



RESEARCHPAPER

Alpha-glucosidase and DPP-IV inhibitory activities of Indigenous Red Kandhari cow milk fermented by *Lactobacillus* spp

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Abstract

While current research heavily focuses on the bioactive components of bovine milk from European cattle (*Bostaurus*), limited studies have explored the potential of indigenous cattle (*Bosindicus*) milk. These milk-derived bioactive components hold vast potential for human nutrition and the management of various lifestyle diseases. Diabetes mellitus (DM), a metabolic disorder marked by elevated blood glucose levels (hyperglycemia), has emerged as a critical healthcare challenge in India. The antidiabetic properties of bioactive peptides are specifically linked to their ability to inhibit alpha-glucosidase and dipeptidyl peptidase-IV (DPP-IV)—the primary enzymes driving Type 2 diabetes. Consequently, this study was designed to leverage the proteolytic activity of *Lactobacillus* spp. for the production of alpha-glucosidase and DPP-IV inhibitory milk peptides from the milk of Indigenous Red Kandhari cow breed of Marathwada region of Maharashtra. Fermented Red Kandhari cow skim milk prepared by as many 05 strains of *Lactobacillus* as a single culture was analyzed for peptide content, alpha-glucosidase and DPP-IV enzymes inhibition profiles. Peptide contents (measured by OPA method) were ranged between 2.08 to 2.44 mg of leucine/ml. The alpha-glucosidase and DPP-IV inhibitory activities were found highest in skim milk fermentate with *L. helveticus* NCDC 292 ($31.8 \pm 0.12\%$) and *L. bulgaricus* NCDC 8 ($23.40 \pm 0.22\%$) respectively. The results from this study showed that peptides with α -glucosidase and DPP-IV inhibitory activity can be generated by using *Lactobacillus* spp. from Red Kandhari cow milk proteins.

KEYWORDS:

Diabetes mellitus, Alpha-glucosidase, Dipeptidyl Peptidase-IV, *Lactobacillus*, *Bostaurus*

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Introduction

The profound association between humans and cattle spans millennia, enduring because cattle consistently provide essential resources such as milk, meat, manure, and

draught power to mankind (Sharma et al., 2018). In India, the registration of 50 distinct cattle breeds highlights the country's rich livestock biodiversity (ICAR-NBAGR).

Within human health, bioactive peptides possessing antidiabetic activities hold strong potential as functional foods designed to mitigate widespread lifestyle disorders. Diabetes mellitus (DM), a metabolic disorder characterised by persistent hyperglycemia (elevated blood glucose), has escalated into a major epidemic across India.

The antidiabetic efficacy of milk-derived bioactive peptides is directly linked to their ability to inhibit alpha-glucosidase and dipeptidyl peptidase-IV (DPP-IV)—the key enzymes responsible for postprandial hyperglycemia in Type 2 diabetes (Patil et al., 2015). These functional peptide fragments are liberated either via the degradation of native milk proteins by gastrointestinal digestive enzymes or through proteolysis by lactic acid bacteria (LAB) during milk fermentation. Consequently, targeting the inhibition of alpha-glucosidase and DPP-IV represents an exceptionally effective strategy for managing Type 2 diabetes (Lacroix and Li-Chan, 2013).

However, there is a pronounced dearth of empirical data regarding the bio-functional attributes of milk from indigenous cattle breeds compared to their European counterparts. The Red Kandhari is an agile, indigenous breed native to western and central India, found principally in the Latur and Nanded districts of Maharashtra. Addressing this research gap, the present study was designed to explore the milk proteins of the indigenous Red Kandhari cow breed to maximize the production of alpha-glucosidase and DPP-IV inhibitory peptides using precisely selected proteolytic *Lactobacillus* spp.

Material and Method

Culture & Milk: Lactic acid bacteria cultures (freeze dried ampoules) were procured from the National Collection of Dairy Culture (NCDC), ICAR-National Dairy Research Institute (ICAR-NDRI), Karnal, India. Red Kandhari cow milk was procured from the private dairy farm from Wardha District of Maharashtra.

Proteolytic activity of *Lactobacillus* cultures: Proteolytic activity was qualitatively determined on skim milk agar (Lim et al., 2019). Active culture was inoculated (@1%, v/v) in sterile Red Kandhari skim milk and incubated for 24 h at 37°C. The fermented milk was stored at 4°C for

further analysis. Sterile skim milk incubated under similar condition was taken as control. Whey fraction was prepared from fermented skim milk by centrifugation at 10000 rpm for 10 min at 4°C. Proteolytic activity of fermented skim milk was evaluated by determining free NH₂ group using Ortho-phthaldialdehyde (OPA) method (Church et al., 1983).

Alpha-glucosidase inhibitory activity

The method of Ramchandran and Shah (2009) was adopted with some modifications to measure alpha-glucosidase inhibitory activity of the whey samples.

$$\text{Alpha-glucosidase inhibition (\%)} = [(A-B) - (C-D)] / (A-B) * 100$$

Where, A = O.D. of substrate and enzyme at 10 min

B = O.D. of substrate and enzyme at 0 min

C = O.D. of substrate, enzyme and whey sample at 10 min

D = O.D. of substrate, enzyme and whey sample at 0 min

Dipeptidyl peptidase IV inhibitory activity

DPP-IV inhibitory activity was measured according to the method described by Lacroix and Li-Chan (2012) with some modifications.

Statistical analysis

Statistical analysis was performed using MS-Excel and Graphpad Prism. Results were presented as Means ± Standard Error of Mean (SEM), and statistical significance was set at p<0.05 or p<0.01

Result and Discussion

Cultures:

Purity of *Lactobacillus* cultures was evaluated by morphology, Gram staining and catalase test. All the cultures were catalase negative, gram-positive rods.

Evaluation of *Lactobacillus* cultures for proteolytic activity

Proteolytic activities of 21 strains of *Lactobacillus* cultures were evaluated on skim milk agar (37°C for 24 h). All cultures showed zone of clearances on skim milk agar indicating proteolytic activities (Table 1). Size of clear

zone in skim milk agar indicated the extent of proteinases produced by *Lactobacillus* cultures (Pailinet *et al.*, 2001). *L. helveticus* NCDC 288 and *L. helveticus* NCDC 292 showed

Peptide content

During fermentation, milk proteins were hydrolyzed by *Lactobacillus* proteinases and peptidases, resulting in the release of various peptides. Peptides content in whey fraction of fermented skim milk by *Lactobacillus* cultures was measured by OPA method (Fig. 1). Peptide contents were ranged between 2.08 to 2.44 mg of leucine equivalent/ ml, whereas unfermented skim milk had a peptide content of 0.15 mg leucine/ml. The free amino group content in fermented milk was higher due to the proteolytic activity of microorganisms compared to unfermented skim milk (Donkor, 2007). Proteolytic activity was maximum for *L. helveticus* NCDC 288 (2.44 ± 0.03 mg leucine/ ml) followed by *L. helveticus* NCDC 292 (2.42 ± 0.02 mg leucine/ ml). Ramesh *et al.* (2012) reported that *L. rhamnosus*, *L. helveticus*, and *L. delbrueckii* ssp. *bulgaricus* were the highest protease producers.

DPP-IV inhibitory activity

DPP-IV inhibition potential of peptides in whey fraction was also determined (Fig. 2). Higher inhibition was observed in milk fermentate with *L. bulgaricus* NCDC 8 ($23.40 \pm 0.22\%$) and *L. helveticus* NCDC 288 ($22.5 \pm 0.10\%$), followed by *L. helveticus* NCDC 292 ($21.6 \pm 0.15\%$). The lowest inhibition was found in *L. casei* NCDC 17 ($20.2 \pm 0.10\%$). Casein (CN) hydrolysates enriched in short peptides have showed DPP-IV inhibitory activity (Boots, 2006). DPP-IV inhibitory activity was reported in water-soluble extract of a Gouda-type cheese (Uenishiet *al.*, 2012).

Alpha-glucosidase inhibitory activity

Whey fractions of all fermented skim milks of *Lactobacillus* spp. were evaluated for inhibition of alpha-glucosidase (Fig. 3). The inhibitory activities were higher for the fermentates from *L. helveticus* NCDC 292 ($31.8 \pm$

the larger zones of clearances. Percival *et al.* (1999) reported a wide clear zone for the whole culture compared with supernatant fluid on skim milk agar. 0.12%) and *L. helveticus* NCDC 288 ($30.7 \pm 0.21\%$), followed by *L. rhamnosus* NCDC 19 ($29.3 \pm 0.25\%$). The lowest inhibitory activity was observed with *L. bulgaricus* NCDC 8 ($20.6 \pm 0.20\%$). Unfermented milk displayed miniscule inhibition (3.20%). Ramchandran and Shah (2008) reported the inhibition of alpha-glucosidase by filtrate of LAB fermented skim milk. Cell-free excretory supernatant (CFS) obtained from the *Lactobacillus* sp. showed alpha-glucosidase inhibitory activity (Zenget *al.*, 2016). The inhibitory activity might be due to the specific peptide-cleavage and fragmental release in CFS.

Conclusion:

The findings of this study demonstrate that functional peptides with potent alpha-glucosidase and DPP-IV inhibitory properties can be successfully generated through the fermentation of indigenous Red Kandhari cow milk using *Lactobacillus* spp. Notably, this bioactivity was shown to be a strain-specific characteristic, with *L. bulgaricus* NCDC 8 and *L. helveticus* NCDC 292 emerging as the most effective cultures for bioactive peptide production.

Moving forward, further structural and functional characterisation of these liberated peptides is warranted. Exploring bioactive peptides within fermented products derived from indigenous (desi) cattle milk presents a highly promising opportunity for the dairy sector. Specifically, it offers a strategic pathway for commercial expansion and the value-added promotion of traditional dairy products enhanced with evidence-based health benefits.

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