Bactericidal activities in a new bacterial strain isolated from iron mine.

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Abstract

A new bacterial strain was isolated from an iron and potassium rich soil of Odisha and was found to grow in presence of various carbon sources. The bacterial strain was found to produce some extra cellular antibacterial agent that can kill other Gram positive and Gram negative bacteria. The cell free culture broth can distinctly showed the bacteriocidal effects and the Gram positive bacteria were found to be more susceptible than the Gram negative strains tested.

Key words: bactericidal activity, bacteriocin **,** *Klebsiella sp.*

Introduction

Antibiotics have been utilized in animal research almost from the time of their discovery and quickly found widespread use in the farm environment as therapeutic agents and as growth promotants. Solutions are urgently required for the growing number of infections caused by antibiotic-resistant bacteria. (Cotter *et al,* 2013). Moreover, since the rate of development of new antibiotics has severely declined, alternatives to antibiotics must be considered in both animal agriculture and human medicine (Allen *et al*, 2014)

There are certain types of antibacterial proteins produced by bacteria that can kill or inhibit the growth of other bacteria. Bacteriocins, antimicrobial peptides, and bacteriophage have attracted attention as potential substitutes for, or as additions to, currently used antimicrobial compounds (Joerger, 2003).

Bacteriocins, extracellular proteinaceous bactericidal substances that are produced by many species of bacteria, trigger the killing of strains or species closely related to their producers. Their narrow specificity of action and their proteinaceous nature distinguish them from other antibiotics (Daw and Falkiner, 1996). In contrast to the currently used antibiotics, bacteriocins are often considered more natural because they are thought to have been present in many of the foods eaten since ancient times (Cleveland *et al*., 2001)

Bacteriocins, in the present perspective, might warrant serious consideration as alternatives to traditional antibiotics. These molecules exhibit significant potency against other bacteria (including antibiotic-resistant strains), sufficient stability and can have narrow or broad activity spectra.(Joerger, 2003)

The present study deals with the production of extra cellular bactericidal agent (bacteriocin) by a newly

isolated bacterial strain and elucidation of its bactericidal effect on other bacterial strains.

Materials and methods

Collection of soil: Soil samples were collected from the top soil of Khonbond iron mine (at 21°57 min 18.02 sec North and 85°23 min 15.4 sec East) located at an altitude of 676 meter in Keonjhar district of Odisha, India and were taken in sterilized polyethylene bags using sterilized spatula and stored at 4ºC until examination.

Isolation of the bacterial strain: The bacterial strain was isolated by cultivating in basal medium (BM) composed of (g/L): peptone, 0.9; $(NH_4)_2HPO_4$, 0.4; KCl, 0.1; $MgSO_4 \cdot 7H_2O$, 0.1 (pH-8.0).and 0.1 glucose at for 24-36 hours.

Identification of the bacterial strain: The bacterial strain was characterized by routine biochemical tests. The strain was deposited to NCIM, Pune for identification through 16s rRNA.

Photomicrographic study: The crystal violet-saffranine stained bacterial cells were visualized under Axioscop-40 (Zeiss) microscope at 1009.For SEM, paraformaldehyde–glutaraldehyde fixed and totally dehydrated specimens were sputter coated with gold palladium under vacuum and observed and photographed in a scanning electron microscope (FEI Quanta-200 MK 2).

Testing of soil: The soil sample collected was tested with soil testing kit.

Other indicator organisms: Bacterial strains were collected from various sources, soil, septicemic neonates, milk samples and laboratory culture collections.

Determination of bactericidal activities: The isolated strain was grown in basal medium (pH-8.0) seeded with 5% inoculum at 34ºC for 24 h.The cells were removed from the growth medium by centrifugation (10,000×g for 10 min, 4ºC). The cell-free supernatant was aseptically soaked in sterile filter paper discs and was used as the source of antibacterial agent (Benkerroum et al, 1993). The indicator organisms were grown on a plate containing their respective growth medium on Petri dishes and the discs were seeded on the plate. Kirby-Bauer disc diffusion method (Collee *et al*, 1996) was followed to detect if the strain possessed any bactericidal activity.

All experiments were done in triplicate and their values were averaged.

Results and discussion

The soil from which the working strain was isolated was found to have very low nitrogen (50-99Kg/Acre), medium phosphorous (8-10 Kg/Acre) and very high potassium (>150 Kg/Acre) content. The soil had enormous amount of iron that imparted a typical red colour. The pH was found to be 6.5 and carbon content was very low. The soil showed almost no other micro organism other than the working strain.

The present strain PRBC 14 was a Gram negative, catalase negative non motile rod and under scanning electron microscope it was found to have a length of 3.5 µm each cell.(Fig 1) and was identified as *Klebsiella* sp.

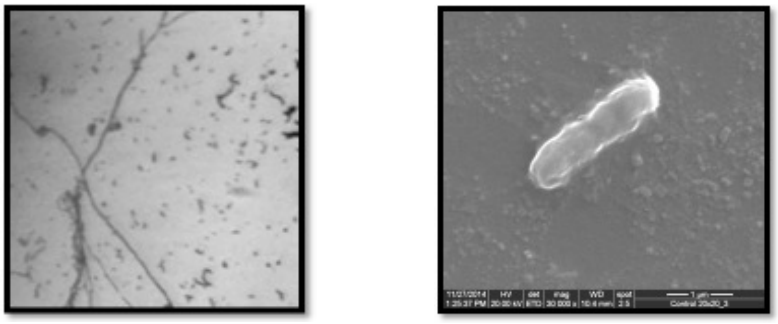

Fig 1. Morphology of *Klebsiells* sp PRBC 14 A. under compound microscope B under scanning electron microscope.

The strain was found to grow in presence of various carbon sources (Fig 2) and amazingly grew well in absence of any major carbon source also. Probably this ability enabled the strain to thrive in an environment with almost no vegetation and carbon residues.

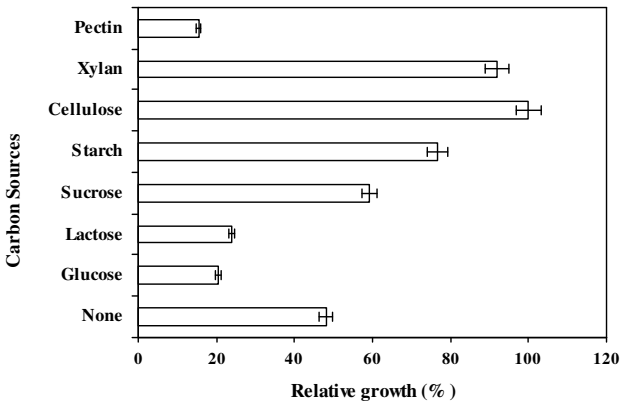

Fig 2. Relative growth of *Klebsiells* sp PRBC 14 in presence of various mono and polysaccharides

The strain was found to produce some active extra cellular agent, which could check the growth of a number of gram positive and gram negative bacteria (Fig 3). It was found that the bactericidal effect was more pronounced against Gram positive bacteria.

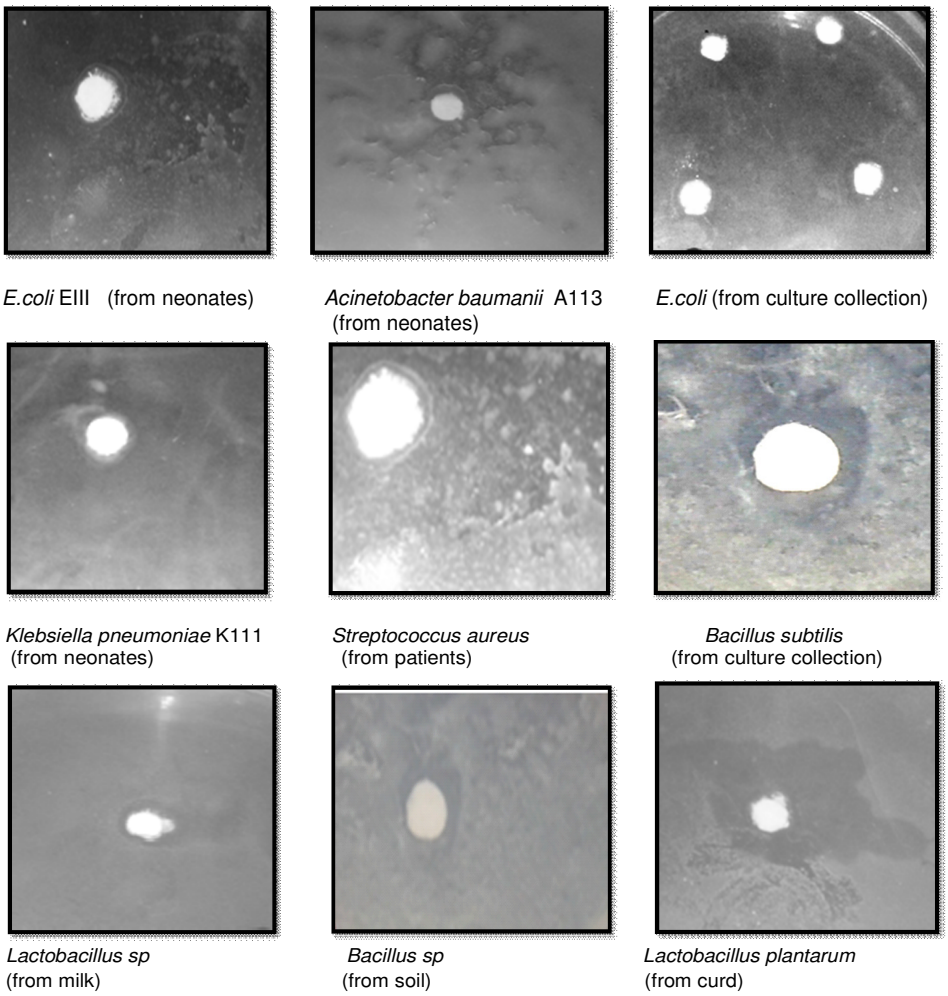

Fig 3. Zone of inhibition of bactericidal agents produced by *Klebsiella* sp PRBC 14 against indicator organisms mentioned in parentheses.

Conclusion

Bacteriocins, which are clearly distinguishable from clinical antibiotics, should be safely and effectively used to control the growth of target pathogens in foods. (Cleveland *et al*, 2001). The bactericidal compound produced by the present strain, after purification, might be utilized for food preservation.

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References

Allen, HK, Trachsel J, Looft T, and Casey TA (2014) Finding alternatives to antibiotics. Annals of the New York Academy of Sciences 1323 : 91–100

Benkerroum N, Ghouati Y, Sandine WE, Tantaoui-Elaraki A (1993) Methods to demonstrate the bactericidal activity of bacteriocins. Letters in Applied Microbiology,17(2):78-81

Collee J.G., Miles R.S., Watt B. Tests for identification of bacteria. In: Collee J.G., Fraser A.G., Marmion B.P., Simmons A. (eds): Mackie and McCartney Practical Medical Microbiology, 14th edition. Churchill Livingstone, New York, 1996, pp 131-49.

Cotter PD, Ross RP, Hill C. (2013) Bacteriocins - a viable alternative to antibiotics? Nat Rev Microbiol.11(2):95-105.

Daw, MA, and Falkiner FR (1996). Bacteriocins: nature, function and structure. Micron 27**:**467-479..

Jennifer Cleveland J, Montville TJ, Nes IF, Chikindas ML (2001) Bacteriocins: safe, natural antimicrobials for food preservation. International Journal of Food Microbiology 71: 1–20

Joerger, RD (2003). Alternatives to antibiotics: bacteriocins, antimicrobial peptides and bacteriophages. Poultry Science.; 82(4):640-7.