Screening of Bacterial Isolates for Bacteriocin Production from Different Bacterial Ecosystems

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Abstract—*There are variety of bacteria in our surrounding environment. Bacteriocins are small protein molecules secreted by some specific kind of bacteria to protect themselves from other types of bacteria. Commercially Bacteriocins are exploited as food bio preservatives as they are having bactericidal effects. The present study deals with primary screening of bacteriocinogenic activity of bacteria isolated from different bacterial ecosystems like Garden soil, Polluted river water, food and vegetable waste from home, hospital canteen and sewage. A total number of 106 isolates were checked for their bacteriocinogenic activity. Out of 106 bacterial isolates 28% (30 bacterial strains) showed positive bactericidal effect to their closely related bacterial species. The result suggests that we can go for more different bacterial habitat to isolate bacteria and check their bacteriocinigeny and able to identify the habitat in which large number of bacteria are able to produce bacteriocin.*

Keywords: *Bacteriocinogenic activity, Bacterial ecosystems, Bacteriocin.*

"1. Introduction"

Our environment is inhabited by various types of micro organism. Some are pathogenic and some are non pathogenic. Some are good for our health some causes toxicity. We need to find out those micro organisms as well as bacteria which are beneficial to us for our wellbeing. Bacteriocins are bacterial exotoxin produce by bacteria itself to kill simillar types of bacteria for their survival in unfavourable condition (1, 2, 3, 4). This is how bacteriocin can be used as therapeutic agent (5, 6, 7), bio preservatives (8) and dairy food preservative (9, 10) as they are having bactericidal activity. In our study we covered some bacterial ecosystems to screen bacteriocin producing isolates. This is the first step to find our bacteriocin from available bacteria in the surrounding environment.

."2. Material and Methods"

With the objectives of adopting standard methods, excessively 106 bacterial isolates were obtained from diverse bacterial ecosystem of Faridabad and New Delhi such as garden soil, polluted river water, food and vegetable waste for case studies as detailed below

2.1 From garden soil

Samples from garden soil having 1 gram of soil was mixed in de-ionized water and diluted till 10^{-10}. Pour plate culture were done in nutrient agar media with 10^{-9} and 10^{-10} dilution. Inoculated culture media were incubated overnight to get bacterial growth.

2.2 From polluted river water

Polluted Water samples were taken from different sites of Yamuna river and Hindon river. The Polluted water samples were processed by 10 fold dilution in sterile d/w and 100 micro litre of diluted samples were spread over nutrient agar and Mac Conkey agar plates followed by overnight incubation at 37^{o}C.

2.3 From food and vegetable waste

The raw food and vegetable samples were collected from home, hospital canteen, garbage and sewage in sterilized bottles and diluted 10 fold in sterile deionised water till 10^{-14}. Sample is collected in sterile bottle which is tenfold diluted in sterile water blanks further diluted to 10-14. Mac Conkey agar plates were spread with 0.1ml of samples obtained from the final dilution and kept in incubator at 37^{o}C for 24 hours for observing bacterial growth..

2.4 Identification of Bacterial isolates

The strains were identified by their cultural characteristics, microscopic examination after gram staining and different biochemical reactions. Bacterial genus were identified properly where as the species level characterization were done in very few isolates through this method (11).

2.5 Culture media

Muellar Hinton Agar Media were used for checking bacteriocinogenic activity. Nutrient Agar media were used as a general purpose media and Brain Heart Infusion agar media were used to grow fastidious organism and MacConkey agar plates were used to grow lactose fermenting bacteria. All those media are used to obtain growth of both producer and indicator strain. The Bacterial strains were stored in 20% glycerol stock at - 4^{o}C. The cultures were incubated in Nutrient broth media for 2 successive days to obtain its log phase for further use.

2.6 Growth parameters

For the growth of bacterial strains overnight incubation at 37^{o}C was monitored.On the other hand for the growth of fungal isolates media plates were kept at room temperature (25oC) for a week. Both the isolated strains and indicator cultures were sub cultured in liquid broth media.

2.7 Indicator bacteria/ fungal strains

American Type Culture Collection strains are used as indicator strains.

2.8 Primary screening for bacteriocinogenic effect

Soft agar overlay was used to observed the Bacteriocin or BLIS production by different strains.

2.8.1 Spot Agar Assay

In this method the isolates were spotted on BHI agar media and incubation done at 37^{o}C for 18 - 24 hours. The inhibition zone around the spot was observed and measured.

"3. Results and Discussion"

This study deals with the bacteria producing bactertiocin inhabiting different habitats. Different bacterial species were isolated from various habitats like garden soil, polluted water, food and vegetable waste and they were screened for their bacteriocin production. The bacteriocin producing strains were identified by their morphology , culture characteristics and biochemical reactions. In our primary screening a total of 106 bacterial strains were screened for their bacteriocinogenic activity by spot agar techniques among them 28% exhibited bacteriocinogenic activity where as 62% of bacterial isolates did not possesses any bacteriocin activity.

Gram positive bacteria such as Corynebacterium and Staphylococcal bacteriocin were isolated and purified genetically and enzymatically. On the other hand, there were several gram negative bacteria like Pseudomonas spp. and Escherichia coli known for their bacteriocin production capability. The study showed that most of the bacteria secreted at the minimum a single type of bacteriocin (12).

It has been reported that most of the bacteria which produces bacteriocins showed very narrow spectrum of activity against other similar bacterial species (13) .Very few researchers have claimed finding bacteria producing bacteriocineg. *L. monocytogenes*active against a broad spectrum of bacterial species (14).

A previous study showed that among 51 environmental bacterial isolates 41% showed bacteriocinogenic activity where as 59% did not possess any kind of inhibitory activity through spot agar and well diffusion methods (15).

There are few limitations in spot overlay techniques such as some bacteria that could produce acidity resulting of zone of inhibition of indicator bacteria. Bacteriocin or bacteriocin like inhibitiry substance sceretion are confrimed by agar well diffusion techniques that some researchers used that technique for the primary screening (16, 17).

"4. Conclusion"

The study concluded that obtaining bacteria from sources such as garden soil, polluted river water, vegetable and food waste source is not enough to find out bacteria producing bacteriocin with broad spectrum of antimicrobial activity. We should isolate bacteria from more habitats and check their bacteriocinogenic activity. Bacteriocin with broad spectrum antimicrobial activity can be used as alternative theraputic agent and can be also used in bio preservation.

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