International Journal of Life Science Research Archive

ISSN: 0799-6640 (Online)

Journal homepage: https://sciresjournals.com/ijlsra/

(RESEARCH ARTICLE)

Extraction of bioactive compounds from *Lactobacillus gasseri* and its food applications

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International Journal of Life Science Research Archive, 2024, 2024, 07(02), 100-104

Publication history: Received on 05November 2024; revised on 12 December 2024; accepted on 14 December 2024

Article DOI: https://doi.org/10.53771/ijlsra.2024.7.2.0081

Abstract

Our project involves extracts derived from *L.gasseri*, characterization of secondary metabolites using UV visible spectroscopy and thin layer chromatography. Antimicrobial activity of the extracted metabolites are determined using well diffusion method and the results indicates that these secondary metabolites have the potential to inhibit the growth of gram positive bacteria such as Staphylococcus aures and *Bacillus cerus* and gram negative bacteria such as *Shigella dysenteriae* and Salmonella typhi. Antifungal activity was performed against Aspergillus oryzae and Aspergillus flavus and the results indicate that these extracted secondary metabolites doesn't have antifungal property. MIC was determined against the pathogen using broth dilution method. Moreover, the applications of extracted secondary metabolites in improving the shelf life of the fruits are determined. Plum fruit and Avacado fruits are taken into study for our project and results indicates that coating of secondary metabolites over the fruits improve the shelf life.

Keywords: *Lactobacillus gasseri;* Bioactive compounds; Food applications; Anti-fungal; Anti-microbial; Shelf life

1 Introduction

Lactobacillus gasseri is a species in the genus *Lactobacillus* identified in1980 by François Gasser and his associates. It has been widely used for various purposes. In our project we have estimated the biomass content present in the *Lactobacillus gasseri* culture. Secondary metabolites present in the crude sample of *Lactobacillus gasseri* was extracted using ethyl acetate precipitation and Ammonium sulphate precipitation. Extracted metabolites were characterized using Thin layer Chromatography. Crude sample along with extracted metabolites were then tested for Antibacterial activity and Antifungal activity. Applications of crude sample and extracted metabolites in extending the shelf life of fruits were analysed.

2 Material and methods

2.1 Materials used

Lactobacillus gasseri was procured from Centre for Bioscience and Nanoscience Research, Coimbatore, Tamil Nadu, India. We have collected the fruits such as Plum (*Prunus domestica)* for the study.

2.2 Chemical sused

The chemical slike MRS brothmedia, Maltagar, Nutrientagar, Ethylacetate, Ammonium sulfate, N-Butanol, Aceticacid, Ninhydrinin acetone, Molten agar, Phosphate buffer saline, 2% Na_2CO_3 in 0.1 N NaOH, 0.5% $CuSO_4$ were used in sufficient quantity for the project.

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2.2.1 Estimation of Biomass

Lactobacillus gasseri was grown on MRS broth media. The broth media were incubated at 37 °C, under shaking for 24 hours. After incubation, biomasswas estimated and centrifuged at 10,000 rpm for 10 min to collect the cell-free supernatant. Biomass production in gramsdry weight (gdw) was estimated in flask cultures by collecting 1ml cultures in a pre-weighed 2 ml eppendorf tube. Cells were pelleted at 5000rpm for 5 min, and the supernatant was discarded. Tubes were dried well and were weighed on a precision balance to estimate the weight of the dried biomass. Biomass was calculated using the following equation: Biomass Estimation = Weight of the tube containing the pellet – Weight of the pre-weighed tube.

2.3 Extraction of Secondary Metabolites

After desired biomass growth was obtained, the cell free supernatant was collected by centrifuging the growth medium at 10000rpm for 10 minutes in a REMI centrifuge. After the cell free supernatant collection Ammonium sulfate was gently added to it with continuous stirring and mixing with a saturation of80%. Then it was kept in 4 °C for overnight for the precipitation. After overnight incubation the accumulated pellet was collected by centrifugation and then theobtainedpelletwasdissolvedinminimumamountofPhosphatebuffersaline.

2.4 Characterization of Secondary Metabolite

The extracted secondary metabolite was analyzed using Labtronics, LT-291microprocess UV– Spectrophotometer to characterize the formed secondary metabolites in the range of 200 to 600 nm. The peaks extracted were noted.

2.4.1 Thin Layer Chromatography of Secondary Metabolite

For the extracted metabolites, a thin layer chromatography (TLC)was carried out using silica gel sheets (Silica gel 60 F 254 20 x 20 cm gel thickness: 0.25mm, Merck) with a mobile phase made up of (N- Butanol: Acetic acid: Water) at a ratio of 3:1:1. The compound was applied to the silica plate using atooth pick until it becomes thickens. Then it was placed in chromatographic chamber for the development of the spot. After the development of the mobile phase, thespotwasvisualizedusing0.2%ninhydrininacetoneandtheRetentionfactoroftheseparatedcompoundwascalculatedusingtheformula:

Rf=(distance travelled by the sample)/(distance travelled by solvent).

2.5 Antimicrobial activity of Secondary Metabolites

2.5.1 Antibacterial Activity

The antibacterial efficacy of the extracted metabolites were evaluated using different bacterial cultures by well diffusion method. Nutrient agar (28 grams in1000 ml of distilled water were sterilized under autoclave at 121 °C for 15 lbs pressure for 15 minutes) was prepared and poured to the sterilized petri plates when the media cooled to room temperature.

Two strains of Gram positive (Staphylococcus aureus and Bacillus cereus) and two strains of Gram negative (Shigella dysenteriae and Salmonella typhi) bacteria were used to swab. The seeded medium was swabbed with each bacterial suspensions (80µL) and the wells were cut with Cork borer. Each well was loaded with extract (20µL), an antibiotic disc (Azithromycin AZM 30 mcg) as a positive control and DMSO as a negative control. The plates were kept for incubation at 37 °C for 24 hours. Extracted metabolite 1 and Extracted metabolite 2, crude sample were taken in different concentration in the well. The formed inhibition zones were measured by antibiotic zone scale, recorded and considered as indication for antibacterial activity.

2.5.2 Antifungal Activity by well diffusion Method

To identify the potency of extracted secondary metabolites, different fungal suspensions were used against the extract. Malt Agar Medium (45 gramsin1000 mL) was autoclaved at 121 °C for 15 psi for 15 minutes and poured to the sterile petri plates after cooled to room temperature. After solidification, fungal suspensions (80µL) of each Aspergillus oryzae and Aspergillus flavus were spread throughout the plate using cotton swabs. Sample was loaded in the cutwells (20µL) and incubated2-3daysatroomtemperature.20 µl of Fluconazole also was used as a standard. Extracted metabolite 1 and Extracted metabolite 2, crude sample were taken in different concentration in the well. After incubation, zone of inhibition was measured in mm.

2.5.3 Minimal inhibitory concentration-MIC by Broth dilution method

Nutrient broth (13 gmin 1000mLwaspreparedandsterilizedunderautoclave at 121 °C for 15 psi for 15 minutes). Sterile 96 well plate was taken and the wells were filled with 50µL sterilized nutrient broth. Along with 10µLof Bacillus cereus, samples such as Secondary metabolite 1, secondary metabolite 2, crude sample were added in different concentration of 10, 20, 30, 40 and 50µl. Incubated the well plate for 24 hours at 37 °C. After 24 hours, MIC was determined by optical density readings at 600 nm with an ELISA plate reader (Robonik).

MIC was calculated using the formula:

% of cell death= Control OD– Sample OD/ControlODX100

2.6 Shelf-Life Analysis of fruit

For shelf-Life analysis, Nutrient Broth (13 grams in 1000 mL) was prepared and sterilized at 121ºC for 15 minutes. Sterile cotton swabs were prepared and swabbed on each of the plum fruits. Coating were done suing crude sample and extracted secondary metabolites. Uncoated and plum and coated plum were observed for 7 days. Decrease in weight because of deterioration is noted by visual observation and decrease in weight of the fruit.

3 Results and Discussion

3.1 Biomass Estimation

Table 1 Biomass Estimation

Microfuge tubes	Initial weight (g)	Final weight (g)	Weigh to fthe dried biomass(g)
Tube1	1.032	1.083	0.051
Tube2	0.853	0.892	0.039

Our findings suggest that the majority of cell mass is composed of proteins and nucleic acids, which are essential components for cellular function. Extraction of Secondary Metabolites

Theethylacetateextractionyieldedasignificantamountofsecondarymetabolites from the *L. gasseri*, as evidenced by the distinct color and aroma of the extract. Ammoniumsulfateprecipitationprovedtobeaneffectivemethodforisolating secondary metabolites from the *L. gasseri*. The addition of ammonium sulfate to the extract resulted in the formation of a white color precipitate, which was enriched in secondary metabolites.

3.2 Thin Layer Chromatography

Table 2 R_f values

No. of Compounds	RfValue ofSecondarymetabolites1	RfValue ofSecondarymetabolites2	RfValue ofCrude
First compound	0.28	0.28	0.2
Second compound	0.42	0.42	0.44
Third compound	0.56	0.56	0.62

TLC analysis revealed the presence of multiple compounds with varying Rf values, suggesting a diverse range of secondary metabolites. This analysis further confirmed the identification of several compounds, including flavonoids, terpenoids, and alkaloids. These compounds are known for their various biological activities, such as antioxidant, anti-inflammatory, and antimicrobial properties.

3.3 Anti-bacterial activity

Table 3 Zone of Inhibition values

S. No	Name of test organism	Zone of inhibition (in mm)				
		Secondary metabolite 1	Crude	Secondary metabolite 2	Disc (positive control)	DMSO (Negative Control)
1.	*B.cereus*	20	21	21	27	NIL
2.	*S.aureus*	19	14	16	18	NIL
3.	*S. typhi*	14	18	23	13	NIL
4.	*S.dysenteriae*	15	15	16	18	NIL

The results of our study demonstrated the significant antibacterial activity of secondary metabolites, crude samples against various pathogenic bacteria such as *Staphylococcus aureus*, *Shigella dysenteriae, Bacillus cereus and Salmonella typhi*. The zone of inhibition produced by secondary metabolite 2 was significantly greater than the control, indicating its potent antimicrobial properties. These compounds can disrupt the cell walls and membranes of pathogenic bacteria, leading to cell death. Moeover all the extracts show more inhibition against B, cereus. Thus *Bacillus cereus was further used in* MIC study.

3.4 MIC against B. Cereus

Table 4 Inhibitory Percentage Values for different Concentration

Conc. Sample	10(µg/ml)	20(µg/ml)	30(µg/ml)	40(µg/ml)	50(µg/ml)
Crude	39.45%	49.63%	20.92%	54.00%	46.78%
Secondary metabolites1	16.65%	45.30%	50.31%	50.88%	50.88%
Secondary metabolites2	37.12%	90.8%	54.80%	62.19%	55.42%

The MIC values determined in this study revealed antimicrobial susceptibility among the tested bacterial strain B. cereus. Inhibitory Concentration was found at maximum in the secondary metabolite concentration of 40µg/ml.

3.5 Shelf life analysis of Plum Fruit

Table 5 Weight of Plum fruit with coating and without coating

S. No	Coating	Initial weight of plum(gm)Day 1	Final weight of plum(gm)Day 7
1.	Control	29.20	24.70
2.	Crude	23.56	21.98
3.	Secondary metabolite 1	27.06	26.19
4.	Secondary Metabolite 2	30.98	29.07

Fruits were kept in normal room condition for 7 days. After 7 days weight of the fruit is tested and values are tabulated. We can see there is huge decrease in weight of the fruit in control specimen after 7 days whereas coated fruits show only minimal weight loss. Results indicate that shelf life of fruits can be improved by coating and secondary metabolite 1 provides better results.

3.6 Anti-fungal activity

The results of our study demonstrated the significant anti-fungal activity of *crude sample and extracted metabolite* against fungal species such as *Aspergillus oryzae* and *Aspergillus flavus*. We conclude that our sample doesn't inhibit the growth of fungus. Zone of inhibition values are not found.

4 Conclusion

This study successfully demonstrated the potential of extracts obtained from *Lactobacillus gasseri* in extending shelf life of fruit and also their antibacterial and anti-fungal property. Extraction of metabolites from *Lactobacillus gasseri* was done using Ethyl acetate and Ammonium sulphate precipitation method. Characterisation of extract was determined using TLC. Antibacterial activity of crude sample of *Lactobacillus gasseri,* extracted secondary metabolites were assessed using disc diffusion method against various pathogens. Results infer that extracted metabolites perform better against *B. Cereus.* MIC values were determined against *B. Cereus* using the extracts in different concentration. *Results from* Antifngal activity of extract against fungal species reveal that there is no significant improvement in inhibition of fungal species. Shelf life analysis of fruits reveal that fruits coated with extracted metabolites improve the shelf life to a considerable extent

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

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