

Cactus pear (*Opuntia ficus-indica*) cladode extracts as a growth medium for lactobacillus species: The case of *Lactobacillus reuteri* and *Lactobacillus mali*

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Abstract

Different commercially available culture media have been in use for the isolation and identification of microorganisms for many years. However, the high cost and limited availability culture media has been a problem in the local market of Tigray, Ethiopia that hinder researchers from carrying out their study. Therefore, the present study aimed to develop locally available cheap plant-based culture medium from cactus pear (*Opuntia ficus-indica*) cladodes extract for the growth of *Lactobacillus* species. For this purpose, free external injuries of cactus pear cladode samples were collected from the vicinity of Adigrat, Tigray, Ethiopia. For the test two *Lactobacillus* species (*Lactobacillus reuteri* and *Lactobacillus mali*) were used. The growth of *Lactobacillus* species on cactus pear cladode agar and broth (4:100, 4:200 and 4:300 w/v) were evaluated and compared with de Man, Rogosa, and Sharpe (MRS) medium. All prepared cactus pear cladode agar (CPCA) media were transparent and free from particles and turbidity. Both tested *Lactobacillus* species were produced small, white and round colonies on CPCA at 72 hours without bad odor and any pigments. Exponential growth was observed in cactus pear cladode broth (4:200 w/v) in short time than other cactus pear cladode broth (4:100 and 4:300 w/v) medium. When compared with MRS medium (control), the colony counts of *Lactobacillus* species in cactus pear cladode broth (4:200 w/v) medium did not differ significantly ($P < 0.05$). The present study clearly showed that the cactus pear cladode (4:200 w/v) medium could be used as alternative culture medium to support growth of *Lactobacillus* species.

1. Introduction

The cactus pear plants are belonging to the genus *Opuntia* of the *Cactaceae* family that is widely distributed and grown in arid and semi-arid regions throughout the world, where few other plants can survive because of harsh conditions such as extremely high temperatures and limited water supply (Ramoba and Monyama, 2022). Among the family species, *Opuntia ficus-indica* is the most known widespread species in arid and semi-arid regions of the world that has an important source of income and animal feed (Nancib et al., 2017). Cactus pear (*Opuntia ficus-indica*) is originated in Mexico and has been taken by humans to other areas of the world such as south and central America, Africa and the Mediterranean countries (Coquimbo and Chile, 2017; Filannino et al., 2016). Mexico is the largest producer and consumer that has largest cultivation area in the world. Other countries such as Italy, South Africa, Chile, Israel, and Spain are also known producers. Cultivation of *Opuntia ficus-indica* has a wide range of applications some of them are it uses as a forage supplement, consumption of cladodes, medical uses and carmine production (Andreu-Coll et al., 2021). In addition to the excellent quality and flavor of the fresh fruit, its young leaves serve as nutritious vegetable and salad dish.

Cactus Pear (*Opuntia ficus-indica*) could contribute a sustainable food and feed production in various countries, like Ethiopia that has large areas of semiarid and arid lands. Tigray region which is located in north Ethiopia has a semi-arid area which is suitable environmental condition to cultivate *Opuntia ficus-indica*. The eastern and southern zones of Tigray region are the most known and main production areas of Cactus Pear (Andreu-Coll et al., 2021). The Cactus Pear (*Opuntia* species) is known by different names

in various countries wherever it is found. In Tigray region the cactus pear plant is locally known as “Beles” and has become the integral part of the people’s economy. More than 85% of the population of Tigray lives in rural areas and their main source of livelihood are directly from agriculture. However, there is limited agricultural potential and the animal production in the area also faces challenges due to feed shortage. The cactus pear in Tigray is a source of food, animal feed and it is a means of additional income and economic viability of small and low-income farmers to ensure food security. Currently, cactus pear is widely spread throughout the region and is believed to cover about 80 000 km² and is estimated to have more than 360 000 ha of cactus pear, two-thirds of which are spiny plants (Coquimbo and Chile, 2017). In fact, the plant is widely distributed throughout the region and beyond the region.

Since ancient times, Cactus Pear (*Opuntia ficus-indica*) was used as folk medicine for human. Recently, the cactus cladodes increased its popularity in developed countries because of high potential source of phytochemicals and prebiotics. Cladodes are a cheap and suitable substrate for functional foods or dietary supplements and easily harvest high annual production per hectare (Filannino et al., 2016). The cactus cladodes contain all the necessary nutrients and growth factors such as carbohydrates, proteins, amino acids, vitamins, and minerals which are required for the growth of microorganisms. The most abundant carbohydrates in cladodes are soluble fibers and low-molecular-weight carbohydrates (monosaccharides). It also contains citric, malic, and succinic acids that may vary according to the cladode age (Magarelli et al., 2022). In addition, cladodes are a source of carotenoids, fats, oil, polyunsaturated fatty acids, polyphenols, alkaloids and various flavonoids. The cactus cladodes have a potential to treat different diseases, including inflammation diabetes, cancers, viral and bacterial infections and remarkably, improves wound healing on dermal tissues (El-mostafa et al., 2014). according to research finding of Ortega-ortega et al. (2017), the cactus pear seed oil has good antioxidant and antimicrobial properties against bacterial pathogen.

Microbial study depends on culture media that provide suitable environmental condition to culture and maintain the microorganism under laboratory optimum conditions (Pathmanathan and Ravimannan, 2016). All microorganism’s growth requirements vary and therefore, cannot grow in a single culture medium formulation (Izebe et al., 2020). Generally, the availability of nutrients in culture media could be a good source of carbon, nitrogen, inorganic salt, sulphur, phosphorus, vitamins and various minerals to support the growth of bacteria and fungi which is also important in the development of microbiological studies (Syamsia et al., 2021; Oledibe et al., 2023). Different raw materials from local plants such as, orange peel, vegetable waste, lentils, cane molasses, bran, sorghum, corn, millet, onion, and garlic peel powder etc. are exist to use in the composition of different alternative culture media for microbial growth or to stimulate microbial compound production (Penha et al., 2022).

Recent scientific research has been focused on developing the culture media with alternatives cheap and widely available raw materials (Izebe et al., 2020). This is because microbial culture media are expensive and microbiological researches are carried out at high cost and scarcity of culture media in developing country. Therefore, the use of locally formulated media can help reduce the dependence on imported media and contributes for developing effective microbiological research (Pathmanathan and

Ravimannan, 2016). In Ethiopia, the high cost of commercially available synthetic culture media is a serious problem that affects from carrying out microbiological research. Currently, in Tigray, Ethiopia microbial culture media are very expensive and not easily available in the local market which can hindered microbiology researchers to carry out their study. Accordingly, there is a strong justification to develop cost-effective culture media from simple, accessible, and potential natural resources viz cactus pear (*Opuntia* species) cladodes in order to make them affordable and readily available in the country. Therefore, the study is aimed to develop locally available culture medium from cactus pear (*Opuntia ficus-indica*) cladodes extract for the growth of *Lactobacillus* species.

2. Materials and methods

2.1 Plant material collection

Twenty-five kgs of samples of full-grown cladodes of cactus pear (*Opuntia ficus-indica*) which is free of external injuries were collected from around Adigrat town, Tigray, Ethiopia. Mature cladodes of the cactus pear were excised and directly inserted into sterile plastic bags after removing thrones and other unnecessary materials. The samples were transported in an ice box to Biotechnology laboratory, Mekelle university in Ethiopia. Samples were kept in the refrigerator at 4°C prior to processing.

2.2 Preparation of cactus pear cladode powder

Cactus pear cladodes were washed three times by dipping in a container filled with pure fresh water to remove impurities and residues (Magarelli et al., 2022). Then, cladodes were sliced transversely into small size with a sterilized sharp knife and sun-dried for 5-8 days at a clean area. Dried cladodes were mechanically grinded using a mortar and pestle and further reduced into powder using an electric blender to a size that pass through a one-millimeter sieve to get a fine dehydrated powder (Youssef et al., 2016). The dehydrated powder was transferred into sterile plastic bags and stored at room temperature and dark conditions until further analysis (Ortega-ortega et al., 2017).

2.3 Preparation of cactus pear cladode culture media

Culture media preparation was done according to the method described by Hossain et al. (2020) and Youssef et al. (2016) with slight modification. Cactus pear cladode broth (CPCB) preparation was done by dissolving of an amount of dehydrated powder of cactus pear extract in gram with distilled water in different proportions to formulate the suitable medium (4/100, 4/200, and 4/300 w/v) in separate conical flask. Thereafter, the medium was heated on hot plate at 100°C for 10 - 15 minutes with stirring until the solution to achieve homogeneity and filtered using muslin filter (1 mm). The filtered and clear cactus pear cladode broth (CPCB) was transferred to a clean flask. Furthermore, each proportion of pure CPCB was used to prepare cactus pear cladode agar (CPCA) with 2% (w/v) agar according to agar dilution method. The pH of the media was adjusted to 6.5 which is the suitable growth condition of *lactobacillus* species (Shareef, 2019). The CPCA were homogenized and sterilized using autoclave at 121 °C at 15 psi for 15 mins. The culture media was removed from the autoclave and allowed to cool

down at room temperature. Finally, after 45 mins the Petri dishes containing the medium were turned upside down and evaluated the characteristics of CPCA by observing (Oledibe et al., 2023). In general, the CPCA culture media preparation protocol was summarized as the following procedure (**Fig. 1**).

Fig. 1 A workflow of cactus pear cladode media development

CPCA represents for cactus pear cladode agar, CPCB represent for cactus pear cladode broth, w/v represents for weight/volume measured by gram and milliliters.

2.4 Collection of test *Lactobacillus* species and their growth characteristics on CPCA

All tested *Lactobacillus* species were obtained from the college of veterinary sciences in Mekelle University, Ethiopia. The tested bacterial species were *Lactobacillus reuteri* and *Lactobacillus mali*. The examinations were done in the biotechnology laboratory college of veterinary sciences in Mekelle University. The bacterial species examined in the present study were reactivated in de Man, Rogosa and Sharpe (MRS) broth (HiMedia), by incubating at 37 °C for 24 hours (h). Thereafter, 0.1 ml fresh culture was inoculated using spread plate method on MRS agar (HiMedia) and incubated at 37 °C for 48 h. Their purities were examined by gram staining and catalase activity. The sterilized cactus pear cladode agar medium was poured into a sterile Petri dish and allowed to solidify under biological safety cabinet. It was incubated at 37 °C for 24 h to check the contamination. The pure colony of *Lactobacillus* species were taken and serially diluted in sterile normal saline solution (0.85% (w/v) NaCl). Following this, 0.1 mL of the fourth dilution (10^{-4} dilution) was taken and inoculated using spread plate method on each proportion CPCA medium and MRS agar (control), and incubated anaerobically at 37 °C for 48 hours. Finally, the growth and morphological characteristics of the colonies of *Lactobacillus reuteri* and *Lactobacillus mali* were observed by naked eye on cactus pear cladode agar medium and compared with the controlled MRS agar (Somnath and Shyama, 2015). The experiments were carried out in triplicates (Colombo et al., 2014).

2.5 Growth characterization of *Lactobacillus* species in CPCB

Lactobacillus reuteri and *Lactobacillus mali* were inoculated separately in MRS broth and incubated anaerobically at 37°C for 18 h. One ml of the 18 h old LAB culture was centrifuged at 5000 rpm for 10 min at 4°C and the resulted pellet was washed once with phosphate buffer saline (PBS, pH 7.0). The cell pellets were inoculated into 10 ml cactus pear cladode broth (CPCB) with different proportions (4/100, 4/200, and 4/300 w/v) and MRS broth used as a control and then, incubated anaerobically at 37°C for 72 h. Growth characteristics were determined by taking samples at 12 h, 24 h, 36 h, 48 h, 60 h and 72 h of incubation, spread plated and incubated anaerobically at 37°C for 48 h. Then growth cell counts were carried out using a colony counter to determine the colony forming unit of bacterial cells per milliliter log(CFU/mL) (Wambugu 2015; Hawaz 2016). According to the method described by Lingoh et al. (2020), Petri dishes that contained colony-forming units (CFU) ranging from 30-300 colonies were used to determine cell count. The experiment was repeated for triplicate. The growths were also characterized by

plotting normal growth curves. The bacterial cell density in CPCB compared with MRS broth were determined by their optical density (OD) using spectrophotometer at 600 nm after 48 h incubation time (Hossain et al., 2020). In all growth characterizations MRS medium was used as control of optimal growth.

3. Data Analysis

Before data analysis, the total counted viable colony forming units (CFU/mL) was converted to logarithmic value (logCFU/mL). Then, the experimental data were analyzed using Minitab software version 19 and one-way analysis of variance (ANOVA) with Fisher's Least significance Difference (LSD) test ($P < 0.05$) was used to show significant differences between the means of treatments.

4. Result

4.1 Culture media preparation and bacterial growth onCPCA

In this study, dried cladode powder was produced (**Fig. 2**). According to the result, from dehydrated powder of CPC three type of CPCA culture media proportion (4/100 w/v, 4/200 w/v and 4/300 w/v) were developed using 2% agar. The petri dishes containing the CPCA medium were turned upside down and evaluated the characteristics of CPCA by observing. The appearance of all prepared CPCA culture media were found light brown color, clear and transparent. This indicated that the medium is free from presence of particles and turbidity as shown on the image below (**Fig. 3**).

In the present result, both tested *Lactobacillus* species were produced small white and round colonies after 72 h incubation without bad odor and any pigments. However, *Lactobacillus reuteri* and *Lactobacillus mali* were shown large colony size on MRS agar medium after 72h incubation as compared their growth on the surface of cactus pear cladodes agar medium (**Fig. 4**).

Fig. 2 Cladode powder of *Opuntia ficus-indica*

Fig. 3 CPCA and MRS agar culture media

Fig. 4 *Lactobacillus* species growth on culture media: (A) *Lactobacillus* growth on MRS agar medium; (B) *Lactobacillus* growth on cactus pear cladode agar (CPCA) medium.

4.2 Growth characterization of *Lactobacillus* species in CPCB

Growth curve of *Lactobacillus* species: the growth characteristics of *Lactobacillus reuteri* and *Lactobacillus mali* were evaluated in CPCB with different proportion as compared with the standard commercial MRS broth medium to determine in which medium shows optimum timing for the maximum growth. Depending the time of incubation, all *Lactobacillus* species were not grown equally in all prepared types of media. According to the result below in the figure, *Lactobacillus reuteri* and *Lactobacillus mali* were shown exponential growth (log phase) from 24 h to 36 h in culture medium

proportion of CPCB (4:200 w/v) which was exhibited fast growth in a short time of incubation than the other formulation of CPCB medium. In addition, all bacterial species were shown log phase from the hours of 24-48 in medium CPCB (4:100 w/v) and from the hours of 36-48 in medium CPCB (4:300 w/v). from 48-72 hours there is no bacterial growth increase and decrease in cell number which is in stationary growth phase. In the present result, *Lactobacillus reuteri* was exhibited comparably excellent growth in CPCB and MRS media than *Lactobacillus mali*. In this study, commercially standard MRS medium were used as control to determine the growth characteristics (Fig. 5).

Fig. 5 Growth curve profiles of *Lactobacillus reuteri* and *Lactobacillus mali* in cactus pear cladode broth culture medium compared to synthetic standard MRS culture medium.

cfu/mL represent for colony forming unit per milliliter, MRS represent for de Man, Rogosa, and Sharpe medium, h represents for hours, cactus pear cladode culture medium formulation indicated by 4/100, 4/200 and 4/300 w/v.

Determination of bacterial cells density: the result of this study showed that the cactus pear (*Opuntia ficus-indica*) cladodes media can promotes the growth of *Lactobacillus reuteri* and *Lactobacillus mali*. The optical density value at 600 nm after 48 h incubation was given 0.183 and 0.172 for *Lactobacillus reuteri* and *Lactobacillus mali*, respectively in the CPCB (4:200 w/v) medium which shown high cell density than any other media proportion of CPCA (4:100 w/v and 4:300 w/v) (Fig. 6).

Fig. 6 Cell density of *Lactobacillus* species growth

CPCB represent for cactus pear cladode broth medium, MRS represent for de Man, Rogosa, and Sharpe standard medium

Enumeration of bacterial cell growth: In the present result, the increase bacterial colonies counts were exhibited in the CPCB (4:200 w/v) than in the other formulation of CPCB (4:100 and 4:300 w/v) which is comparable with standard commercial MRS medium. Growth of each *Lactobacillus reuteri* and *Lactobacillus mali* in all formulation of CPCB (4:100 w/v, 4:200 w/v and 4:300 w/v) were observed no significant differences ($P<0.05$) at 24h incubation. However, significant growth variations of *Lactobacillus reuteri* and *Lactobacillus mali* in all formulation of CPCB were exhibited at 36h, 48h, 60h and 72h incubation. Growth of *Lactobacillus reuteri* in both CPCB (4:200 w/v) and MRS (control) medium were shown no significant differences ($P<0.05$) colonies count at 36h, 48h, 60h and 72h incubation. Furthermore, growth variation of *Lactobacillus mali* were observed in each CPCB (4:200 w/v) and MRS (control) medium at 36h, 48h, 60h and 72h incubation (table. 1).

Table. 1 *Lactobacillus* species growth colony counts (logCFU/mL)

<i>Lactobacillus</i> species	Bacterial growth media				
	Time (h)	CPCB (4:100 w/v)	CPCB (4:200 w/v)	CPCB (4:300 w/v)	MRS (control)
<i>Lactobacillus reuteri</i>	0h	2.10±0.10 ^a	2.11±0.08 ^a	2.12±0.01 ^a	2.11±0.02 ^a
	12h	2.12±0.08 ^a	2.13±0.10 ^a	2.13±0.01 ^a	2.20±0.04 ^a
	24h	2.15±0.06 ^b	2.17±0.06 ^b	2.14±0.01 ^b	2.26±0.01 ^a
	36h	2.23±0.03 ^b	2.37±0.01 ^a	2.17±0.01 ^c	2.40±0.03 ^a
	48h	2.34±0.01 ^b	2.40±0.01 ^a	2.24±0.01 ^c	2.42±0.02 ^a
	60h	2.37±0.03 ^b	2.42±0.01 ^a	2.25±0.01 ^c	2.44±0.02 ^a
	72h	2.38±0.02 ^b	2.43±0.01 ^a	2.26±0.01 ^c	2.45±0.02 ^a
<i>Lactobacillus mali</i>	0h	2.10±0.02 ^a	2.10±0.05 ^a	2.05±0.05 ^a	2.12±0.03 ^a
	12h	2.11±0.02 ^b	2.13±0.03 ^{ab}	2.11±0.02 ^b	2.16±0.01 ^a
	24h	2.14±0.01 ^b	2.15±0.02 ^b	2.13±0.01 ^b	2.25±0.01 ^a
	36h	2.17±0.01 ^c	2.30±0.02 ^b	2.15±0.01 ^c	2.37±0.01 ^a
	48h	2.25±0.01 ^c	2.32±0.02 ^b	2.22±0.03 ^c	2.41±0.01 ^a
	60h	2.28±0.02 ^c	2.35±0.03 ^b	2.24±0.02 ^d	2.43±0.02 ^a
	72h	2.30±0.02 ^c	2.36±0.02 ^b	2.25±0.01 ^d	2.43±0.01 ^a

Number of colony counts (logCFU/mL) expressed as mean ± standard deviation (SD) for three independent experiments performed in triplicate, CPCB (Cactus pear cladode broth), w/v (weigh/ volume), MRS (de Man, Rogosa, and Sharpe), h (hours). different superscripts letters (a, b, c, d) in the same row for each *Lactobacillus* species indicate significant differences among means ($P<0.05$).

5. Discussion

In the world researchers are continuously searching for various feasible initiatives to reduce their research expenditures (Hanisah et al., 2022). Therefore, the use of cheap and locally available source microbial culture media is necessary to reduce the culture medium cost as this can be important to support microbial researchers in developing country. In the present study, locally available plant extracts obtained from cactus pear (*Opuntia ficus-indica*) cladode was used as growth culture medium for *Lactobacillus* species. According to the finding of Reda et al. (2020) that cactus pear cladode extract in the form of dehydrated powder revealed a potential nutrient composition required for microbial growth. Similarly,

recent scientific reports indicated that dehydrated powder of cactus pear cladode extract were good microbial growth promoters as they contained all the necessary nutrients as well as growth factors such as carbohydrate, vitamins, fibers, amino acids, proteins, macro nutrients and micronutrients which is very much comparable to that commercial culture medium (Ibrahim et al., 2021; Filannino et al., 2016; Kolniak-ostek et al., 2020). Preparation of culture medium from *Opuntia ficus-indica* cladodes do not need pre-treatments as they show a lower lignin content that is easily usable by microorganism (Magarelli et al., 2022). The present research demonstrated that dehydrated powder of cactus pear (*Opuntia ficus-indica*) cladode extract was exhibited excellent growth promoters for *Lactobacillus reuteri* and *Lactobacillus mali* which is comparable with the commercially synthetic standard MRS medium (control). Commercially available MRS growth media are selective media that contains all the bacterial growth requirement nutrients specifically for the growth of *Lactobacillus* species, and it is suitable for laboratory growth of these bacterial species. However, at industry the large-scale production of *Lactobacillus* species in MRS is not profitable, expensive and non-consumable that used for fermented foods. Therefore, an alternative cost-effective growth medium prepared from edible plant source is necessary (Bolivar-Jacobo et al., 2023; Berisvil et al., 2021). In the case of present study, the evaluation of the costs of all the preparation and availability CPCA, they turned out to be significantly cheaper than the commercial MRS medium. According to the result of current study showed that, cactus pear (*Opuntia ficus-indica*) cladode powder could be used as alternative culture media to the MRS medium for the growth of *Lactobacillus* species as it is suitable growth medium, locally available and obtained from cheap edible plant source. Similar study reported by (Magarelli et al., 2022) that production of alternative culture medium from locally available of cladodes of *Opuntia ficus-indica* extracts to grow *Bacillus amyloliquefaciens*, *Burkholderia ambifaria*, *Pseudomonas fluorescens* and *Azotobacter chroococcum*. Furthermore, mass production of *Lactobacillus* species using low-cost hydrolyzed sugarcane molasses medium have been reported by Acosta-Piantini et al (2023). According to the finding of Tamine et al (2017) that *Opuntia ficus-indica* cladodes used as a raw material for lactic acid production by *Lactococcus lactis subsp. Lactis*. In the present study shown that the morphological of the *Lactobacillus* species colonies grown on both CPCA and MRS media were similar in terms of margin, color and shape. However, colonies formed on MRS medium after 48 h incubation period slightly larger in size compared to CPCA medium. This is may be due to the nutrient complexity of CPCA that do not easily available for the growth of the bacteria than MRS (Acosta-Piantini et al, 2023). During microbial growth, pigment formation relies on a dynamic metabolic equilibrium provided by medium nutritional content such as peptones, minerals, and different ions (Shareef, 2019). However, in the present study, pigment formation by *Lactobacillus* species were not affected by the CPCA medium.

Bacterial growth curves can provide important information for studying microbial growth characterization and selecting optimal growth (Jannah et al, 2021). In the present study, different proportion of cactus pear (*Opuntia ficus-indica*) cladode culture media (4/100, 4/200, and 4/300 w/v) were evaluated considering the growth characteristics of *Lactobacillus reuteri* and *Lactobacillus mali*, which manifested different growth ability and reached different viable cell counts. Accordingly, the CPCA medium were produced different results that a high growth was obtained for *Lactobacillus* species in 4:200 w/v proportion than

other CPCB (4/100 and 4/300 w/v) which is comparable to MRS broth (control) medium. Similar finding reported by Youssef et al. (2016) that *Klebsiella oxytoca* and *Enterobacter agglomerans* exhibited excellent growth in broth culture medium prepared from dehydrated powder of cactus pear (*O. ficus-indica*) which is very much comparable to the synthetic standard N-deficient combined carbon source medium (CCM). In bacterial growth study, it is known that the lag phase is the time in which a bacterial growth passes from the adaptation phase (Lag) to the exponential growth phase (Log) (Berisvil et al., 2021). According to the current research, *Lactobacillus* species were exhibited higher lag phase CPCB medium than standard MRS medium. This difference may be due to the nutrient complexity and suppressive metabolites of CPCB medium that contributes to take long time to adapt the medium. It is known that bacterial growth with a higher lag phase require longer time to grow. The present growth curve for *Lactobacillus reuteri* and *Lactobacillus mali* showed the logarithmic growth phase (Log) at 24-36h in CPCB medium and at 12-36h incubation time in MRS medium (control). In this study, high *Lactobacillus* species growth observed in CPCB (4:200 w/v) proportion in which their growth accelerates and cell number (log CFU/mL) rapidly increased within the time exponential growth phase. This medium (4:200 w/v) proportion is very comparable to commercial MRS medium and have a potential to replace it. Growth of bacteria in broth medium can be monitored using turbidity or light scatter measurements using spectrophotometer. As the number of cell density increases, the broth medium becomes increasingly cloudy or turbid because light passing through it is scattered by the bacteria present (Held, 2021). Based on the present study, the OD values at 600 nm after 48h growth exhibited higher cell density in CPCB (4:200 w/v) proportion medium than other CPCB (4/100 and 4/300 w/v) medium proportion.

Growth of *Lactobacillus reuteri* and *Lactobacillus mali* were exhibited higher number of colonies in the chemical synthetic MRS broth medium (control) compared to different formulation of cladode pear (*Opuntia ficus-indica*) based culture broth medium. However, growth of *Lactobacillus reuteri* in CPCB (4:200 w/v) culture medium showed no significant difference ($P < 0.05$) in colony counts (log CFU/mL) compared to the MRS medium. Additionally, in this study, it was observed that the growth of *Lactobacillus mali* in CPCB (4:200 w/v) significant difference in bacterial count compared to MRS medium. In the present study, among all formulated media, CPCB (4:200 w/v) culture medium were best comparable with MRS medium which could serve as viable alternatives to support *Lactobacillus* species growth. Moreover, CPCB (4:200 w/v) medium were found as the best medium that supported bacterial growth in short incubation time compared to CPCA (4:200 w/v) medium. *Lactobacillus* species growth on the surface of CPCA (4:200 w/v) medium were taken above 48h to see visible colony whereas growth in CPCB (4:200 w/v) medium were taken above 24h to increase their number of cells. This may be the nutrient content within cactus pear cladode broth medium easily utilize by the bacteria they grow compared with cactus pear cladode agar medium.

Declarations

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Statements and declarations

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Competing interest

The authors declare that they have no financial interest to disclose.

Author contribution

Material preparation, data collection, and analyses were performed by Ashenafi Teklay Yaekob, Etsay Mesele Egigu, and Melaku Mekonen Kasegn. Tesfakiros Semere, Samson Zemikael Haftu and Asqual Zeslassie Gebremeskel contributed in manuscript editing, reviewing and data analysis. The first draft of the manuscript was written by Ashenafi Teklay Yaekob and all authors commented on previous versions of the manuscript. All the authors have read and approved the final manuscript.

Data Availability

All data generated or analyzed during this research are available in this article.

Ethics approval

Not applicable and not related with human or animal subjects.

Consent to participate

Consent to participate does not apply to this study

Consent to publish

Not applicable

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Figures

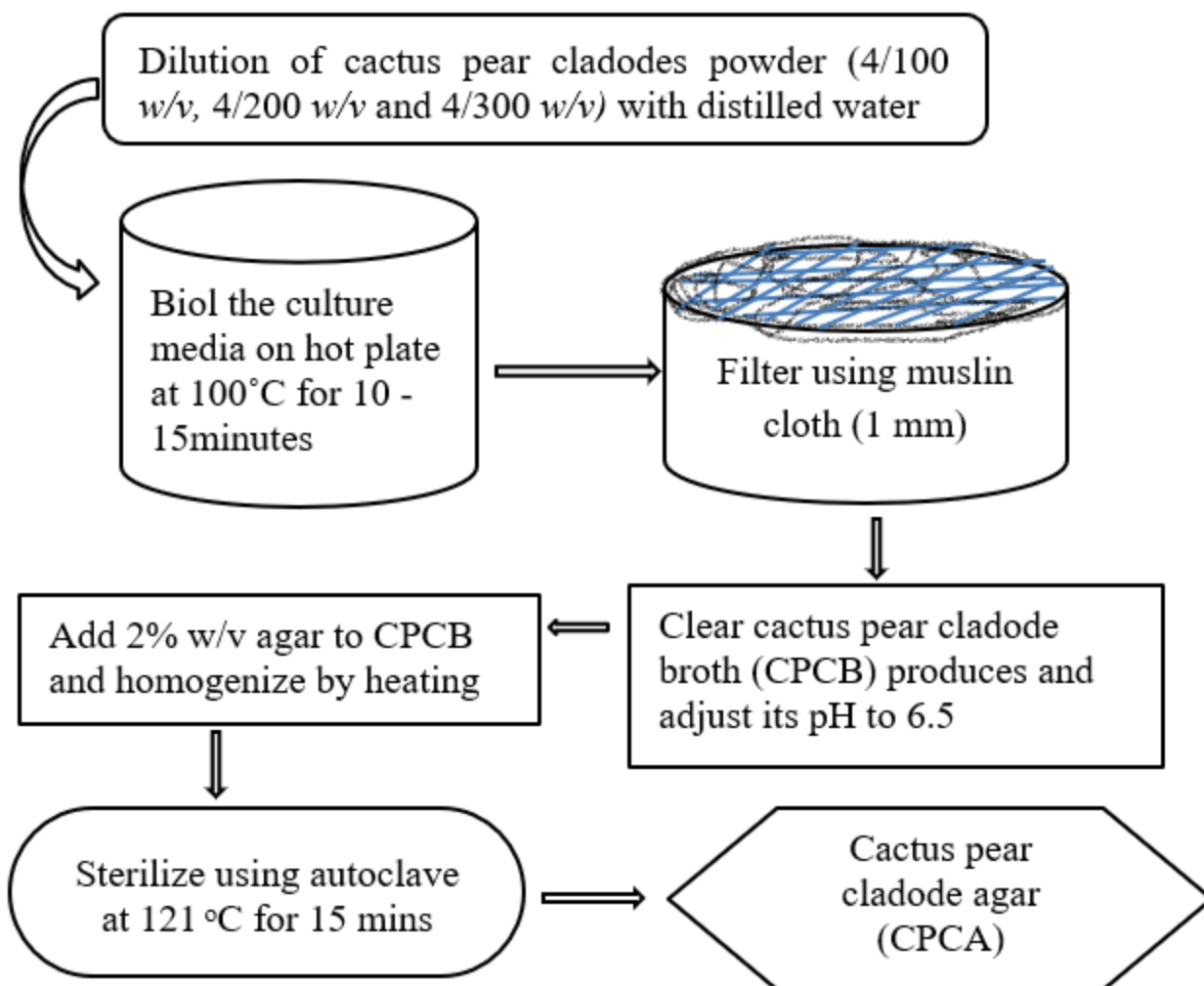


Figure 1

A workflow of cactus pear cladode media development

CPCA represents for cactus pear cladode agar, CPCB represent for cactus pear cladode broth, w/v represents for weight/volume measured by gram and milliliters.



Figure 2

Cladode powder of *Opuntia ficus-indica*

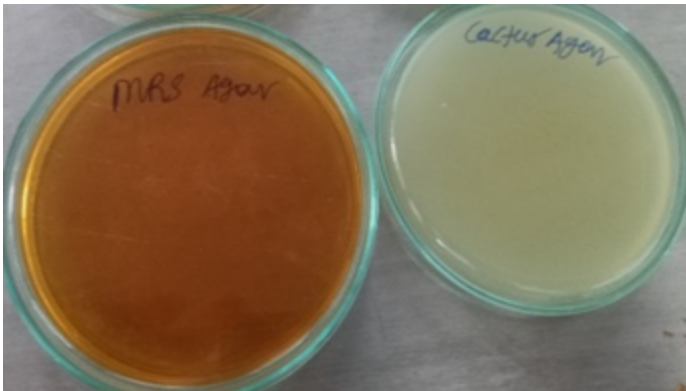


Figure 3

CPCA and MRS agar culture media

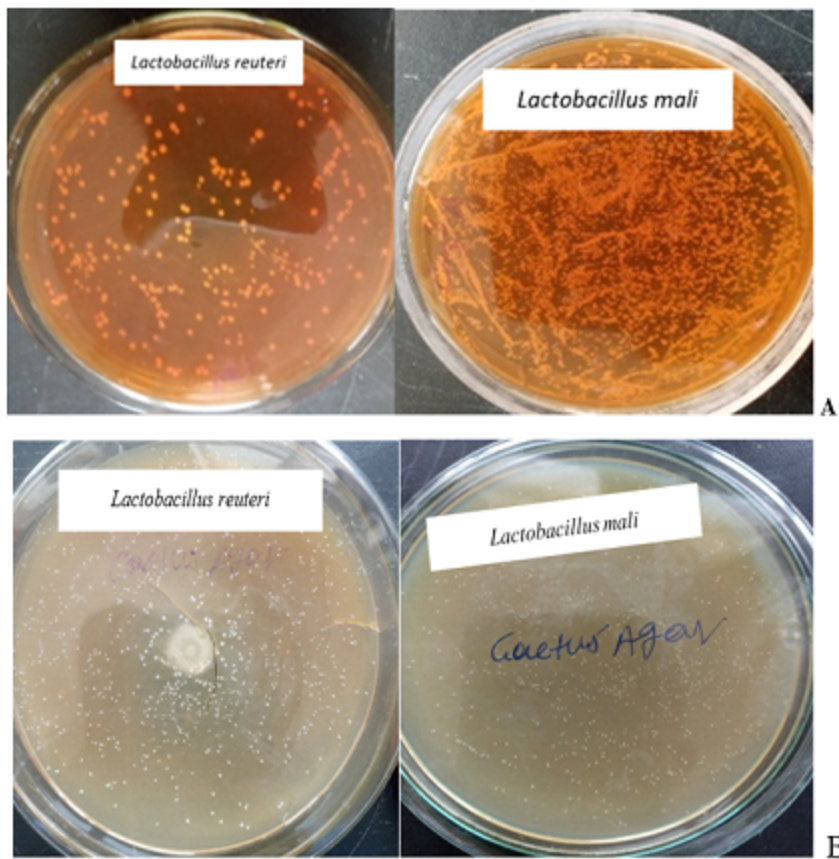


Figure 4

Lactobacillus species growth on culture media: (A) Lactobacillus growth on MRS agar medium; (B) Lactobacillus growth on cactus pear cladode agar (CPCA) medium.

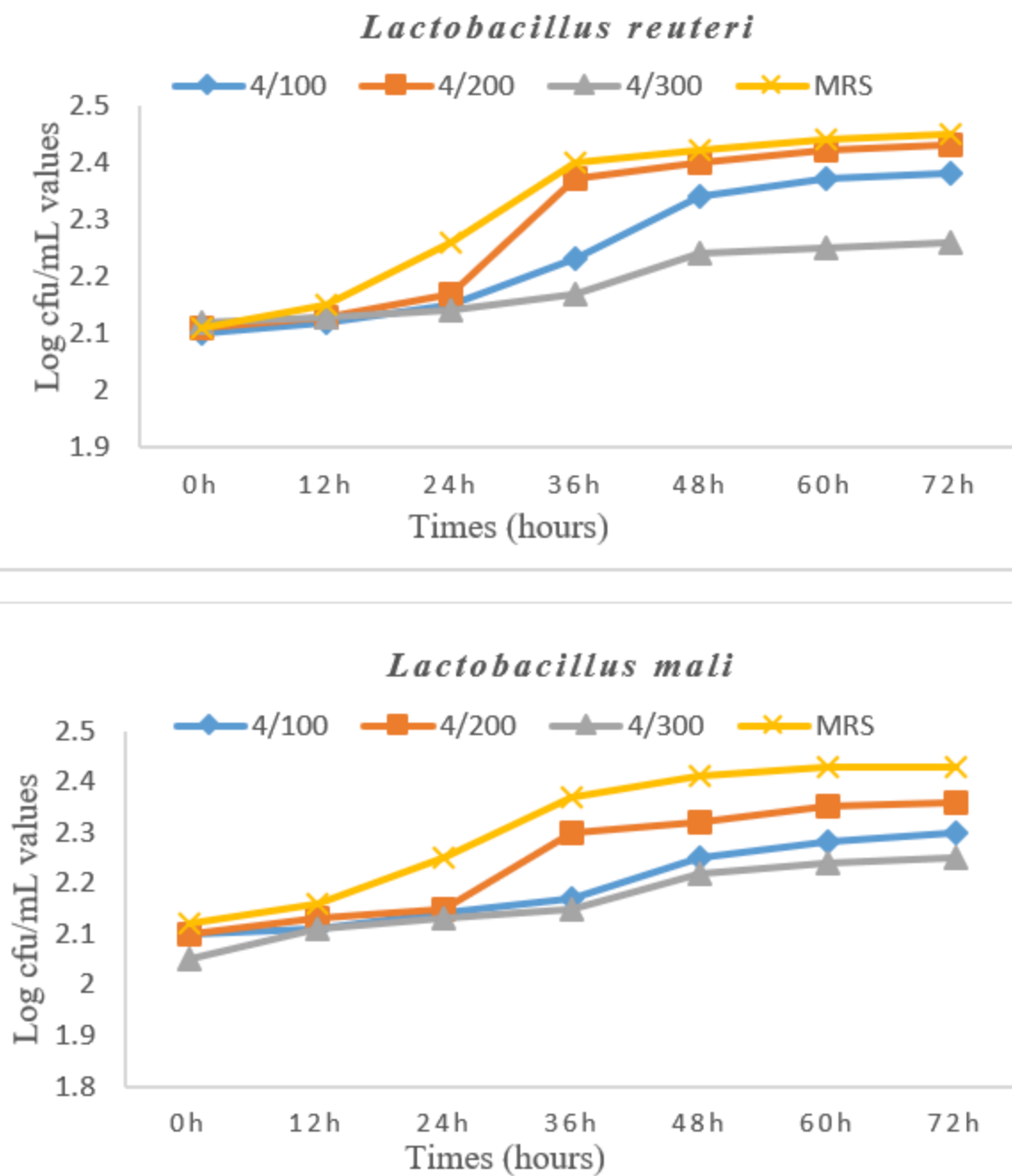


Figure 5

Growth curve profiles of *Lactobacillus reuteri* and *Lactobacillus mali* in cactus pear cladode broth culture medium compared to synthetic standard MRS culture medium.

cfu/mL represent for colony forming unit per milliliter, MRS represent for de Man, Rogosa, and Sharpe medium, h represents for hours, cactus pear cladode culture medium formulation indicated by 4/100, 4/200 and 4/300 w/v.

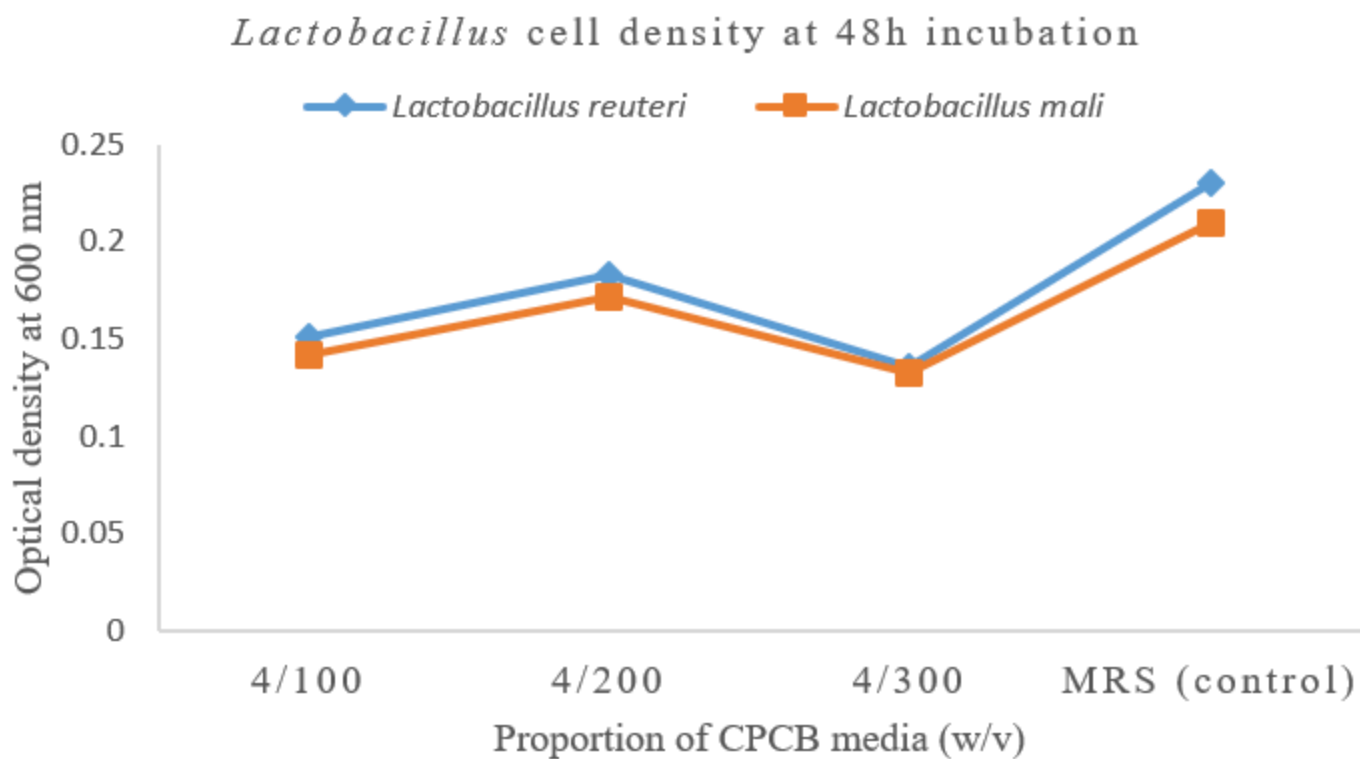


Figure 6

Cell density of *Lactobacillus* species growth

CPCB represent for cactus pear cladode broth medium, MRS represent for de Man, Rogosa, and Sharpe standard medium