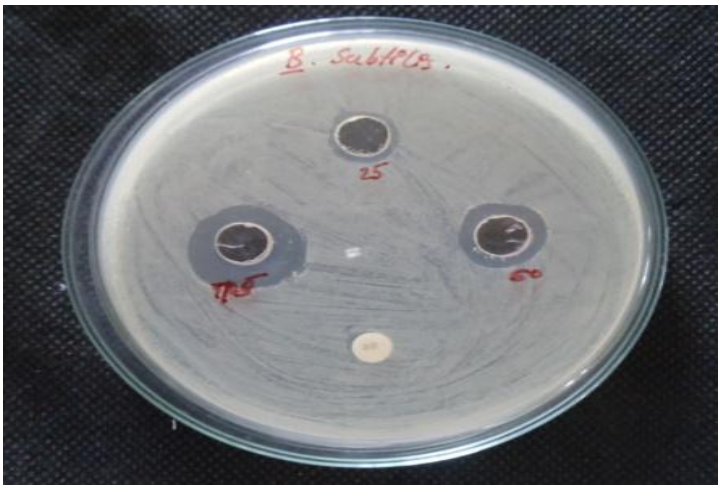

Figure 5: Bacteriocin Vs *Bacillus subtilis*

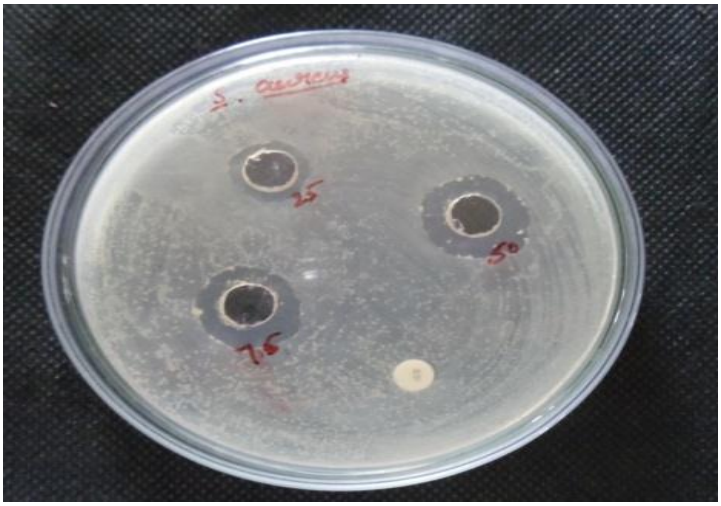

Figure 6: Bacteriocin Vs *Staphylococcus aureus*

Figure 7: Bacteriocin Vs *Klebsiella pneumoniae*

The present investigation highlights the isolation, characterization and activity of bacterocin produced by *Bradyrhizobium japonicum* from maize field. To state that the isolate *Bradyrhizobium japonicum* was tested for antibacterial activity against gram-positive and gram-negative bacteria such as *E.coli, Klebsiella pneumoniae, Staphylococcus aureus* and *Bacillus subtilis*. The inhibitory effect demonstrated bv *Bradyrhizobium japonicum* against these bacteria is an indication of possession of antibacterial activity. Bacteriocins have been reported to be inhibitory against several other bacteria.[16,17,18,19,20] Bacteriocin, production was strongly dependent on pH, nutrients source and temperature as claimed by Todorov and Dicks (2004). Bacteriocin production was influenced when incubated in different enzymes α-amylase, DNase, RNase and lipase resulted in greater bacteriocin production.[22]

Purified bacteriocin from *Bradyrhizobium japonicum* revealed homogeneity of a single protein band on 10% native PAGE. Its molecular weight was estimated as 28KDA by SDS-PAGE.

4. CONCLUSION

The bacteriocin produced by *Bradyrhizobium japonicum* was assayed by agar well diffusion method and the highest dilution gave a define zone of growth inhibition. Mode of action of bacteriocin produced by *Rhizobium* was tested and the behaviour of the bacteriocin produced by isolated strain was considered as bactericidal.

In conclusion therefore, the peculiar antimicrobial characteristics of *Bradyrhizobium japonicum* can positively have impact on their use as starter cultures for bioinoculants production, with a view to improving the growth and productivity of the various food crops.

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