International Journal of Plant & Soil Science

Volume 35, Issue 19, Page 1803-1811, 2023; Article no.IJPSS.106423
ISSN: 2320-7035

Exploring Lactic Acid Bacterial Metabolites for Antimicrobial Activity Against Spoilage Organisms of Grapes and Potato

P Christina Agnelo [a++*], R. Subhashini [b#], K. Kumutha [a^], A. Subbiah [c†] and M Ilamaran [d‡]

[a] Department of Agricultural Microbiology, Agricultural College and Research Institute, Madurai, TNAU, Tamil Nadu, India.
[b] Department of Agricultural Microbiology, Agricultural College and Research Institute, Coimbatore, TNAU, Tamil Nadu, India.
[c] Grapes Research Station, Anaimalayanpatty, Rayappanpatty post, Theni District, Tamil Nadu, India.
[d] Department of Food Science and Nutrition, Community Science College and Research Institute, Madurai, Tamil Nadu, India.

Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/IJPSS/2023/v35i193731

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://www.sdiarticle5.com/review-history/106423

Original Research Article

Received: 02/07/2023
Accepted: 07/09/2023
Published: 09/09/2023

[++] PG Scholar;
[#] Professor;
[†] Associate Professor (Horti.) and Head;
[‡] Associate Professor (Food science and Nutrition);
[^] Professor and Head,;
**Corresponding author: E-mail: christinaagnelo@gmail.com;*

ABSTRACT

Biopreservation of foods is an alternative and novel method of preservation with increased special interest from consumers.Lactic acid bacteria (LAB) has the potential to restrict the microbial proliferation of foods, thus preventing spoilage and extending their shelf life. The antimicrobial capabilities of lactic acid bacteria are linked to various actions by the production of antimicrobial compounds such as bacteriocins, hydrogen peroxide, organic acids, etc. The study aims to explore LAB from traditional foods for the biopreservation of grapes and potatoes.LAB were isolated from twelve different food samples *viz.,* mango pickle, curd, cumbu gruel, neera, cold rice, finger millet, infant feces, milk, lime pickle, fermented cumbu (grain), fermented cumbuflour and whey water and their cell free metabolites were extracted. A total of thirteen bacteria and seven fungal cultures were isolated from spoiled grapes and potato. An *in vitro* experiment was conducted to study the antimicrobial activity of LAB metabolites by performing an agar well diffusion assay against the spoilage cultures. The results revealed varied antimicrobial activity of LAB isolates to different spoilage cultures from potato and grapes. Among the LAB isolates, metabolites from curd, cumbu gruel, fermented cumbuflour, finger millet and neera cultures were observed to inhibit more number of spoilage organisms and produced maximum inhibition zone. A consortium was prepared by pooling the metabolites from these promising cultures and assayed for antimicrobial activity which confirmed the above results.

Keywords: Lactic acid bacteria; metabolite; spoilage organisms; consortium.

1. INTRODUCTION

Biopreservation,a new and innovative approach to control food spoilage is growing significance for several industries and consumers. The use of coatings on fruits and vegetables for extending the shelf life is a practice followed for decades. Edible coatings in foods include organic or inorganic compounds, proteins, lipids, waxes, and resins either alone or in combination [1]. Biopreservation could be highly helpful in extending the shelf life of fresh-cut fruits and vegetables by the use of safe, natural or controlled microflora and biologically active compounds of non-toxic nature with food safety.

Lactic acid bacteria constitute a ubiquitous bacterial group that is widespread in nature in niches of dairy (fermented), meat and vegetable origin, gastrointestinal and urogenital tracts of humans and animals and soil and water. LAB are Gram-positive, non-respiring and non-motile microorganisms with an optimumpH range between 5.5 and 5.8 that help in extending the shelf life of products. LABmainly produce lactic acid as a by-product during metabolic activities. They play a multifaceted role in agriculture, food and medical fields.

LAB is referred to as probiotics due to their capacity to resist in the gastrointestinal tract or to produce exopolysaccharides, biological preservatives and bacteriocins. These antimicrobial properties can extend the shelf life of fruits and vegetables [2]. The antimicrobial capabilities of LABs are linked to various actions by the production of antimicrobial compounds such as bacteriocins, hydrogen peroxide, organic acids etc*.,* [3].

Grapes (*Vitis vinifera L*.) are one ofthe most widely consumed fruits in the world. They are highly perishable. Fungal contamination is one of the main causes of economic losses worldwide in the food industry and agriculture [4]. Post-harvest decay of grapes is due to grey mold *(Botrytis cinerea*), sour rot (*Aspergillus carbonarius*), *Rhizopus* rot (*Rhizopus stolonifer*) and blue mold (*Penicillium expansum*).

Potato (*Solanum tuberosum* L.) is the world's number one non-grain food commoditypredominantlycultivated in Asia and Europe. Difficulties in growing this crop commercially can be partially attributed to its susceptibility to disease, of which late blight caused by the oomycete *Phytophthora infestans* is considered the most important.

There are several drawbacks in using chemical pesticides, particularly for vegetable and fruit production which include fear of general public in using hazardous chemicals, risks of toxic residues in treated products as well as contamination of soil and water. LAB is used as a natural food grade biopreservative against abroad spectrum of undesirable microorganisms.

This indicates the potentiality of these bacteria in restricting microbial proliferation of foods, thus preventing spoilage and extending their shelf life. Hence the objective of my study is to identify the potential LAB cultures metabolites that hinder the growth of spoilage organisms.

2. MATERIALS AND METHODS

2.1 Isolation of Lactic Acid Bacteria

Lactic acid bacteria were isolated from mango pickle, cumbu, Neera, cold rice, finger millet, infant faeces, milk, lime pickle and whey water collected from the hostel. Cumbu grains and cumbu flour were soaked in sterile water. Both these samples were allowed to ferment naturally for a day. From the fermented water, 1ml was taken and serially diluted in peptone water blanks and plated using spread plate technique in de Man Rogosa and Sharpe (MRS) agar medium and incubated for 48 to 72 hours.Morphologically different colonies were picked and inoculated in MRS agar to obtain pure cultures.

2.2 Morphological Characters of LAB Cultures

Lactic acid bacterial cultures were morphologically examined forshape, arrangement, surface, margin and elevation. Gram reaction test was conducted for all the bacterial isolates.

2.3 Isolation of Spoilage Organisms

Spoiled samples of grapes and potato were collected from Vegetable and Fruit Market at Matuthavani Madurai, from retail outlets in the locality of Madurai and also from Students Amenity Centre, Agricultural College and Research Institute, Madurai, India. These spoiled samples of potato and grapes were taken as sources for isolating bacterial and fungal cultures. One gram of each sample was serially diluted upto 10^{-5} dilution in peptone water blanks. From 10^{-5} dilution and 10^{-3} dilution, 0.5ml was taken and plated in petriplates containing nutrient agar medium and Rose Bengal agar (RBA) medium respectively for isolating spoilage bacteria and fungi. Morphologically distinct cultures were selected and purified. Both bacterial and fungal spoilage organisms were morphologically characterized.

2.4 Extraction of Cell Free Extracts of LAB

A total of 50 ml MRSroth was inoculated with LAB cultures in 1/100 proportion (v/v) and incubated at 37°C for 72 h. After incubation, broth was centrifuged at 4000 rpm for 15 min, and the supernatant with the secreted metabolites of lactic acid bacteria was separated.

2.5 *In vitro* – Agar Well Diffusion assay

Agar well diffusion method was conducted to test the antimicrobial activity of LAB metabolites against the isolated bacterial and fungal spoilage organisms. 1 ml of the test bacterial and fungal isolates was seeded in Nutrient Agar (NA) and Potato dextrose agar (PDA) medium, plated and allowed to solidify. Using sterile cork borer, wells were made onto the seeded agar plate and 50µl of cell free supernatant was added into the wells. The plates were incubated at 37°C for 48 h and observed for the formation of inhibition zone [5].

2.6 Statistical Analysis

The data collected from the experiments was statistically analyzed using the R software package. At 5% probability level, the critical difference was identified and tabulated (Panse and Sukhatme, 1967).

3. RESULTS AND DISCUSSION

1. Isolation and Morphological characterization of lactic acid bacteria

Lactic acid bacteria were isolated in MRS medium from twelve different sources and they were purified in slant cultures (Fig. 1). Colonies of lactic acid bacteria appeared colorless, raised and with entire margin.Isolates of mango pickle, curd, cumbu, neera, cold rice, finger millet, infant feaces, milk, lime pickle, fermented cumbuflour, fermented cumbu grains and whey water were slimy. Colonies were circular shaped. They stained purple during Gram reacting, confirming their Gram positivebehaviour. Morphological parameters are given in Table 1. Chun-lei et al. [6] had isolated eleven lactic acid bacterial cultures from Inner Mongolian traditional yoghurt which were observed to be Gram positive and their colonies were smooth and sticky. Bogdan et al*.,* [7] isolated five LAB cultures from Kombucha, all the cultures were recorded as Gram positive, with Colorless punctiform colonies and they were

cocci and rod shaped. Ismail et al. [8] isolated and characterized two lactic acid bacteria *viz.*, SK-1 and SK-4 from local cow's milk kefir. Morphology of all the cultures were recorded as coccal shaped, with smooth edges and convex elevation. All the isolates were reported to be Gram positive. Bogdan et al. [7] isolated five LAB cultures from Kombucha, all the cultures were recorded as Gram positive, with Colorless punctiform colonies and they were cocci and rod shaped. Padmavathi et al. [9] isolated 16 LAB cultures from milk, curd and bovine colostrum and their morphological characters were observed. The cells were recorded as coccal to oval or rods, colonies were raised, smooth, margins were entire, with creamy to translucent texture. All the cultures were recorded to be Gram positive.

2. Isolation of Spoilage organisms from grapes and potato

In this study, ten bacterial and ten fungal cultures were isolated from different spoiled potato samples and three bacterial and 7 fungal cultures were isolated from spoiled grapes samples. Colony morphology of the bacterial and fungal spoilage organisms are given in Tables 2 and 3. All the bacterial cultures had translucent colonies withslimy texture. Some cells were cocci shaped whereas some were rods. The cells stained red in gram staining indicating that they are all gram negative in nature. Frances et al. [10] isolated seven spoilage bacterial organisms in grapes which were circular in shape, entire in margin and smooth surfaced. Most of the cultures were found to be gram positive and some where gram negative. Agar well diffusion assay of spoilage organisms with lactic acid bacteria metabolites

3.1 *In vitro* Screening of Cell Free Extracts of LAB for Antibacterial Activity

The present study aimed to investigate the antibacterial activity of lactic acid bacterial metabolites against thirteen spoilage bacteria isolated from grapes and potato by conducting agar well diffusion assay. The results revealed antibacterial activity against four spoilage bacteria *viz.,*POT1B1, POT2B1, POT3B1 and POT8B1 by inhibiting the growth of bacteria with a maximum inhibition zone of 2.8cm diameter (Fig. 2, Table 4**)**. The LAB metabolites of curd, neera, fermented cumbuflour and whey water were found to inhibit bacterial spoilage, of which, neera produced inhibition zone in three bacterial spoilage organisms. This suggested that the lactic acid bacterial metabolites have antibacterial compounds. Research conducted by Jimenezet al., (2017) on safety traits of major LAB species on *Tocosh*(Peruvian traditional fermented potatoes) showed antibacterial activities as well as biogenic amines production capacity. Steglińskaet al. [11] analyzed the metabolic profile of LAB strains isolated from pickled vegetables and milk samples and found that it mainly contained lactic acid, acetic acid, propionic acid and ethanol. They had also registered the strain *Lactiplantibacillus plantarum* KB2 LAB 03 to be highly effective in inhibiting potato phytopathogens namely *Alternaria alternata, Alternaria tenuissima, Fusarium sambucinum, Rhizoctonia solani, Colletotrichum coccodes, Phomaexigua, Streptomyces scabies, Fusarium oxysporum, Alternaria solani* and *Pectobacterium carotovorum*.

3.2 *In vitro* Screening of Cell Free Extracts of LAB for Antifungal Activity

Agar well diffusion method was carried out for testing the antifungal activity of LAB against seventeen isolated fungal spoilage cultures. Results indicated the inhibition of three fungal spoilage organisms namely POT1F3, GRP1F1 and GRP3F2 with a maximum inhibition zone of 2.3 cm diameter (Fig. 3, Table 5). The LAB metabolites of curd, cumbu, finger millet, milk, lime pickle and fermented cumbuflour were found to inhibit the fungal spoilage organisms. Among them, metabolite from cumbu produced inhibition zone in three fungal spoilage organisms. Saladino et al. [12] hadtested sixteen LAB isolates against *Aspergillus parasiticus* and *Penicillium expansum*. At 1:2, 1:5 and 1:10 concentration, they have reported no antifungal activity, whereas at 1:20 concentration, six lactic acid bacterial cultures had exhibited antifungal activity against *Penicillium expansum.* Taurobet al. (2018) reported that *Pediococcuspentosaceus* RG7B strain exhibited antifungal activities against major contaminating fungi *Aspergillus nigeraggrégata* and *Aspergillus carbonarius.* The LAB strain was also identified to have a strong ability to survive in simulated gastric and intestinal fluids and exhibited significant levels of hydrophobicity, making it a promising candidate for a probiotic and ochratoxin A removal capabilities [13,14,15].

Table 1. Morphological characterization of lactic acid bacteria

S.No	LAB cultures	Colour	Margin	Elevation	Texture	Shape	Gram staining
1.	Mango pickle	Colorless	Entire	Raised	Slimy	Round	+ve , Rod shape
2.	Curd	Colorless	Entire	Raised	Slimy, moist	Round	+ve , Rod shape
3.	Cumbu	Colorless	Entire	Raised	Slimy	Round	+ve , Cocci shape
4.	Neera	Colorless	Entire	Raised	Slimy	Round	+ve , Cocci shape
5.	Cold rice	Colorless	Entire	Raised	Slimy	Round	+ve , Rod shape
6.	Finger millet	Colorless	Entire	Raised	Slimy, moist	Round	+ve , Cocci shape
7.	Infant faeces	Colorless	Entire	Raised	Slimy, moist	Round	+ve , Rod shape
8.	Milk	Colorless	Entire	Raised	Slimy, moist	Round	+ve , Rod shape
9.	Lime pickle	Colorless	Entire	Raised	Slimy	Irregular	+ve , Rod shape
10.	Fermented cumbu powder	Colorless	Entire	Raised	Slimy	Irregular	+ve , Rod shape
11.	Fermented cumbu Grain	Colorless	Entire	Raised	Slimy	Irregular	+ve , Rod shape
12.	Whey water	Colorless	Entire	Raised	Slimy	Round	+ve , Rod shape

Table 2. Morphological characterization of bacterial spoilage organism

S. No	Spoilage bacterial cultures	Source	Colour	Margin	Elevation	Texture	Shape	Gram staining & shape
1.	POT1B1	Potato	Translucent	Entire	Raised	Slimy	Round	-ve , Cocci
2.	POT1B2	Potato	Translucent	Entire	Raised	Slimy	Round	-ve , Cocci
3.	POT2B1	Potato	Translucent	Entire	Raised	Slimy	Round	-ve , Cocci
4.	POT3B1	Potato	Translucent	Entire	Raised	Slimy	Round	-ve , Cocci
5.	POT4B1	Potato	Colorless	Entire	Raised	Slimy	Round	-ve , Cocci
6.	POT5B1	Potato	Translucent	Entire	Raised	Slimy	Round	+ve , Rod
7.	POT6B1	Potato	Translucent	Entire	Raised	Slimy	Round	-ve , Cocci
8.	POT7B1	Potato	Translucent	Entire	Raised	Slimy	Round	+ve , Rod
9.	POT8B1	Potato	Translucent	Entire	Raised	Slimy	Round	-ve , Cocci
10.	POT8B2	Potato	Translucent	Entire	Raised	Slimy	Round	-ve , Cocci

S. No	Spoilage bacterial cultures	Source	Colour	Margin	Elevation	Texture	Shape	Gram staining & shape
11.	GRP1B1	Grapes	Translucent	Entire	Raised	Slimy	Round	+ve , Rod
12.	GRP2B1	Grapes	Translucent	Entire	Raised	Slimy	Round	+ve , Rod
13.	GRP2B2	Grapes	Translucent	Entire	Raised	Slimy	Round	+ve , Rod

Table 3. Characterization of fungal spoilage organism

S.No	Spoilage fungal cultures	Source	Colony colour
1.	POTF1	Potato	Whitish colour
2.	POTF2	Potato	Whitish colour with yellow centre
3.	POT1F3	Potato	Whitish and brown in colour
4.	POT3F1	Potato	Black colour
5.	POT4F1	Potato	Whitish colony with slightly pink colour
6.	POT5F1	Potato	Whitish colony
7.	POT6F1	Potato	Purplish Colorless colonies
8.	POT6F2	Potato	Blackish colony
9.	POT8F1	Potato	Whitish colony
10.	POT8F2	Potato	Whitish colony
11.	GRP1F1	Grapes	Whitish colony with black spores
12.	GRP1F2	Grapes	Whitish colony with greyish centre
13.	GRP1F3	Grapes	Black coloured colony
14.	GRP2F1	Grapes	Whitish colony with black colour in middle
15.	GRP3F1	Grapes	Whitish colour colony
16.	GRP3F2	Grapes	Green powdery colony
17.	GRP4F1	Grapes	Blackish colony

Table 4. *In vitro* screening of cell free extracts of LAB for antibacterial activity

Lactic acid bacterial metabolites	Area of Inhibition zone (cm^2) Spoilage bacterial cultures			
	POT1B1*	POT2B1*	POT3B1*	POT8B1*
Mango pickle	0.00	0.00	0.00	0.00
Curd	0.00	2.04±0.008[a]	0.00	0.00
Cumbu	0.00	0.00	0.00	0.00
Neera	1.04±0.024[a]	1.51±0.028[c]	0.00	5.80±0.057[a]
Cold rice	0.00	0.00	0.00	0.00
Finger millet	0.00	0.00	0.00	0.00
Infant faeces	0.00	0.00	0.00	0.00
Milk	0.00	0.00	0.00	0.00
Lime pickle	0.00	0.00	0.00	0.00
Fermented cumbu grain	0.00	0.00	0.00	0.00
Fermented cumbu powder	0.00	1.77±0.008[b]	5.65±0.003[a]	0.00
Whey water	0.00	0.00	0.82±0.005[b]	0.00
CD(P=0.05)	0.021	0.026	0.005	0.048

**Mean for three replications*

Table 5. *In vitro* screening of cell free extracts of LAB for antifungal activity

Lactic acid bacterial metabolites	Area of Inhibition zone (cm^2)		
	Spoilage fungal cultures		
	POT1F3*	**GRP1F1***	**GRP3F2***
Mango pickle	0.00	0.00	0.00
Curd	4.40±0.005[a]	0.00	2.96±0.054[a]
Cumbu	1.04±0.027[e]	1.04±0.008[c]	1.77±0.018[b]
Neera	0.00	0.00	0.00
Cold rice	2.64±0.011[c]	0.00	0.00
Finger millet	2.96±0.029[b]	0.00	0.82±0.006[e]
Infant faeces	2.04±0.037[d]	0.00	0.00
Milk	0.00	1.26±0.029[b]	0.00
Lime pickle	0.00	0.00	1.26±0.003[d]
Fermented cumbu grain	0.63±0.013[e]	0.00	0.00
Fermented cumbu powder	2.96±0.052[b]	0.00	1.51±0.015[c]
Whey water	0.00	0.00	0.00
CD(P=0.05)	0.065	0.025	0.050

**Mean for three replications*

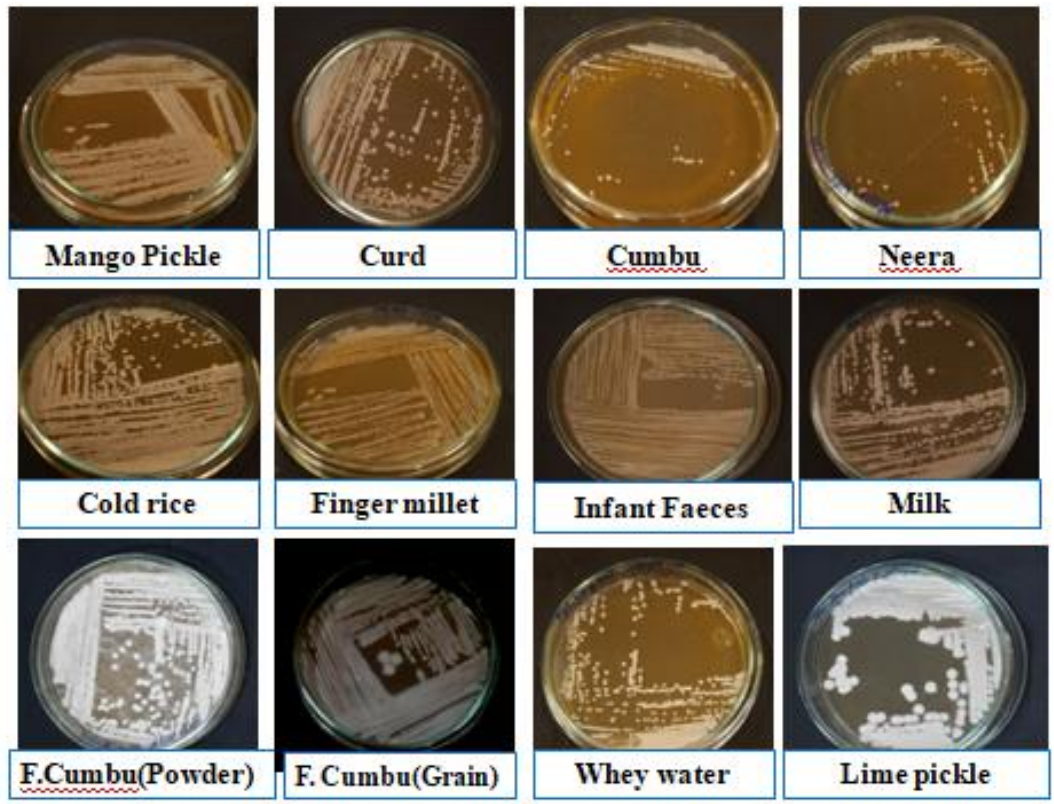

Fig. 1. Lactic acid bacterial cultures isolated from different food samples

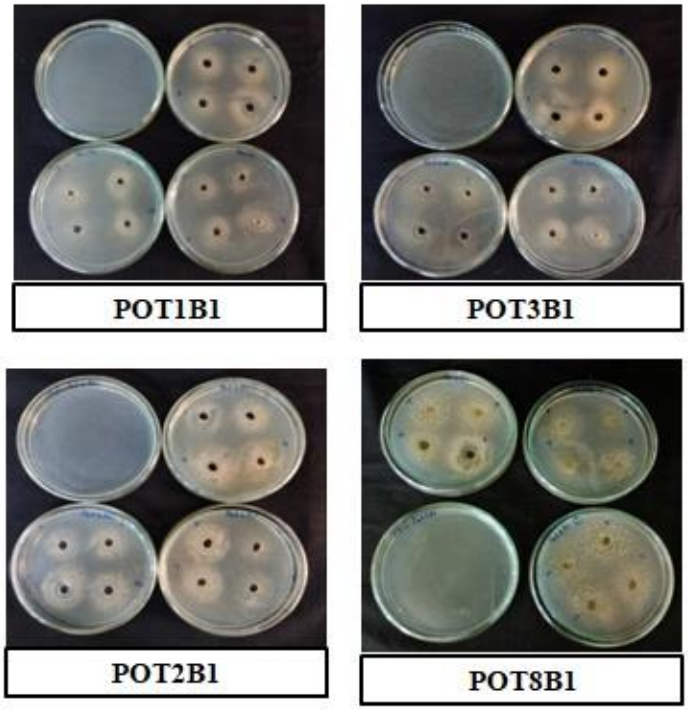

Fig. 2. *In vitro* screening Cell free extracts of LAB for antibacterial activity
(The zone around the well represents the antibacterial activity of LAB metabolites of the cultures)

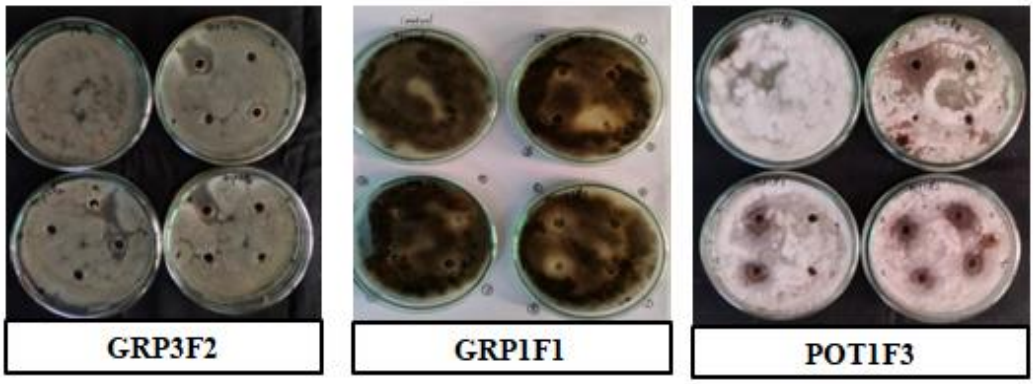

Fig. 3. *In vitro* screening Cell free extracts of LAB for antifungal activity
(The zone around the well represents the antifungal activity of LAB metabolites of the cultures)

4. CONCLUSION

The research paper concludes that the cell free extract containing lactic acid bacterial metabolites exhibited different antimicrobial property against both bacterial and fungal spoilage organisms isolated from spoiled grapes and potato samples.The study could be extended by formulating a product from the metabolites from promising lactic acid bacterial cultures for practical applications for reducing pre and post harvest losses in the crop.

ACKNOWLEDGEMENTS

We would like to express our heartfelt thanks to M/s. Phytotron Research Centre Pvt. Ltd., Bangalore for funding the research work. We would also acknowledge the Department of Agricultural Microbiology, Agricultural College and Research Institute, TNAU, Madurai for provision of facilities during our research work.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Hernandez E. Edible coatings from lipids and resins; Edible Coatings and Films to Improve Food Quality. (JM Krochta; EA Baldwin; and MO Nisperos-Carriedo; eds); 1994.
2. Ranadheera CS, Evans CA, Baines SK, Balthazar CF, Cruz AG, Esmerino EA, Freitas MQ, Pimentel TC, Wittwer AE, Naumovski N, Graca JS. Probiotics in goat milk products: Delivery capacity and ability to improve sensory attributes. Comprehensive Reviews in Food Science and Food Safety. 2019;18(4):867-82.
3. Ramos B, Brandão TR, Teixeira P, Silva CL. Biopreservation approaches to reduce Listeria monocytogenes in fresh vegetables. Food microbiology. 2020;85: 103282.
4. Gerez CL, Torres MJ, De Valdez GF, Rollán G. Control of spoilage fungi by lactic acid bacteria. Biological Control. 2013;64(3):231-7.
5. Adeyemo SM, Agun TF, Ogunlusi ED. Antimicrobial activity of lactic acid bacteria isolated from 'pupuru': an African fermented staple against food borne-pathogens. Journal of Molecular Biology and Biotechnology. 2018;3(1):5.
6. Chun-lei Z, Jia-qi L, Hai-tao G, Jie W, Ri-hua X. Selection of exopolysaccharide-producing lactic acid bacteria isolates from Inner Mongolian traditional yoghurt. Mljekarstvo: časopis za unaprjeđenje proizvodnje I prerademlijeka. 2014; 64(4):254-60.
7. Bogdan M, Justine S, Filofteia DC, Petruta CC, Gabriela LU, Roxana UE, Florentina M, Camelia Filofteia D, CălinaPetruța C, Gabriela L. Lactic acid bacteria strains isolated from Kombucha with potential probiotic effect. Romanian Biotechnological Letters. 2018;23(3): 13592-8.
8. Ismail YS, Yulvizar C, Mazhitov B. Characterization of lactic acid bacteria from local cow s milk kefir. InIOP Conference Series: Earth and Environmental Science. 2018;130:012019 IOP Publishing.
9. Padmavathi T, Bhargavi R, Priyanka PR, Niranjan NR, Pavitra PV. Screening of potential probiotic lactic acid bacteria and production of amylase and its partial purification. Journal of Genetic Engineering and Biotechnology. 2018; 16(2):357-62.
10. Frances EC, Johnson OO, Frances ME. Isolation and identification of microorganisms in wine produced from red muscat grapes. Asian Journal of Plant and Soil Sciences. 2021;6(3):21-7.
11. Steglińska A, Kołtuniak A, Motyl I, Berłowska J, Czyżowska A, Cieciura-Włoch W, Okrasa M, Kręgiel D, Gutarowska B. Lactic acid bacteria as biocontrol agents against potato (*Solanum tuberosum* l.) pathogens. Applied Sciences. 2022;12(15):7763.
12. Saladino F, Luz C, Manyes L, Fernández-Franzón M, Meca G. *In vitro* antifungal activity of lactic acid bacteria against mycotoxigenic fungi and their application in loaf bread shelf life improvement. Food Control. 2016;67:273-7.
13. Panse VG, Sukhatme PV. Statistical methods for agricultural workers. Statistical methods for agricultural workers; 1954.
14. Jiménez E, Yépez A, Pérez-Cataluña A, Vásquez ER, Dávila DZ, Vignolo G, Aznar R. Exploring diversity and biotechnological potential of lactic acid bacteria from tocosh-traditional Peruvian fermented potatoes-by high throughput sequencing (HTS) and culturing. LWT. 2018;87:567-74.
15. Taroub B, Salma L, Manel Z, Ouzari HI, Hamdi Z, Moktar H. Isolation of lactic acid bacteria from grape fruit: antifungal activities, probiotic properties and in vitro detoxification of ochratoxin A. Annals of microbiology. 2019;69(1):17-27.

Peer-review history:
The peer review history for this paper can be accessed here:
https://www.sdiarticle5.com/review-history/106423