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Isolation and functional attributes of probiotic bacteria from chilli and their fermented products

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Abstract

Chilli (*Capsicum annuum* L.) is a widely cultivated vegetable and spice crop that represents a valuable beneficial microorganism with potential probiotic attributes. The present investigation focused on the isolation, characterization, and assessment of probiotic bacteria from fresh chilli and fermented chilli-based products, including mixed pickles, pickled red pepper, and pickled green chilli. A total of 63 bacterial isolates were recovered and screened and its morphological and biochemical characterization revealed that all isolates were Gram-positive and catalase-negative, suggesting their affiliation with lactic acid bacteria. These isolates were further examined for their ability to produce extracellular enzymes, and the selected isolates exhibited notable cellulolytic and proteolytic activities, producing clear hydrolysis zones exceeding 17–18 mm. Among the 63 isolates, 26 demonstrated positive cellulolytic activity, while 32 showed weak cellulolytic responses and 5 exhibited strong activity. Likewise, proteolytic screening indicated that 38 isolates were positive, 20 showed weak activity, and 5 displayed strong protease production. In contrast, amylolytic and lipolytic activities were not detected in the selected isolates. Five promising isolates designated as NCH10, NMP9, NMP19, NPRP^[8], and NPGC^[1] were identified through molecular characterisation. These promising isolates could be explored further for their application in functional food development and probiotic formulations, although molecular characterization and in vivo validation are required to confirm their efficacy and safety

Keywords: Probiotic bacteria, Lactic acid bacteria, Bacterial isolation, Probiotic characterization, extracellular enzyme activity

Introduction

Capsicum annuum L., a popular vegetable and spice crop, is a member of the *Solanaceae* family. Red pepper, spicy pepper and sweet pepper are some other names for it. The Portuguese brought chillies to India, yet they are native to Mexico. The five species of *capsicum* that are primarily grown are *C. annuum*, *C. baccatum*, *C. chinense*, *C. frutescens* and *C. pubescens*. Among these, *C. annuum* L., is the most extensively farmed species, bred for its spicy and sweet pepper fruits, respectively. In tropical regions, chillies are a staple element in curry powder recipes. (Geetha *et al.*, 2017)^[6]. The *Capsicum frutescens* can cultivate the least and *Capsicum annuum* can cultivate the most. Due to their low levels of these nutrients, their contribution to vitamin E, Niacin, vitamin B6 and vitamin A will be minimal. In comparison, it has the highest levels of antioxidants, vitamin E, niacin, vitamin B6, and vitamin A, all of which are beneficial to health. Chillies have a great nutritional value and are high in vitamins, particularly A and C. With 36 g of carbohydrates, 18 g of proteins, 16 g of lipids, 480 mg of Calcium, 3.1 mg of Phosphorus, 31 mg of Iron, 2.5 mg of Niacin, 640 I.U. of vitamin A and 40 mg of vitamin C, vitamin A, vitamin B6, vitamin K and minerals like Calcium, Magnesium, Folate, Potassium, Thiamine, Iron, Copper, etc., are found in chillies every 100 g of dried pods provides roughly 160 calories. Emmanuel *et al.* (2014)

The Capsaicinoids in chilli peppers as a defense mechanism against mammalian predators is an important aspect of fruit quality and one of the primary reasons people consume chilli peppers. Popelka *et al.* (2017)^[14] as per the report it is a highly versatile material used in everything from pharmaceuticals and nutrition to chemical weapons. It has been applied as an analgesic to lessen arthritis-related pain and inflammation. Chilli comes in diverse forms varying in size, colour and spiciness levels. (Azlan *et al.*, 2022)^[1] as per the report additionally, chilli offers various health benefits, serving as a good source of vitamins A and C as well as antioxidants.

Globally, 18.03 lakh hectares were planted with chillies in 2023, yielding an average of 3,229 kg per hectare and 58.22 lakh tonnes. With 27.82 lakh tonnes, India was the world's top producer followed by Bangladesh, Thailand, China, Ethiopia, Myanmar Benin, Cote d'Ivoire, and Pakistan (INDIASTAT, 2021). In Telangana, the production

and productivity of the cultivated chilli area were placed fourth and second, respectively, at 2.66 lakh acres, 6 lakh tons, and 2255 kg per acre. Telangana's chilli production and area make up 13% and 23% of India's total, respectively. Khammam, Mahbubnagar, Gadwal, Suryapet, and Warangal (Rural) are major districts that cultivate chillies. Telangana is expected to produce 6 lakh tonnes of chillies in 2024–2025 compared to 7.94 lakh tonnes in 2023–2024, according to the third advance projections. (Anonymous, 2026).

The chilli has been manifested with a diverse pathogen such as fungal diseases caused by fungi frequently inflict greater harm than those caused by other pathogens. Numerous fungi infect chilli plants with a variety of illnesses. Hussain *et al.* (2011) as per the report these fungi can occasionally produce similar symptoms and be mistaken for one another. Pre-harvest fungal infections include the following: Anthracnose, Fusarium stem rot, Fusarium wilt, Cercospora (frog-eye) leaf spot, Charcoal rot, Choanephora blight (wet rot), Damping-off root rot, Downy mildew and gray leaf.

The lactic acid bacteria are crucial to this process because they generate lactic acid, the primary by product of fermentation. Janiszewska *et al.* (2022) [10] as per the report they are Gram-positive and catalase-negative, and their sugar metabolism is a common characteristic that links them to the right bacteria, most popular *Lactobacillus* cultures for fermenting vegetables are: *Levilactobacillus brevis*, *Limos lactobacillus fermentum* and *Lactiplantibacillus plantarum*. Lactic acid bacteria were isolated and identified by Banik *et al.* (2023) [22] using standard cultural and biochemical assays, which were then followed by molecular identity confirmation.

Lactobacillus one of the most important groups of microorganisms in the transformation and preservation of food such as their tolerance to pH and salinity, wide range temperature, their ability to form biofilms and the production of bioactive compounds (including antimicrobials, antifungal) and the bioactive compound that *Lactobacillus* produce are very well studied in the food processing industry and have resulted in their designation as generally regarded as safe (GRAS) by the United States food and drug administration (US FDA), indicating that they would pose no risks for applications in edible crop production, (Lutz *et al.*, 2012) [11].

Nordin *et al.* (2017) [12] stated that the advantages of lactic acid bacteria (LAB) as biological control agents are well documented. However, little research has been done on its possible use in commercial crop farming, particularly with regard to *Capsicum frutescens*. In order to investigate the impact of lactic acid bacteria (*Lactobacillus plantarum*) on the antioxidative activities of chillies (*Capsicum frutescens*), and above background of is devoted from chilli to isolate and screening LAB for functional products preparation.

Materials and Methods

Sample collection and isolation of Probiotic bacteria

Fresh chilli (*Capsicum annum L.*) and fermented chilli products, including mixed pickles, pickled red pepper, and pickled green chilli, were collected from local markets and transported to the laboratory under aseptic conditions. Ten grams of each sample was homogenized in 90 mL of sterile physiological saline (0.85% NaCl) and subjected to serial dilution up to 10^{-7} . Aliquots (0.1 mL) from appropriate dilutions were spread onto de Man, Rogosa, and Sharpe (MRS) agar plates and incubated at 37 °C for 24–48 h. distinct colonies were selected based on colony morphology and purified by

repeated streaking on fresh MRS agar plates. The purified isolates were maintained on MRS agar slants at 4 °C for further studies.

Morphological and Biochemical Characterization

The bacterial isolates were characterized by observing colony morphology, including size, shape, colour, margin, and elevation. Gram staining was performed to determine the Gram reaction and cellular morphology of the isolates. Catalase activity was assessed by adding a drop of 3% hydrogen peroxide to fresh bacterial cultures and observing bubble formation. Gram-positive and catalase-negative isolates were considered potential lactic acid bacteria and selected for further screening.

Characterization of Functional attributes of probiotic bacteria

Amylolytic Activity

Amylolytic activity was determined by spot inoculating the isolates onto starch agar plates followed by incubation at 37 °C for 48 h. The plates were flooded with iodine solution, and the formation of a clear halo around the colonies indicated starch hydrolysis and positive amylase activity.

Cellulolytic Activity

Cellulase activity was assessed on carboxymethyl cellulose (CMC) agar plates. The isolates were inoculated and incubated at 37 °C for 48 hours. Following incubation, the plates were stained with 0.1% Congo red and subsequently washed with 1 M NaCl solution. The presence of clear zones around the colonies indicated the degradation of cellulose and confirmed positive cellulolytic activity.

Screening for cellulase production revealed variable cellulolytic potential among the isolates. In fresh chilli samples, nine isolates exhibited positive cellulolytic activity, six showed weak activity, and one isolate demonstrated strong cellulase production. Among mixed pickle isolates, six were positive, twelve were weakly positive, and one exhibited strong cellulolytic activity. In pickled red pepper samples, eight isolates showed positive activity, seven exhibited weak activity, and one isolate was strongly positive. Similarly, among isolates from pickled green chilli, six displayed positive cellulolytic activity, three showed weak activity, and one isolate exhibited strong activity. These findings indicate that cellulase-producing bacteria are widely distributed in chilli-based fermented products and may contribute to the degradation of plant cell wall components during fermentation. Zeng *et al.* (2023) [21] as per the report amylolytic and cellulolytic characteristics of lactic acid bacteria, cellulase activity using carboxymethyl cellulose (CMC) agar plates followed by Congo red staining and NaCl washing to visualize cellulose hydrolysis zones. Such enzymatic activities may enhance nutrient availability and facilitate microbial adaptation in plant-based substrates.

Proteolytic Activity

Protease production was evaluated using skim milk agar medium. The bacterial cultures were spot inoculated onto the plates and incubated at 37 °C for 48 hours. Hydrolysis of casein resulted in the formation of clear zones surrounding the colonies, which was considered evidence of proteolytic activity. The diameter of the hydrolysis zones was measured and recorded in millimetres.

Proteolytic screening demonstrated that several isolates possessed the ability to hydrolyze protein substrates. Among

fresh chilli isolates, twelve exhibited positive proteolytic activity, four showed weak activity, and one isolate demonstrated strong protease production. In mixed pickle samples, twelve isolates showed weak proteolytic activity, six were positive, and two isolates exhibited strong activity. Among pickled red pepper isolates, twelve were positive, three were weakly positive, and one isolate demonstrated strong proteolytic activity. In pickled green chilli samples, eight isolates showed positive activity, one was weakly positive, and one isolate exhibited strong protease production. Yoo *et al.* (2026) [20] as per the report proteolytic activity of lactic acid bacteria on skim milk agar and determined enzyme production by measuring the diameter of clear hydrolysis zones surrounding bacterial growth after incubation.

Lipolytic Activity

Lipolytic activity was determined using tributyrin agar medium. The isolates were inoculated onto the agar surface and incubated at 37 °C for 48 h. The formation of transparent zones around the colonies indicated lipid hydrolysis and positive lipase activity.

Results and Discussion

Isolation of Probiotic bacteria

The present investigation involved the characterization of 63 bacterial isolates obtained from fresh chilli, pickled red pepper, mixed pickle, and pickled green chilli samples. Morphological observations revealed considerable diversity among the isolates in terms of colony appearance, elevation, margin, texture, and cellular morphology.

Among the isolates obtained from fresh chilli samples, 11 isolates produced white colonies, while 6 isolates exhibited a creamy-white appearance. Most isolates showed flat elevation, entire margins, and mucoid texture. Microscopic examination indicated that the isolates were predominantly rod-shaped, occurring either singly or in characteristic arrangements. These features are commonly associated with lactic acid bacteria and other fermentative microorganisms.

The mixed pickle samples yielded 20 isolates with diverse morphological characteristics. White colonies were observed in 15 isolates, while 5 isolates showed creamywhite pigmentation. Raised elevation was recorded in 19 isolates and flat elevation in one isolate. Colony margins were predominantly undulated (11 isolates), whereas 9 isolates exhibited curled margins. Smooth texture was observed in 18 isolates, while only 2

isolates displayed a mucoid texture. Microscopic examination revealed that 14 isolates were spherical (cocci), whereas 6 isolates were rod-shaped, indicating the presence of different bacterial groups within the mixed pickle ecosystem.

In pickled red pepper samples, white-coloured colonies predominated, with 12 isolates showing white pigmentation and 4 isolates displaying creamy-white colonies. Colony elevation was mainly raised, observed in 15 isolates, whereas only one isolate exhibited flat elevation. Margin characteristics varied, with 8 isolates showing undulated margins and 8 exhibiting entire margins. All 16 isolates displayed a mucoid texture, suggesting the production of extracellular polysaccharides, which may contribute to adaptation and survival in fermented environments.

In pickled green chilli samples, all 10 isolates produced white colonies with flat elevation and creamy texture. Microscopic observations revealed that all isolates were rodshaped, indicating a relatively uniform bacterial population compared to the other sample sources.

Functional characterisation

Amylolytic and Lipolytic Activities

In the present study, none of the 63 isolates exhibited detectable amylolytic or lipolytic activity under the experimental conditions employed. The absence of clear zones on starch agar following iodine treatment indicated a lack of starch hydrolysis, while the absence of halo formation on tributyrin agar confirmed the inability of the isolates to hydrolyze lipid substrates. Zeng *et al.* (2023) [21] as per the report five isolates of *Lactobacillus plantarum* exhibited positive amylolytic activity in modified MRS medium, with enzyme activities exceeding 4.00 U mL⁻¹ and a maximum value of 4.61 U mL⁻¹. Lipolytic activity of lactic acid bacteria using tributyrin - agar and observed clear hydrolysis zones around lipase-producing colonies. Differences between studies may be attributed to strain variability, substrate composition, and cultivation conditions. Gardoul *et al.* (2026) [5]

The results of the present investigation demonstrate that cellulolytic and proteolytic activities were more prevalent than amylolytic and lipolytic activities among bacterial isolates from chilli and fermented chilli products. These enzymatic traits may contribute to substrate degradation, flavour development, and overall fermentation performance, highlighting the potential application of selected isolates in fermented food production.

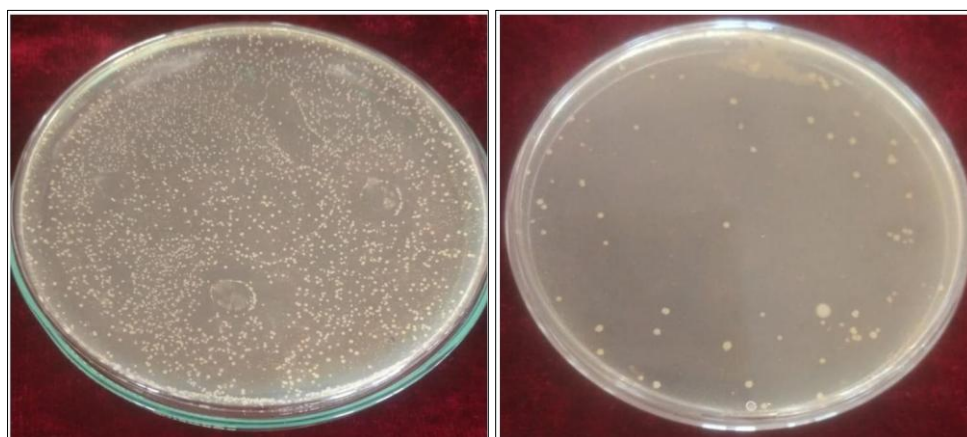


Fig 1: Isolated colonies of chilli and their fermented products



Fig 2: Purified culture of probiotic bacterial isolates

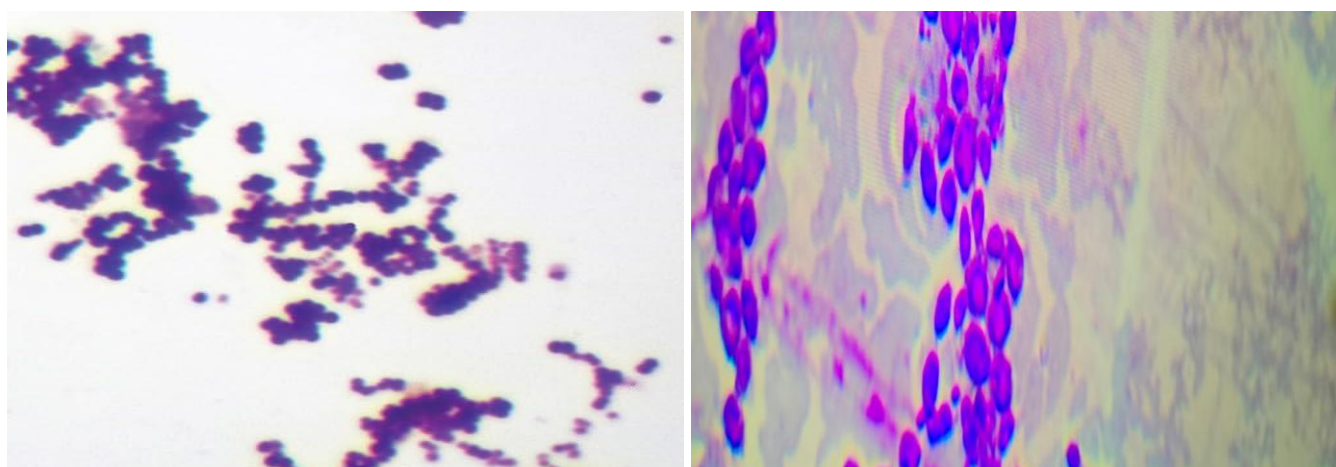


Fig 3: Gram -Positive bacteria appeared purple and violet colour under a microscope

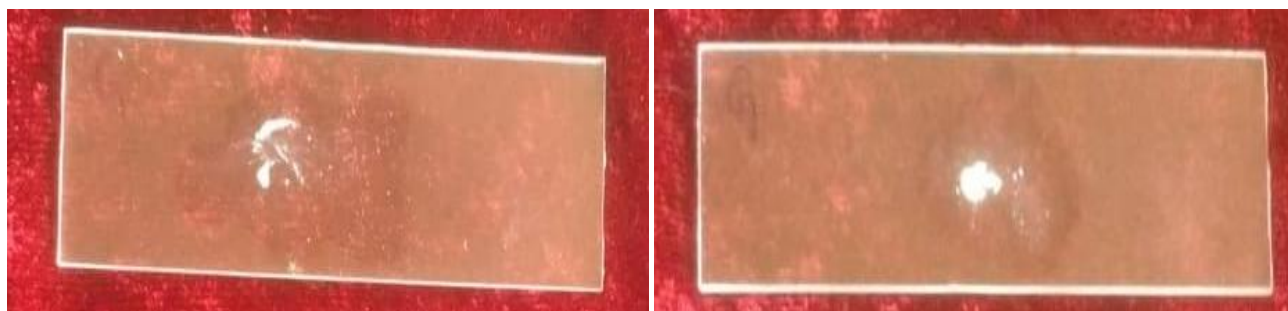


Fig 4: Gram positive bacteria-Catalase negative

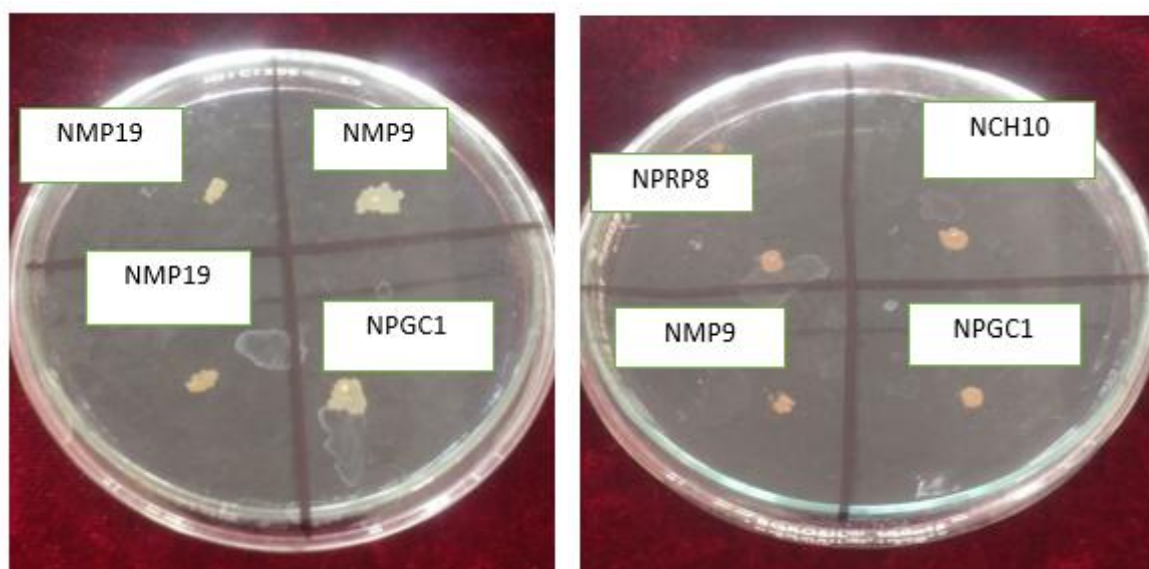


Fig 5: Amylolytic production activity negative

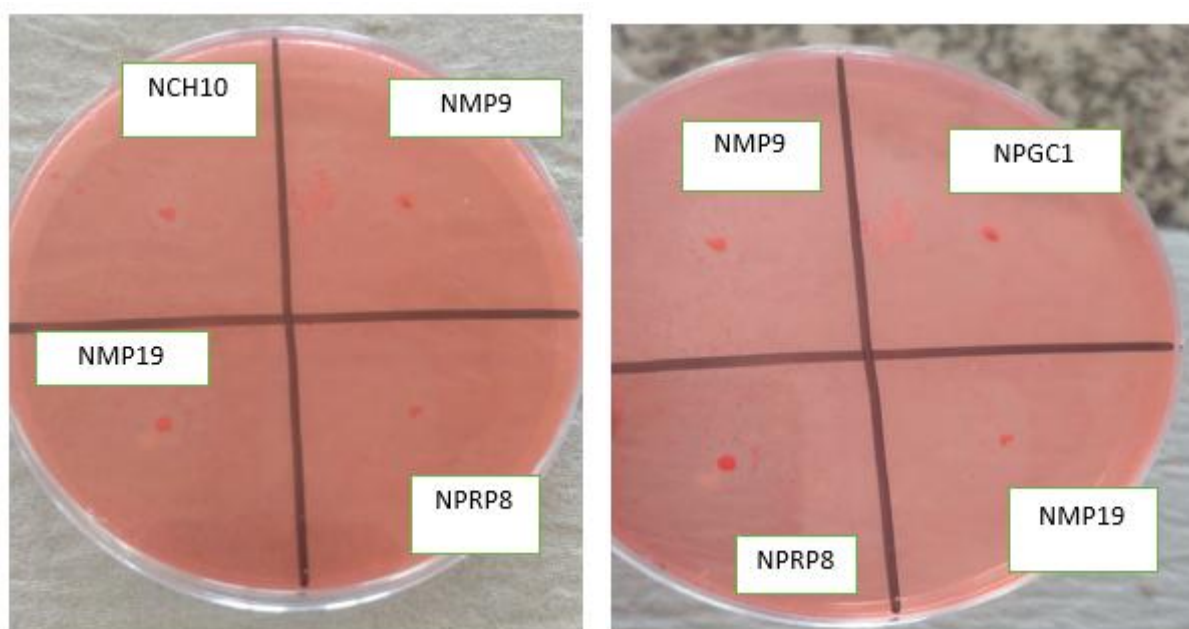


Fig 6: Cellulolytic production activity positive

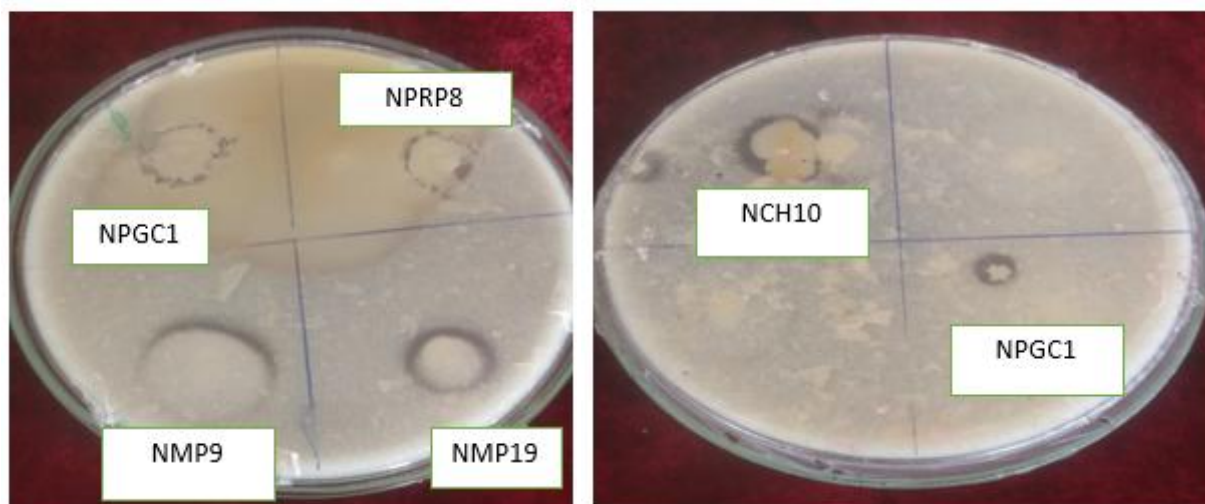


Fig 7: Proteolytic production activity positive

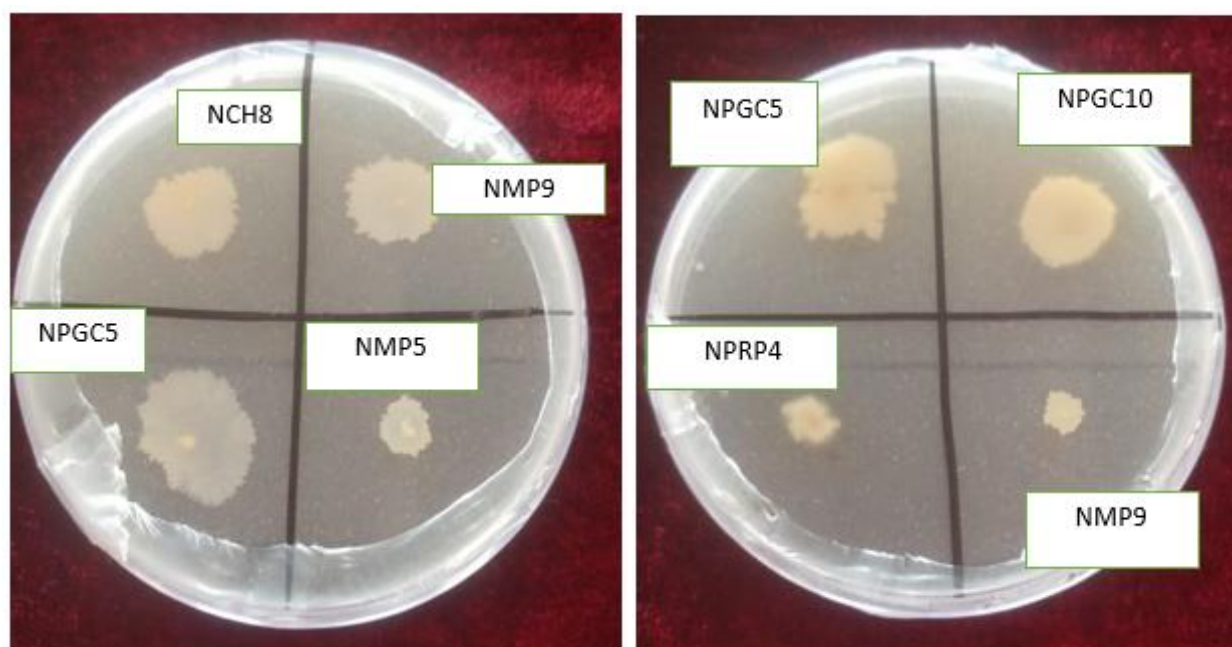


Fig 8: Absence of lipolytic production activity by the isolates, lipolytic activity negative

Conclusion

A total of 63 bacterial isolates were recovered from fresh chilli and fermented chilli products and characterized based on colony morphology, Gram staining, and enzymatic activities. The isolates produced small to medium-sized colonies with variations in shape, margin, elevation, and surface appearance on MRS agar. Gram staining showed that most isolates were Grampositive rods or cocci, commonly associated with beneficial fermentative microorganisms. None of the isolates exhibited amylolytic or lipolytic activity, whereas several

demonstrated cellulolytic and proteolytic activities. Among them, NCH10, NMP9, NMP19, NPRP^[8], and NPGC^[1] showed comparatively higher cellulase and protease activities, indicating greater enzymatic efficiency. These findings suggest that chilli-associated bacteria possess functional properties that may contribute to fermentation and food quality improvement, with potential as probiotic candidates. However, further molecular characterization and *in vivo* studies are needed to confirm their identity, safety, and applications in the food and industrial sectors.

Table 1: Characterization of probiotic bacteria isolated from chilli and their fermented products

Sl.No	Isolate Name	Colony farm	Colour	Elevation	Margin	Texture	Cell and shape arrangements	Grams reactions	Catalase activity
1	NCH1	Circular	White	Flat	Entire	Mucoid	Rod shaped	Gram +	Negative
2	NCH2	Circular	White	Flat	Entire	Mucoid	Rod shaped	Gram +	Negative
3	NCH3	Circular	White	Flat	Entire	Mucoid	Rod shaped	Gram +	Negative
4	NCH4	Circular	White	Flat	Entire	Mucoid	Rod shaped	Gram +	Negative
5	NCH5	Circular	White	Flat	Entire	Mucoid	Rod shaped	Gram +	Negative
6	NCH6	Circular	White	Flat	Entire	Mucoid	Rod shaped	Gram +	Negative
7	NCH7	Circular	White	Flat	Entire	Mucoid	Rod shaped	Gram +	Negative

8	NCH8	Circular	White	Flat	Entire	Mucoid	Rod shaped	Gram +	Negative
9	NCH9	Circular	White	Flat	Entire	Mucoid	Rod shaped	Gram +	Negative
10	NCH10	Circular	White	Flat	Entire	Mucoid	Rod shaped	Gram +	Negative
11	NCH11	Circular	White	Flat	Entire	Mucoid	Rod shaped	Gram +	Negative
12	NCH12	Circular	Creamy white	Flat	Entire	Mucoid	Rod shaped	Gram +	Negative
13	NCH13	Circular	Creamy white	Flat	Entire	Mucoid	Rod shaped	Gram +	Negative
14	NCH14	Circular	Creamy white	Flat	Curled	Mucoid	Rod shaped	Gram +	Negative
15	NCH15	Circular	Creamy white	Flat	Curled	Mucoid	Rod shaped	Gram +	Negative
16	NCH16	Circular	Creamy white	Flat	Curled	Mucoid	Rod shaped	Gram +	Negative
17	NCH17	Circular	Creamy white	Flat	Curled	Mucoid	Rod shaped	Gram +	Negative
18	NMP1	Circular	White	Flat	Curled	Mucoid	Rod shaped	Gram +	Negative
19	NMP2	Circular	White	Raised	Curled	Mucoid	Rod shaped	Gram +	Negative
20	NMP3	Circular	White	Raised	Curled	Smooth	Rod shaped	Gram +	Negative
21	NMP4	Circular	White	Raised	Curled	Smooth	Rod shaped	Gram +	Negative
22	NMP5	Circular	White	Raised	Curled	Smooth	Rod shaped	Gram +	Negative
23	NMP6	Circular	White	Raised	Curled	Smooth	Spherical	Gram +	Negative
24	NMP7	Circular	White	Raised	Curled	Smooth	Spherical	Gram +	Negative
25	NMP8	Circular	White	Raised	Curled	Smooth	Spherical	Gram +	Negative
26	NMP9	Circular	White	Raised	Curled	Smooth	Spherical	Gram +	Negative
27	NMP10	Circular	White	Raised	Undulate	Smooth	Spherical	Gram +	Negative
28	NMP11	Circular	Creamy white	Raised	Undulate	Smooth	Spherical	Gram +	Negative
29	NMP12	Circular	Creamy white	Raised	Undulate	Smooth	Spherical	Gram +	Negative
30	NMP13	Circular	Creamy white	Raised	Undulate	Smooth	Spherical	Gram +	Negative
31	NMP14	Circular	Creamy white	Raised	Undulate	Smooth	Spherical	Gram +	Negative
32	NMP15	Circular	Creamy white	Raised	Undulate	Smooth	Spherical	Gram +	Negative
33	NMP16	Circular	White	Raised	Undulate	Smooth	Spherical	Gram +	Negative
34	NMP17	Circular	White	Raised	Undulate	Smooth	Spherical	Gram +	Negative
35	NMP18	Circular	White	Raised	Undulate	Smooth	Spherical	Gram +	Negative
36	NMP19	Circular	White	Raised	Undulate	Smooth	Spherical	Gram +	Negative
37	NMP20	Circular	White	Raised	Undulate	Smooth	Rod shaped	Gram +	Negative
38	NPRP1	Circular	White	Raised	Undulate	Mucoid	Rod shaped	Gram +	Negative
39	NPRP2	Circular	White	Raised	Undulate	Mucoid	Rod shaped	Gram +	Negative
40	NPRP3	Circular	White	Raised	Undulate	Mucoid	Rod shaped	Gram +	Negative
41	NPRP4	Circular	White	Raised	Undulate	Mucoid	Rod shaped	Gram +	Negative
42	NPRP5	Circular	White	Raised	Undulate	Mucoid	Rod shaped	Gram +	Negative
43	NPRP6	Circular	White	Raised	Undulate	Mucoid	Rod shaped	Gram +	Negative
44	NPRP7	Circular	White	Raised	Undulate	Mucoid	Rod shaped	Gram +	Negative
45	NPRP8	Circular	White	Raised	Undulate	Mucoid	Rod shaped	Gram +	Negative
46	NPRP9	Circular	Creamy white	Raised	Entire	Mucoid	Rod shaped	Gram +	Negative
47	NPRP10	Circular	Creamy white	Raised	Entire	Mucoid	Rod shaped	Gram +	Negative
48	NPRP11	Circular	Creamy white	Raised	Entire	Mucoid	Rod shaped	Gram +	Negative
49	NPRP12	Circular	Creamy white	Raised	Entire	Creamy	Rod shaped	Gram +	Negative
50	NPRP13	Circular	White	Raised	Entire	Creamy	Rod shaped	Gram +	Negative
51	NPRP14	Circular	White	Raised	Entire	Creamy	Rod shaped	Gram +	Negative
52	NPRP15	Circular	White	Raised	Entire	Creamy	Rod shaped	Gram +	Negative
53	NPRP16	Circular	White	Flat	Entire	Creamy	Rod shaped	Gram +	Negative
54	NPGC1	Circular	White	Flat	Entire	Creamy	Rod shaped	Gram +	Negative
55	NPGC2	Circular	White	Flat	Entire	Creamy	Rod shaped	Gram +	Negative
56	NPGC3	Circular	White	Flat	Entire	Creamy	Rod shaped	Gram +	Negative
57	NPGC4	Circular	White	Flat	Entire	Creamy	Rod shaped	Gram +	Negative
58	NPGC5	Circular	White	Flat	Entire	Creamy	Rod shaped	Gram +	Negative
59	NPGC6	Circular	White	Flat	Entire	Creamy	Rod shaped	Gram +	Negative
60	NPGC7	Circular	White	Flat	Entire	Creamy	Rod shaped	Gram +	Negative
61	NPGC8	Circular	White	Flat	Entire	Creamy	Rod shaped	Gram +	Negative
62	NPGC9	Circular	White	Flat	Entire	Creamy	Rod shaped	Gram +	Negative
63	NPGC10	Circular	White	Flat	Entire	Creamy	Rod shaped	Gram +	Negative

Table 2: Functional characterisation of probiotic bacterial isolates

Sl no	Isolate Name	Zone of diameter	Amylolytic activity	Zone of diameter	Cellulolytic activity	Zone of diameter	Proteolytic activity	Zone of diameter	Lipolytic activity
1	NCH1	NO ZONE	Negative(-)	16mm	positive	10mm	Positive(+)	NO ZONE	Negative(-)
2	NCH2	NO ZONE	Negative(-)	10mm	Positive(+)	16mm	Positive(+)	NO ZONE	Negative(-)
3	NCH3	NO ZONE	Negative(-)	12mm	Positive(++)	8mm	Weak positive(+)	NO ZONE	Negative(-)
4	NCH4	NO ZONE	Negative(-)	13mm	positive (+++)	13mm	Positive(+)	NO ZONE	Negative(-)
5	NCH5	NO ZONE	Negative(-)	12mm	Positive(+)	15mm	Positive(+)	NO ZONE	Negative(-)
6	NCH6	NO ZONE	Negative(-)	10mm	Positive(+)	8mm	Weak positive(+)	NO ZONE	Negative(-)
7	NCH7	NO ZONE	Negative(-)	12mm	Positive(+)	9mm	Weak positive(+)	NO ZONE	Negative(-)
8	NCH8	NO ZONE	Negative(-)	14mm	positive	11mm	Positive(+)	NO ZONE	Negative(-)
9	NCH9	NO ZONE	Negative(-)	8mm	Weak positive (+)	13mm	Positive(+)	NO ZONE	Negative(-)
10	NCH10	NO ZONE	Negative(-)	18mm	strong Positive(+)	17mm	strong positive(+)	NO ZONE	Negative(-)
11	NCH11	NO ZONE	Negative(-)	5mm	Weak positive (+)	7mm	Weak positive(+)	NO ZONE	Negative(-)
12	NCH12	NO ZONE	Negative(-)	7mm	Weak positive (+)	12mm	Positive(+)	NO ZONE	Negative(-)
13	NCH13	NO ZONE	Negative(-)	15mm	Positive	11mm	Positive(+)	NO ZONE	Negative(-)
14	NCH14	NO ZONE	Negative(-)	16mm	positive	16mm	Positive(+)	NO ZONE	Negative(-)
15	NCH15	NO ZONE	Negative(-)	4mm	Weak positive (+)	12mm	positive(+++)	NO ZONE	Negative(-)
16	NCH16	NO ZONE	Negative(-)	9mm	Weak positive (+)	16mm	Positive(+)	NO ZONE	Negative(-)

Sl no	Isolate Name	Zone of diameter	Amylolytic activity	Zone of diameter	Cellulolytic activity	Zone of diameter	Proteolytic activity	Zone of diameter	Lipolytic activity
17	NCH17	NO ZONE	Negative(-)	10mm	Weak positive (+)	10mm	Positive(+)	NO ZONE	Negative(-)
18	NMP1	NO ZONE	Negative(-)	16mm	positive	8mm	Weak positive(+)	NO ZONE	Negative(-)
19	NMP2	NO ZONE	Negative(-)	12mm	Positive(+)	7mm	Weak positive(+)	NO ZONE	Negative(-)
20	NMP3	NO ZONE	Negative(-)	9mm	Weak positive (+)	5mm	Weak positive(+)	NO ZONE	Negative(-)
21	NMP4	NO ZONE	Negative(-)	6mm	Weak positive (+)	13mm	Positive(+)	NO ZONE	Negative(-)
22	NMP5	NO ZONE	Negative(-)	12mm	Weak positive (+)	7mm	Weak positive(+)	NO ZONE	Negative(-)
23	NMP6	NO ZONE	Negative(-)	16mm	positive	8mm	Weak positive(+)	NO ZONE	Negative(-)
24	NMP7	NO ZONE	Negative(-)	15mm	positive	9mm	Weak positive(+)	NO ZONE	Negative(-)
25	NMP8	NO ZONE	Negative(-)	5mm	Weak positive (+)	4mm	Weak positive(+)	NO ZONE	Negative(-)
26	NMP9	NO ZONE	Negative(-)	17mm	Strong positive (+++)	18mm	Strong positive(+++)	NO ZONE	Negative(-)
27	NMP10	NO ZONE	Negative(-)	6mm	Weak positive (+)	16mm	Positive(+)	NO ZONE	Negative(-)
28	NMP11	NO ZONE	Negative(-)	12mm	Weak positive (+)	15mm	Positive(+)	NO ZONE	Negative(-)
29	NMP12	NO ZONE	Negative(-)	8mm	Weak positive (+)	12mm	Positive(+)	NO ZONE	Negative(-)
30	NMP13	NO ZONE	Negative(-)	9mm	Weak positive (+)	5mm	Weak positive(+)	NO ZONE	Negative(-)
31	NMP14	NO ZONE	Negative(-)	6mm	Weak positive (+)	6mm	Weak positive(+)	NO ZONE	Negative(-)
32	NMP15	NO ZONE	Negative(-)	14mm	Positive(+)	8mm	Weak positive(+)	NO ZONE	Negative(-)
33	NMP16	NO ZONE	Negative(-)	13mm	Weak positive (+)	9mm	Weak positive(+)	NO ZONE	Negative(-)

SI no	Isolate Name	Zone of diameter	Amylolytic activity	Zone of diameter	Cellulolytic activity	Zone of diameter	Proteolytic activity	Zone of diameter	Lipolytic activity
34	NMP17	NO ZONE	Negative(-)	11mm	Weak positive (+)	10mm	Positive(+)	NO ZONE	Negative(-)
35	NMP18	NO ZONE	Negative(-)	13mm	Weak positive (+)	11mm	Positive(+)	NO ZONE	Negative(-)
36	NMP19	NO ZONE	Negative(-)	18mm	Strong positive (+++)	17mm	Strong positive(+++)	NO ZONE	Negative(-)
37	NMP20	NO ZONE	Negative(-)	11mm	Positive(+)	8mm	Weak positive(+)	NO ZONE	Negative(-)
38	NPRP1	NO ZONE	Negative(-)	12mm	Weak positive (+)	12mm	Positive(+)	NO ZONE	Negative(-)
39	NPRP2	NO ZONE	Negative(-)	5mm	Weak positive (+)	15mm	Positive(+)	NO ZONE	Negative(-)
40	NPRP3	NO ZONE	Negative(-)	6mm	Weak positive (+)	16mm	Positive(+)	NO ZONE	Negative(-)
41	NPRP4	NO ZONE	Negative(-)	7mm	Weak positive (+)	14mm	Positive(+)	NO ZONE	Negative(-)
42	NPRP5	NO ZONE	Negative(-)	8mm	Weak positive (+)	13mm	Positive(+)	NO ZONE	Negative(-)
43	NPRP6	NO ZONE	Negative(-)	6mm	Weak positive (+)	13mm	Positive(+)	NO ZONE	Negative(-)
44	NPRP7	NO ZONE	Negative(-)	4mm	Weak positive (+)	16mm	Positive(+)	NO ZONE	Negative(-)
45	NPRP8	NO ZONE	Negative(-)	17mm	Strong positive (+++)	18mm	Strong positive +++	NO ZONE	Negative(-)
46	NPRP9	NO ZONE	Negative(-)	14mm	positive+	9mm	Weak positive(+)	NO ZONE	Negative(-)
47	NPRP10	NO ZONE	Negative(-)	15mm	positive+	7mm	Weak positive(+)	NO ZONE	Negative(-)
48	NPRP11	NO ZONE	Negative(-)	14mm	positive (+++)	11mm	Positive(+)	NO ZONE	Negative(-)
49	NPRP12	NO ZONE	Negative(-)	14mm	Positive(+)	14mm	Positive(+)	NO ZONE	Negative(-)
50	NPRP13	NO ZONE	Negative(-)	8mm	Weak positive (+)	13mm	Positive(+)	NO ZONE	Negative(-)

SI no	Isolate Name	Zone of diameter	Amylolytic activity	Zone of diameter	Cellulolytic activity	Zone of diameter	Proteolytic activity	Zone of diameter	Lipolytic activity
51	NPRP14	NO ZONE	Negative(-)	6mm	Weak positive (+)	10mm	Positive(+)	NO ZONE	Negative(-)
52	NPRP15	NO ZONE	Negative(-)	7mm	Weak positive (+)	9mm	Weak positive(+)	NO ZONE	Negative(-)
53	NPRP16	NO ZONE	Negative(-)	11mm	Weak positive (+)	12mm	Positive(+)	NO ZONE	Negative(-)
54	NPGC1	NO ZONE	Negative(-)	18mm	Strong positive+++	18mm	Strong positive(+++)	NO ZONE	Negative(-)
55	NPGC2	NO ZONE	Negative(-)	4mm	Weak positive (+)	14mm	Positive(+)	NO ZONE	Negative(-)
56	NPGC3	NO ZONE	Negative(-)	10mm	Positive(+)	16mm	Positive(+)	NO ZONE	Negative(-)
57	NPGC4	NO ZONE	Negative(-)	11mm	positive+	12mm	Positive(+)	NO ZONE	Negative(-)
58	NPGC5	NO ZONE	Negative(-)	15mm	positive (+++)	13mm	Positive(+)	NO ZONE	Negative(-)
59	NPGC6	NO ZONE	Negative(-)	14mm	Positive(+)	14mm	Positive(+)	NO ZONE	Negative(-)
60	NPGC7	NO ZONE	Negative(-)	9mm	Weak positive (+)	12mm	Positive(+)	NO ZONE	Negative(-)
61	NPGC8	NO ZONE	Negative(-)	8mm	Weak positive (+)	14mm	Positive(+)	NO ZONE	Negative(-)
62	NPGC9	NO ZONE	Negative(-)	11mm	Positive(+)	15mm	Positive(+)	NO ZONE	Negative(-)
63	NPGC10	NO ZONE	Negative(-)	12mm	positive+	10mm	Weak positive(+)	NO ZONE	Negative(-)

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