# Exploring lactic acid bacterial metabolites for antimicrobial activity against spoilage organisms of grapes and potato

**Abstract:**

Biopreservation of foods is an alternative and novel method of preservation with increased special interest from ~~the~~ consumers.Lactic acid bacteria (LAB)~~has~~ havethe potential~~ityin restricting~~to restrictthe 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 ~~at exploring~~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



## Introduction:

Biopreservation, ~~is~~a new and innovative approach to control food spoilage is growing significance for several industries and consumers. ~~Use~~ 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 (Hernandez, 1994). 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~~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 (Ranadheera*et al.*, 2019). The antimicrobial capabilities of LABs are linked to various actions by the production of antimicrobial compounds such as bacteriocins, hydrogen peroxide, organic acids etc**.,** (Ramos *et al.*, 2020).

Grapes (*Vitis vinifera L.*) are one ~~among~~ 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 (Gerez*et al.*, 2013). 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.

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**Materials and methods:**

**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. Cumbu grains andcumbuflourwere

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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. 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.

**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.

**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.

**5. *Invitro* – 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 (Adeyemo *et al.*, 2018).

**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).

**Result and discussion**

**1. Isolation and Morphological characterization of lactic acid bacteria**

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Lactic acid bacteria were isolated in MRS medium from twelve different sources and they were purified in slant cultures. 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 **Table1**. Chun-lei *et al.* (2014) 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.,* (2018) 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.* (2018) 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.,* (2018) 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.* (2018) 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 ~~the~~ **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.* (2021) 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.

## 3. 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 (**Fig1**, **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 Jimenez*et 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. Steglińska*et al.* (2022) analyzed the metabolic profile of LAB strains isolated from 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*Pectobacteriumcarotovorum.*

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### 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.2, 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.* (2016) 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.*Taurob*et 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.

**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.

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**Tables**

**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 |
| 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 | Colorless colour |
| 2. | POTF2 | Potato | Colorless colour with yellow centre |
| 3. | POT1F3 | Potato | Colorless and brown in colour |
| 4. | POT3F1 | Potato | Black colour |
| 5. | POT4F1 | Potato | Colorless colony with slightly pink colour |
| 6. | POT5F1 | Potato | Colorless colony |
| 7. | POT6F1 | Potato | Purplish Colorless colonies |
| 8. | POT6F2 | Potato | Blackish colony |
| 9. | POT8F1 | Potato | Colorless colony |
| 10 | POT8F2 | Potato | Colorless colony |
| 11 | GRP1F1 | Grapes | Colorless colony with black spores |
| 12 | GRP1F2 | Grapes | Colorless colony with greyish centre |
| 13 | GRP1F3 | Grapes | Black coloured colony |
| 14 | GRP2F1 | Grapes | Colorless colony with black colour in middle |
| 15 | GRP3F1 | Grapes | Colorless 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*** |
| **MP** | 0.00 | 0.00 | 0.00 | 0.00 |
| **Curd** | 0.00 | $2.04\pm0.008^{a}$ | 0.00 | 0.00 |
| **Cumbu** | 0.00 | 0.00 | 0.00 | 0.00 |
| **Neera** | $1.04\pm0.024^{a}$ | $1.51\pm0.028^{c}$ | 0.00 | $5.80\pm0.057^{a}$ |
| **CR** | 0.00 | 0.00 | 0.00 | 0.00 |
| **FM** | 0.00 | 0.00 | 0.00 | 0.00 |
| **IF** | 0.00 | 0.00 | 0.00 | 0.00 |
| **Milk** | 0.00 | 0.00 | 0.00 | 0.00 |
| **LP** | 0.00 | 0.00 | 0.00 | 0.00 |
| **FCG** | 0.00 | 0.00 | 0.00 | 0.00 |
| **FCP** | 0.00 | $1.77\pm0.008^{b}$ | $5.65\pm0.003^{a}$ | 0.00 |
| **WW** | 0.00 | 0.00 | $0.82\pm0.005^{b}$ | 0.00 |
| **S. Ed** | 0.300 | 0.810 | 1.627 | 1.666 |
| **CD(P=0.05)** | 0.021 | 0.026 | 0.005 | 0.048 |

*Mean for three replications

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**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*** |
| **MP** | 0.00 | 0.00 | 0.00 |
| **Curd** | $4.40\pm0.005^{a}$ | 0.00 | $2.96\pm0.054^{a}$ |
| **Cumbu** | $1.04\pm0.027^{e}$ | $1.04\pm0.008^{c}$ | $1.77\pm0.018^{b}$ |
| **Neera** | 0.00 | 0.00 | 0.00 |
| **CR** | $2.64\pm0.011^{c}$ | 0.00 | 0.00 |
| **FM** | $2.96\pm0.029^{b}$ | 0.00 | $0.82\pm0.006^{e}$ |
| **IF** | $2.04\pm0.037^{d}$ | 0.00 | 0.00 |
| **Milk** | 0.00 | $1.26\pm0.029^{b}$ | 0.00 |
| **LP** | 0.00 | 0.00 | $1.26\pm0.003^{d}$ |
| **FCG** | $0.63\pm0.013^{e}$ | 0.00 | 0.00 |
| **FCP** | $2.96\pm0.052^{b}$ | 0.00 | $1.51\pm0.015^{c}$ |
| **WW** | 0.00 | 0.00 | 0.00 |
| **S. Ed** | 1.546 | 0.450 | 1.002 |
| **CD(P=0.05)** | 0.065 | 0.025 | 0.050 |

Please add the full name of the abbreviations

*Mean for three replications

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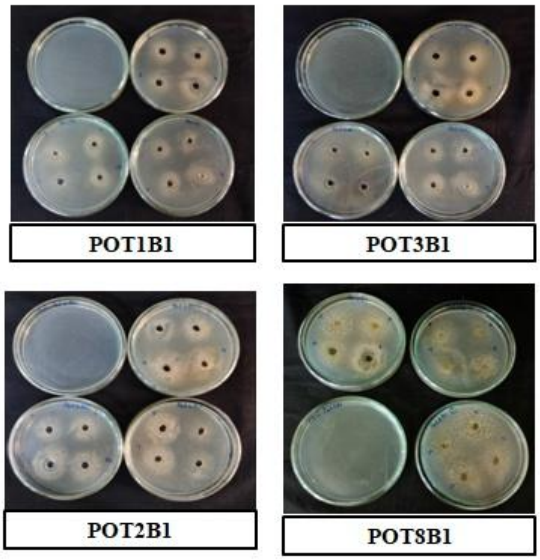


**Fig.1. *In vitro* screening Cell free extracts of LAB for antibacterial activity**

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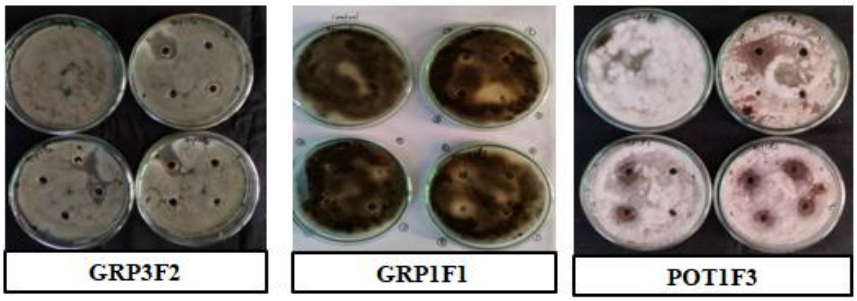


**Fig.2. *In vitro* screening Cell free extracts of LAB for antifungal activity**

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