INNSPUB

International Journal of Microbiology and Mycology | IJMM |
pISSN: 2309-4796
http://www.innspub.net
Vol. 18, No. 2, p. 1-7, 2024

Open Access RESEARCH PAPER

Bacteriocin and its effect against skin pathogens

Aparna Ravi*[1], M. Thankavel[2]

[1]*Department of Microbiology, Nehru Arts and Science College, Coimbatore, India*

[2]*Deen School of Life Sciences, Nehru Arts and Science College, Coimbatore, India*

Keywords: Bacteriocin, Inhibitory effect, Antagonistic activity, Skin pathogens, Kirby Bauer disc diffusion

Publication date: February 22, 2024

Abstract

This paper aims to examine the isolation of *Lactobacillus* from dairy products (milk, curd, and yogurt), extraction of Bacteriocin from it as well as to determine their inhibitory effect against few fungal skin pathogens and bacterial skin pathogens such as*: Candida albicans, Aspergillus* sp., *Malassezia* sp., *Fusarium* sp., and *Penicillium* sp., *Staphylococcus aureus, Streptococcus pyogens, Klebsiella* sp. The antagonistic activity of *Lactobacillus* sp. is mainly due to the bacteriocin present in it, therefore in this study, the bacteriocin is extracted and checked against the indicated microorganisms. Kirby Bauer Disc Diffussion method is used and zone of clearance is observed around the pathogenic species indicating that they shows some kind of antagonistic activities which are further measured and noted, which gives a clear picture about the degree of resistance of bacteriocin against the pathogenic microorganisms.

* **Corresponding Author:** Aparna Ravi ✉ aparnaravimicrophd20@gmail.com

Introduction

Skin is an important organ that represents the first line of defense against the external environment. Some microorganisms are pathogenic but some are usually present on the skin does not causing damages but during adverse condition like immunosuppressant phases the organisms develop infections in the host (Hall and Dorsch, 2002). Some of such cases like; Primary cutaneous aspergillosis usually involves site of injury, at or near intravenous catheter sites, occlusive dressings, burns, or surgery (Walsh and Groll, 1999). Secondary infections infect the underlying structures or from wide spread blood borne seeding of the skin. Superficial infections including keratitis, otomycosis are commonly caused by *Penicillium* sp., *Malassezia* sp. are another example of normal skin flora colonizes as commensals and during adverse conditions develops diseases at the head, neck dermatitis, and malassezia folliculitis (Gupta and Kohli, 2004).

Fusarium sp. Causes infections in patients in conditions like hematologic malignant or bone marrow transplant (Nucci and Anaissie, 2007). Candida nail infections occur in patients with chronic mucocutaneous candidiasis caused by *Candida albicans*, they invade the entire nail plates (Kirkpatrick, 2001). *Staphylococcus aureus* is a major cause of bacterial skin infections namely, abscesses in boils, furuncles, Cellulitis (Prendiville, 1989). *Streptococcus pyogens* are also bacterial species causing infections in the superficial keratin layer called impetigo, the superficial epidermis layer-erysipelas, the subcutaneous tissue layer-cellulitis, fascia called necrotizing fasciitis or in the muscle-myositis, myonecrosis (Stevens and Bryant, 2017). *Klebsiella* causes surgical wound infections, they usually enters through break in the skin and gradually leads to soft tissue infections (Paterson and Bonomo, 2005).

Balanced skin is crucial for maintaining healthy skin functioning; but changes in the skin microbes are associated with skin diseases such as those caused by *Candida albicans*, *Aspergillus*, *Malassezia furfur*, *Fusarium*, *Penicillium*, *Staphylococcus aureus*, *Streptococcus pyogens*, and *Klebsiella* sp. Lactobacilli are probiotic belonging to the group of lactic acid bacteria; they are Gram-positive, non-sporulating, anaerobic or facultative anaerobic rods. They are commonly present in dairy products, soil, lakes, and the intestinal tract of humans and animals. They possess antagonistic activity against various pathogenic microorganisms (Salminen *et al.*, 2004). In the present study, the Lactobacillus sp. from dairy products like milk, curd, and yogurt are isolated; then bacteriocin is extracted from it and checked their efficacy against fungal and bacterial pathogens using the technique of Kirby Bauer disc diffusion method (Aasen and Moretro, 2018). Bacteriocins are low molecular weight peptides secreted by the bacterial cells to kill sensitive cells present in the same ecosystem competing for food and other nutrients. Bacteriocins, along with their native antibacterial property, also exhibit additional antiviral and antifungal properties nowadays (Riley and Wertz, 2002). The dairy samples (milk, curd, yogurt) were collected in sterile containers from different places of Vandithavalam, Palakkad, Kerala. The samples were analyzed microbiologically, identified, and confirmed by biochemical tests. The study aimed to extract bacteriocin from *Lactobacillus* sp. and to see whether its effect against the mentioned microorganism.

Materials and methods

Collection of milk samples

Raw milk samples of cow and goat were collected in a sterile container in the month of February 2022 at various places of Vandithavalam, Palakkad, Kerala for the isolation *of Lactobacillus* species and examined under aseptic conditions.

Collection of curd samples

Homemade curd samples and commercially available curd samples of two different brands were collected for the isolation of *Lactobacillus* sp.

Collection of Yogurt samples

Yogurt samples of two different commercial brands were collected for the isolation of *Lactobacillus* sp.

Collection of microorganisms

Candida albicans

Swabs were taken from patients with toe nail and skin infections characterized by fluid discharge , Colour changes of the nail, Pain in the nail and toe.

Malassezia

Malassezia samples were collected by taking the Scrappings from various patients of head and neck dermatitis characterized by white patchy scales present in the affected areas with itching and dryness.

Aspergillus

Swabs were collected from patients with skin injury and burns.

Fusarium

Swabs were collected from skin lesions through aseptic techniques.

Penicillium

Swabs were taken from patients of skin lesions with swelling and redness in the skin with discolouration.

Staphylococcus aureus

Swabs were taken from the pimples of patients with granules and pus.

Streptococcus pyogens

Discharges from the wound is collected through sterile cotton swabs.

Klebsiella

Swabs were taken from patients with boils and wound with discoloration.

Isolation and characterization of Lactobacillus from dairy samples

1. The milk, curd and yogurt samples were serially diluted in various test tubes.
2. MRS (de Man, Rogosa, and Sharpe agar-selective media for LB) agar plates were prepared.
3. Swabs from various dilutions from each of the samples of milk, curd and yogurt were swabbed onto MRS agar plates using sterile techniques and control plates were maintained.
4. All the inoculated plates of three different samples of different dilutions were incubated at 37 degree Celsius for exactly 24 hours and then plates were observed for growth.
5. Colonies formed in the plates were subjected for Staining and biochemical tests for the confirmation of the species of microorganisms present.

Grams staining

A clean grease free glass slide was taken, washed thoroughly, surface sterilised using ethanol and smear of single colony has been made on the slides and allowed to dry, the smear is heat fixed by passing the slide above flame of Bunsen burner for 2-3 times. The smear was cooled, flooded with the primary stain -crystal violet, then waited for one minute was washed it under running tap water. Then added the Mordant- Gram's iodine, waited for one minute, decolorized it with 95% ethyl alcohol, washed under running tap water. Finally, added few drops of the counter stain-Safranin, waited for one minute and washed under running tap water, air dried and observed under oil immersion of 100X (Buchanan, 1982).

Catalase test

A clean glass slide was taken, washed and added a drop of hydrogen peroxide to the center of the slide, then fresh culture of test organism is mixed properly to it using a tooth pick or inoculation loop and observed for the presence of bubble formation (Schlegel, 1976).

Oxidase test

Oxidase disc was placed in a moisture free area and a small portion of test culture is spreaded on to it using a toothpick or inoculation loop, observed for the presence of colour change (Beveridge, 2001).

Indole test

Tryptophan broth was prepared, poured into test tubes, sterilized, cooled and inoculated with the test organisms, incubated at 37°C for 24 hours. After incubation few drops of Kovac's reagent as an indicator was added to the test tubes and observed the colour change (Buchanan, 1982).

Methyl red test

MR-VP broth was prepared, poured into sterile test tubes and sterilized. Broth was inoculated with the test organisms and incubated at 37°C for 24 hours. After incubation Methyl red reagent was added and observed the colour change (Beveridge, 2001).

Voges-Proskauer test

MR-VP broth was prepared and poured into sterile test tubes and sterilized, inoculated with the test organism and incubated at 37°C for 24 hours. After incubation few drops of Barrit's reagent A and Barrit's reagent B, is added, Shaked well and observed the colour change (Buchanan, 1982).

Citrate utilization test

Simmon citrate agar medium was prepared and sterilised, poured into test tubes and slants were prepared by keeping the test tubes by sliding above a glass rod. Then streaked with the test organism and incubated at 37°C for 24 hours (Schlegel, 1976).

Carbohydrate fermentation test

Glucose, Fructose, Sucrose, Xylose, Lactose fermentations were checked. The test was performed by using 1% sugar in MRS broth. Media was prepared and poured into test tubes and Durham's tube was inserted invertably into it. Test culture is inoculated and the tubes were incubated at 37°C for 24 hours. Phenol red was used as an indicator and observed the colour changes and formation of gas bubbles (Buchanan, 1982).

Extraction of Bacteriocin

Extraction of bacteriocins from *Lactobacillus* was done using chloroform extraction method (Beveridge, 2001).

Isolation of microorganism

Candida albicans

Sabouraud Dextrose Agar (SDA) media was prepared and the Samples collected from patients were swabbed onto it. Plates were incubated at 37°C for 2-5 days and growth was observed (Buchanan, 1982).

Malassezia

Dandruff like flaky scrapings from patients were introduced to Dixon agar and SDA agar plates and incubated at 37°C for 3-5 days and observed growth (Buchanan, 1982)..

Aspergillus, Penicillium and Fusarium

Swabs taken from patients were introduced to SDA plates and incubated at 35°C for 2-5 days (Buchanan, 1982).

Staphylococcus aureus

Swabs from patients were inoculated into HiCrome Staph Selective Agar plates and incubated at 37°C for 24 hours (Buchanan, 1982).

Streptococcus pyogens

Swabs from patients were inoculated into Nutrient agar plates, incubated at 37°C for 24 hours (Buchanan, 1982).

Klebsiella

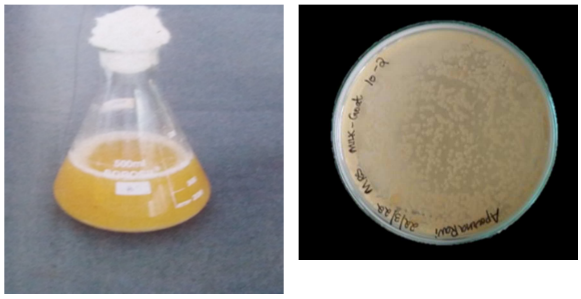
Swabs from patients were inoculated into MacConkey agar plates, incubated at 37°C for 24 hours (Buchanan, 1982).

Checking the antagonistic activity

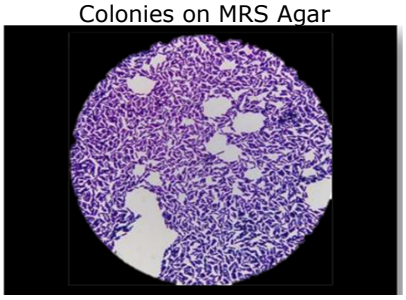
Antimicrobial activity was tested by disc diffusion method (Beveridge, 2001). *Candida albicans, Aspergillus, Malassezia, Fusarium* and *Penicillum, Staphylococcus aureus, Streptococcus pyogens and Klebsiella* samples were swabbed onto separate Muller Hinton Agar plates, sterile discs coated with bacteriocins were placed on it. The plates were incubated and observed the zone of inhibition around the discs. The diameter of zone was measured.

Results

In the MRS agar plates, small to medium white colonies were observed. Gram staining showed purple rods indicating Gram-positive rod shaped bacteria (Fig. 1, Table 1).



Colonies on MRS Agar



Microscopic observation of LB

Fig. 1. Colonies on MRS Agar and microscopic observation of LB

Table 1. Morphological characteristics of *Lactobacillus*

Characteristics	LB -1 observations	LB -2 Observations
Colony morphology on Nutrient agar	Creamy pale white colonies were seen	Small white raised colonies were seen
Colony morphology on MRS agar	Pale white coloured colonies were seen	Pale white coloured round colonies were seen
Gram staining	Purple coloured rods in chains were observed	Purple coloured rods in chains were observed

Table 2. Biochemical characteristics of *Lactobacillus*

Name of tests	Results (LB-1)	Results (LB-2)
Indole	Positive	Positive
Methyl red	Positive	Positive
Voges-proskauer	negative	negative
Citrate utilization	Positive	Positive
Oxidase	Negative	Negative
Catalase	Positive	Positive
Triple sugar iron agar	Negative	Negative
Carbohydrate utilisation	Glucose positive Lactose positive Sucrose positive	Glucose positive Lactose positive Sucrose positive

Table 3. Measurement of zone of inhibition against bacteriocin of *Lactobacillus*

Organisms	Diameter of zone (mm)
Candica albicans	14mm
Aspergillus sp.	15mm
Malassezia furfur	14.5mm
Fusarium sp.	13mm
Penicillium	12mm
Staphylococcus aureus	12.5mm
Streptococcus pyogens	13mm
Klebsiella	13mm

Biochemical tests showing: oxidase- positive, catalase- positive, Indole -positive, MR -positive, Citrate- positive, lactose fermentation- positive indicated that the isolated microorganism is confirmed as the (LAB) Lactic acid bacteria- *Lactobacillus* sp. (Table 2).

Bacitracin was extracted from the organism. *Candida albicans, Aspergillus, Malassezia, Fusarium* and *Penicillum, Staphylococcus aureus, Streptococcus pyogens, Klebsiella* swabbed plates were inoculated with the bacteriocin

coated discs, zone of clearance was observed. The diameter of zone was measured and tabulated (Fig. 2, Table 3).

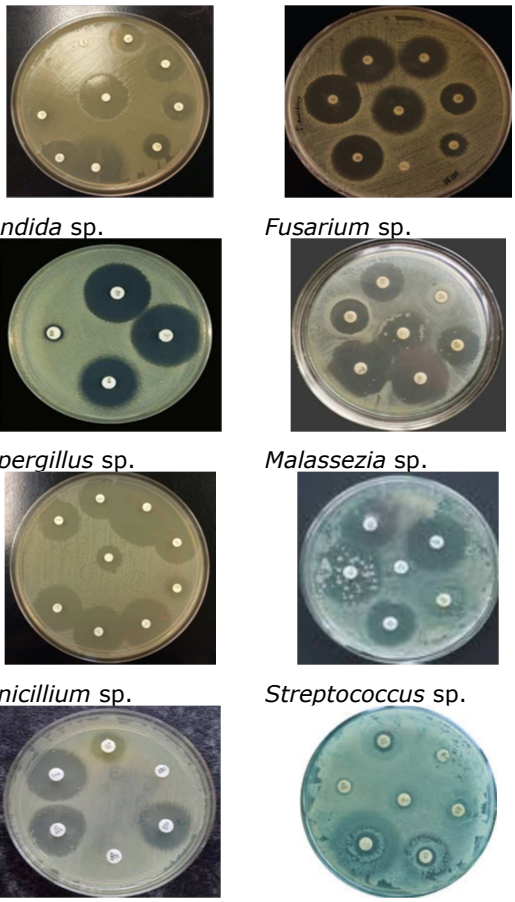


Fig. 2. Zone of inhibition against bacteriocin of *Lactobacillus*

Discussion

One of the major challenges that microbiologists are facing nowadays is the multidrug resistance shown by various pathogenic species (Ventola, 2015). If the compound from a microorganism itself is efficient to control the growth and adverse effects of pathogens, it will be a revolutionary moment (Fernández, 2019). The use of probiotics is one example of such an application. Probiotics have been considered effective in maintaining gut health for many years, and now they are being explored for more possible applications (Hill *et al.*, 2014). The probiotic Lactic acid bacteria, *Lactobacillus*, is isolated, and its virulent compound, Bacteriocin, is extracted, purified, and checked. It is confirmed that it has the ability to kill or suppress the growth of various fungi and bacteria (Cotter *et al.*, 2005). Antagonistic activity of Bacteriocin of Lactobacillus sp isolated from dairy samples was subjected to the study and observations against fungal and bacterial pathogens, showing good degrees of inhibition (Mokoena, 2017). Considering this as a reference, recent studies are ongoing, expecting the formulation of new therapeutics from bacteriocins, which could be a broad-spectrum drug and a solution for the challenge of multidrug resistance shown by various pathogens (Perez *et al.*, 2014).

Conclusion

A total of two raw milk samples from cow and two from goat, two curd samples (homemade, commercially available), two yogurt samples were collected, Morphological, physiological, microscopic observation, biochemical characterisations were made and confirmed as *Lactobacillus* species. The antagonistic activity of bacteriocin is checked. They are found to be sensitive against human *pathogen Candida albicans, Aspergillus* sp., *Malassezia* sp., *Fusarium* sp., *Penicillium* sp., *Staphylococcus aureus, Streptococcus pyogens, Klebsiella* sp. causing various skin infections in human beings. This has an important role in medical microbiology and human health.

References

Aasen IM, Moretro T. 2018. Extraction and Purification of Bacteriocins from Lactic Acid Bacteria. In Bacteriocins: Production, Applications and Safety (pp. 73-91). Springer.

Beveridge TJ. 2001. Use of the Gram stain in microbiology. Biotechnic & Histochemistry **76**(3), 111-118.

Buchanan RE. 1982. Microbial staining methods. ASM Press.

Cotter PD, Hill C, Ross RP. 2005. Bacteriocins: Developing innate immunity for food. Nature Reviews Microbiology **3**(10), 777-88. DOI: 10.1038/nrmicro1273.

Fernández L. 2019. Fighting Fire with Fire: Exploiting Bacterial Antagonism to Combat Drug Resistance. Trends in Microbiology **27**(4), 168-174.

Gupta AK, Kohli Y. 2004. Prevalence of Malassezia species on various body sites in clinically healthy subjects representing different age groups. Med Mycol. **42**(1), 35-42. DOI: 10.1080/13693780310001610056.

Hall GS, Dorsch MM. 2002. Skin and soft tissue infections. In Principles and Practice of Infectious Diseases (6th ed., Vol. 1, pp. 309-331). Elsevier.

Hill C, Guarner F, Reid G, Gibson GR, Merenstein DJ, Pot B, Sanders ME. 2014. Expert consensus document: The International Scientific Association for Probiotics and Prebiotics consensus statement on the scope and appropriate use of the term probiotic. Nature Reviews Gastroenterology & Hepatology **11**(8), 506-514. DOI: 10.1038/nrgastro.2014.66.

Kirkpatrick CH. 2001. Chronic mucocutaneous candidiasis. Pediatr Infect Dis J. **20**(2), 197-206. DOI: 10.1097/00006454-200102000-00017.

Mokoena MP. 2017. Lactic acid bacteria and their bacteriocins: classification, biosynthesis and applications against uropathogens: a mini-review. Molecules **22**(8), 1255.

Nucci M, Anaissie E. 2007. *Fusarium* infections in immunocompromised patients. Clin Microbiol Rev. **20**(4), 695-704. DOI: 10.1128/CMR.00014-07.

Paterson DL, Bonomo RA. 2005. Extended-spectrum beta-lactamases: a clinical update. Clin Microbiol Rev. **18**(4), 657-86. DOI: 10.1128/CMR.18.4.657-686.2005.

Perez RH, Zendo T, Sonomoto K. 2014. Novel bacteriocins from lactic acid bacteria (LAB): various structures and applications. Microbial Cell Factories **13**(Suppl 1), S3. DOI: 10.1186/1475-2859-13-S1-S3

Prendiville JS, Hebert AA, Greenwald MJ, Esterly NB. 1989. Management of Stevens-Johnson syndrome and toxic epidermal necrolysis in children. J. Pediatr. **115**(6), 881-7. DOI: 10.1016/s0022-3476(89)80736-x.

Riley MA, Wertz JE. 2002. Bacteriocins: Evolution, Ecology, and Application. Annual Review of Microbiology **56**, 117-137. DOI: 10.1146/annurev.micro.56.012302.161024.

Salminen S, von Wright A, Ouwehand A. 2004. Lactic Acid Bacteria: Microbiological and Functional Aspects (4th ed.). CRC Press. https://doi.org/10.1201/b11503

Schlegel HG. 1976. General Microbiology. Cambridge University Press.

Stevens DL, Bryant AE. 2017. Necrotizing Soft-Tissue Infections. N. Engl. J. Med. **377**(23), 2253-2265. DOI: 10.1056/NEJMra1600673.

Ventola CL. 2015. The antibiotic resistance crisis: part 1: causes and threats. P&T: A Peer-Reviewed Journal for Formulary Management **40**(4), 277-283.

Walsh TJ, Groll AH. 1999. Emerging fungal pathogens: evolving challenges to immunocompromised patients for the twenty-first century. Transpl Infect Dis. **1**(4), 247-61. DOI: 10.1034/j.1399-3062.1999.010404.x.