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Unveiling *kinema*: blending tradition and science in the Himalayan fermented soya delicacy

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Abstract

Kinema, a sticky-textured fermented soybean food with *umami* flavour, is originated in the historical Limbuwan region, now situated in the eastern districts of Nepal. It has since expanded to different areas in Darjeeling Hills and Sikkim in India and Bhutan. The Limboo community, one of the oldest indigenous groups in the Himalayas, has developed a method for fermenting soybeans to produce *kinema*. *Bacillus* is the main bacterial genus responsible for fermenting *kinema*, along with lactic acid bacteria, yeasts, and fungi. *Kinema* is full of nutrients like protein, fats, carbohydrates, amino acids, vitamins, and minerals and offers health benefits through its antioxidant, anti-inflammatory, antiobesity, and antidiabetic properties. A novel strain of *Bacillus subtilis* Tamang has been identified in *kinema* samples from Darjeeling Hills, India, exhibiting elevated levels of γ-PGA and various biomarker genes for health benefits and biological functionalities. *Kinema* contains secondary metabolites including several bioactive compounds, immunomodulators, vitamin B-complexes, and others that help promote good health. The transcriptome of the *B. subtilis* Tamang strain reveals genes associated with GABA production, Vitamin B12 synthesis, and fibrinolytic activity. Safety evaluations have demonstrated that the strain and the production of kinema do not pose any toxicity risks based on animal studies. Despite facing challenges in terms of its strong taste and slimy consistency for broader acceptance, the bioactive elements and safe starter cultures found in *kinema* hold promise for the development of functional food.

Keywords Kinema, Fermented soybean, Food heritage, Ethnic food, *Bacillus subtilis*, The Himalaya, Metagenomics

Introduction

The Himalaya is believed to have originated around 5 million years ago, during the Eocene or Paleocene period [1]. It is divided into four distinct geographical regions based on elevation: snow-capped mountains, alpine and temperate zones with rich forests, sub-temperate zones for agriculture and pastoralism, and foothills with fertile lands for agriculture and livestock. These regions cover the Western, Central, and Eastern Himalayas, spanning across India, Nepal, China (Tibet), and Bhutan. The food heritage of the Himalayan community is influenced by the regions' climatic conditions, agricultural resources, and ethnic origins. Himalayan ethnic foods, known for their unique taste, flavour, and health benefits, including fermented foods and alcoholic beverages, are gaining popularity within tourists, nature enthusiasts, food enthusiasts, and hikers [2]. The ancient Himalayan people developed fermentation methods to preserve perishable raw materials, creating flavourful fermented foods using their knowledge of ethno-microbiology [3]. The diverse ethnic fermented foods and alcoholic beverages of the Himalayas play a significant role in the traditional

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gastronomy of the region, influenced by ethnicity, dietary culture, religious practices, and climatic diversity [4]. Fermented soybean dishes are a key part of the diet for Mongoloid ethnic communities in the Eastern Himalayan regions of India, Nepal, and Bhutan. These dishes are part of a wide variety of fermented foods in the Himalayas, showing an important development in human history. There are three types of fermented soybean foods in Asia, categorized by the fermenting organisms involved in fermentation: (1) fermented by filamentous fungi, yeast, and lactic acid bacteria; (2) fermented by bacteria, mostly *Bacillus* spp.; and (3) fermented by filamentous fungus [5, 6]. In the regions of Eastern Himalayas, people traditionally make and eat soybean foods that are fermented using bacteria [7]. The Himalayan people do not have a history of consuming soybean foods fermented with fungi. These bacterial-fermented soybean foods have a sticky texture and a savoury taste, known by different names in various regions such as *kinema* in eastern Nepal, Darjeeling Hills, and Sikkim in India, southern Bhutan, *bemerthu* and *bekanthu* in Assam, *grep-chhurpi, peha, peron namsing*, and *peruñyaan* in Arunachal Pradesh, *axone/aakhonii* in Nagaland, *hawaijar* in Manipur, *tungrymbai* in Meghalaya, *bezeithu* in Tripura, and *bekang* in Mizoram (Fig. 1). This review discusses the food heritage, microbiology, and health advantages of *kinema*, a traditional fermented soybean dish enjoyed as a side dish curry by the Nepali/Gorkha community in the Eastern Himalayan regions of North East India, eastern Nepal, and southern Bhutan.

The origin and history of soybean and *kinema* in the Eastern Himalayas

The origins and history of soybean cultivation in the Eastern Himalayas are still debated because there are no archaeological studies or historical documents available. It is believed that wild soybeans were first domesticated in the eastern part of Northern China during the Shang dynasty in 1100 BCE, which is considered the primary soybean gene pool [8]. The cultivation of domesticated soybeans expanded from northern China to central and southern China, Southeast Asia, and the Korean peninsula during 300 BCE and then to Japan by the seventh century CE during the Zhou dynasty [9]. Genetic analysis has confirmed that soybeans originated from Manchuria-Mongolia-Korea [10]. Some hypothetical analyses suggest that soybeans, known locally as *bhaṭamāsa* in the Hindi/Nepali language, were potentially introduced to the Eastern Himalayan regions from China [11], possibly Yunnan Province, or via traders from Indonesia through Myanmar to India

Fig. 1 In the Eastern Himalayan regions of North East India, Nepal, and Bhutan, there is a distribution of diverse naturally fermented soybean foods with a gooey texture and *umami* flavour. These include *kinema* in eastern Nepal, Darjeeling Hills, and Sikkim in India, southern Bhutan, *bemerthu* and *bekanthu* in Assam, *grep-chhurpi, peha, peron namsing*, and *peruñyaan* in Arunachal Pradesh, *axone* in Nagaland, *hawaijar* in Manipur, *tungrymbai* in Meghalaya, *bezeithu* in Tripura, and *bekang* in Mizoram

[12]. North East India is viewed as a passive microcenter within the soybean secondary gene centre [13], while a tertiary soybean gene centre may be in central India, particularly representing Madhya Pradesh state as the largest soybean producer. The origins of soybeans often have roots in myths and legends, with beliefs that the Limboo people, one of the oldest aboriginal Kirat races of the Gorkha community in the Eastern Himalayas [14, 15], were among the first to cultivate soybeans. According to folklore and myths of the Limboo community, soybeans, referred to as "chembi" in their language, were domesticated by them between 2500 and 100 BCE [16].

India is the fifth largest producer of soybeans globally, but historically, soybeans were not widely embraced as a traditional food in Indian cuisine due to their distinct taste and local dietary preferences [17]. Fermented soybean products are traditionally eaten by predominantly Mongoloid communities in the Eastern Himalayan areas of North East India, Nepal, and Bhutan (Fig. 1). These groups consist of the Gorkha, Lepcha, Bhutia, Mizo, Hmar, Lakher, Pawi, Meiti, Kuki, Angami, Chakhesang, Ao, Sema, Rengma, Lotha, Chang, Konyak, Sangtam, Phom, Zeliang, Mao, Maram, Tangkhul, Maring, Anal, Mayao-Monsang, Lamkang, Nockte, Haimi, Htangun, Ranpan, Kolyo, Kenyu, Kacha, Yachimi, Kabui, Uchongpok, Makaoro, Jeru, Somra, Chakmas, Monpa, Sherdukpen, Memba, Khamba, Khampti, Singpho, Adi, Aka, Apatani, Bangni, Nishing, Mishmi, Miji, Tangsa, Nocte, Wancho, Bodo, Karbi, Miri, Khasi, Garo, Lhotshamp, Drukpa/Ngalop, and Sharchop. In the Eastern Himalaya, two main types of soybeans, the 'yellow cultivar' and the 'dark brown/black cultivar', are typically grown in the sub-Himalayan regions as a standalone crop or alongside rice and maize. Harvesting of these soybeans usually takes place between November and December.

The Limboo community has a reputation for mastering the technique of fermenting soybeans to produce kinema, a sticky and flavourful delicacy. The term *kinema* is derived from the Limboo word kinamba,' with ki' indicating fermented and 'namba' meaning flavour. This practice is believed to have started around 600 BCE during the Kirat dynasty in Limbuwan, the historical realm of the Limboo [18], which now comprises the nine districts of modern Nepal including Jhapa, Ilam, Panthar, Taplejung, Morang, Sunsari, Dhankuta, Terhthum, and Sankhuwashava. Although there is limited archaeological and historical proof regarding the origins and age of *kinema*, it is likely one of the oldest fermented soybean foods in the Himalayan culinary heritage. During a visit to the mountainous regions of Ilam, Dhankuta, and Terhthum in 1991, the author encountered mainly Limboo communities. Through surveys and interviews with elderly residents of these areas, it was suggested that *kinema* originated in the historical Limbuwan.

Kinema is similar to other bacterial-fermented sticky and *umami*-flavoured soybean foods from Southeast Asia, like *pe poke* in Myanmar, *thua nao* in northern Thailand, *sieng* in Cambodia, *douchi* in Yunnan Province of China, *cheonggukjang* in Korea, and *natto* in Japan. A concept known as the 'natto triangle' [19], later renamed as the *'kinema-thua-nao-natto* triangle (KNT)' [18], illustrates the consumption of these types of fermented soybean foods in unique dishes specific to the countries within this KNT triangle, not found elsewhere in the world. The KNT triangle represents a significant theoretical development in the dietary evolution of Asian populations, now supported by sequence-based metagenomics research and multi-omics approaches. However, the preparation of fungal-fermented soybean products like *miso, soy sauce, tofu*, and *tempeh* is not a traditional practice in the Himalayas.

Traditional fermentation and culinary

In eastern Nepal, local dark brown soybean seeds (Fig. 2a) are used to make *kinema*. In the Darjeeling Hills and Sikkim in India, yellow soybean seeds (Fig. 2b) are preferred for *kinema* production. In the southern region of Bhutan, small-seeded soybean seeds are commonly used. The process of making *kinema* in India, Nepal, and Bhutan follows similar steps (Fig. 3). The traditional method involves selecting, cleaning, washing, and soaking soybean seeds in water overnight. The soaked seeds are drained, placed in a container of fresh water, and boiled over a firewood flame in an earthen oven for 2–3 h until they become soft. After boiling, the seeds are cooled on a bamboo mat for a few minutes, then moved to a wooden mortar, where they are gently split using a wooden pounder to separate the cotyledons. From a scientific perspective, the ethnographic understanding of the producers from different ethnic backgrounds suggests that breaking apart the cotyledons of soybean seeds could potentially enhance the surface area to accelerate the fermentation process by aerobic spore-forming bacteria, a crucial and distinct phase in *kinema* production when compared to similar Asian sticky fermented soybean products. Adding a small amount of wood ash to the cooked soybeans is a unique practice in *kinema* production, not found in other fermented soybean foods. Approximately 1% or less of fresh wood ash is added to the cooked soybeans and thoroughly mixed in, a practice exclusively seen in *kinema* production. Additionally, wood ash is a great source of potassium, calcium, and other essential nutrients [20] and is also used for neutralization to increase the pH to an alkaline level. The traditional *kinema* producers may not know the

Fig. 2 **a** Seeds of a dark brown or black variety of locally grown soybeans; **b** seeds of a yellow variety of locally grown soybeans; **c** freshly produced *kinema*; **d** *Kinema*-infused curry; **e** pickle made from *kinema*; **f** chunky *kinema* soup

scientific mechanism behind it, but it is understood that *kinema* fermentation is alkaline in nature. Split soybeans are placed in a basket lined with fern fronds, or fig plant, or banana leaves and left to ferment above an earthen kitchen oven at a temperature of around 25–40 °C for 1–3 days. The fermentation duration varies depending on the season and temperature, as well as the location. Fermentation is considered complete when a whitish sticky substance appears on the soybeans, indicating the presence of *Bacillus* spp. [2] with a slightly ammoniacal taste ($pH > 8.0$). When a wooden pestle or a spoon touches the whitish mucilaginous materials appeared on fermenting soybeans, it produces a sticky thread-like material, which nothing but a poly-γ-glutamic acid (γ-PGA) [21]. Traditional consumers prefer sticky *kinema* (Fig. 2c) for its sensory quality. Fresh *kinema* remains edible for three days'; however, sun-drying can prolong its storage duration. Sun-dried *kinema* is capable of being kept at room temperature for around 1 month.

In the Himalayas, the culinary preparation of *kinema* differs slightly from other Asian sticky fermented soybean foods. This variation is likely due to the local preference for curry-type side dishes. To make *kinema* curry, the kinema is sautéed in hot vegetable oil and then mixed with chopped onions, tomatoes, 1–2 sliced green chilies, a pinch of salt, and turmeric powder. The mixture is blended for 3–5 min before adding a little water to create a thick curry consistency. It is then cooked for an additional 5–7 min. *Kinema* curry is typically served as a side dish alongside the main course or entrée (Fig. 2d).

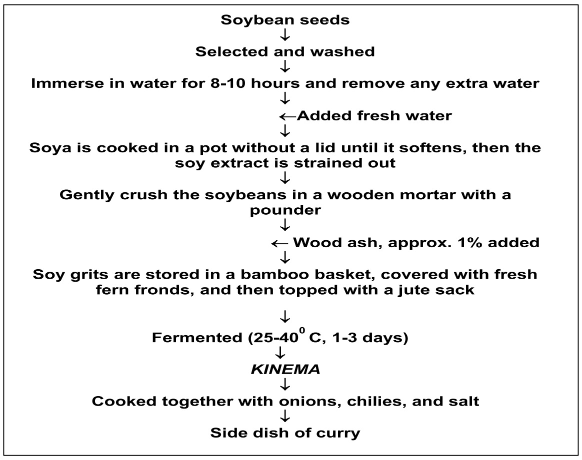

Fig. 3 Flow chart showing the traditional process of making *kinema* in the Eastern Himalayas

The process of making *kinema* at home differs depending on the region and community, with mountain women using their ethno-microbiological knowledge. This traditional skill has been passed down through generations, with mothers teaching their daughters for over 3000 years [18]. *Kinema* production serves as a small cottage industry for income generation, particularly among rural women in mountainous areas. It is commonly sold in local markets across eastern Nepal, North East India, and Bhutan.

Ethno-microbiology and metagenomics of *kinema*

The Himalayan fermented soybean food undergoes spontaneous fermentation, mainly by rod-shaped, spore-forming, and semi-aerobic bacteria. Studies using phenotypic, biochemical, and molecular identification techniques have shown that *Bacillus subtilis* is the primary fermenting bacterium in *kinema* [22–24]. Additionally, *Bacillus licheniformis, B. sonorensis, Enterococcus faecium, Candida parapsilosis,* and *Geotrichum candidum* have also been identified in the fermentation process [22, 25]. Soybeans, unwashed wooden mortar and pounder, fern fronds, and other leaves can help introduce necessary microorganisms for natural fermentation in the making of *kinema* [26]. Culture-independent methods have led to a significant change in taxonomy and identification of various microorganisms in *kinema*. A study analysed bacterial and fungal communities in kinema samples from the Eastern Himalayan regions, specifically collected from different locations in Darjeeling Hills and Sikkim in India, eastern Nepal, and southern Bhutan using high-throughput sequence analysis [27]. The results showed that Bacillota was the most abundant phylum in *kinema*, followed by Pseudomonadota and other phyla. The study revealed a high diversity of microorganisms in *kinema* samples from the Eastern Himalayan regions, identifying 277 species of bacteria (99% culturable and 1% unculturable) and 80 fungal species (33.72% culturable and 66.28% unculturable). The most abundant bacterium found in *kinema* was *Bacillus subtilis*, consistent with previous findings from culture-dependent methods. *Bacillus subtilis* is listed as a safe microorganism in the microbial food cultures safety inventory of the International Dairy Federation [28]. Other bacteria detected in *kinema* included *B. licheniformis, B. thermoamylovorans, B. cereus,* and others. The genus *Bacillus* exhibited diversity with 27 species at varying abundances. Ascomycota was the predominant fungal phylum in *kinema*, comprising species such as *Wallemia canadensis, Penicillium* spp., *Aspergillus* spp., and other filamentous moulds, as well as *Pichia sporocuriosa, Trichosporon spp., Saccharomycopsis malanga,* and *Rhodotorula cycloclastica* as abundant yeasts in *kinema* [27]. The complete microbial community, which includes bacteria, eukaryotes (yeast and fungi), viruses, archaea, and an unclassified domain, can be analysed through shotgun metagenomic sequencing. Hence, we used shotgun metagenomic sequencing tool and profiled 47 phyla, 331 families, 709 genera, and 1560 species of domains in *kinema* samples from Nepal, India, and Bhutan [29]. Bacteria were the most prevalent group (97%), followed by viruses, eukaryotes, and archaea [29, 30].

In comparison with HTS, where only 27 species of *Bacillus* were found [27], over 280 *Bacillus* species were identified in *kinema* samples through shotgun metagenomic sequencing [29]. *Bacillus subtilis* was the most prevalent species, along with other abundant species like *Bacillus glycinifermentans, B. cereus, Brevibacillus borstelensis, B. licheniformis, B. thermoamylovorans, B. coagulans, B. circulans, B. paralicheniformis*, and others (Fig. 4). Deep metagenomic analysis of *kinema* revealed

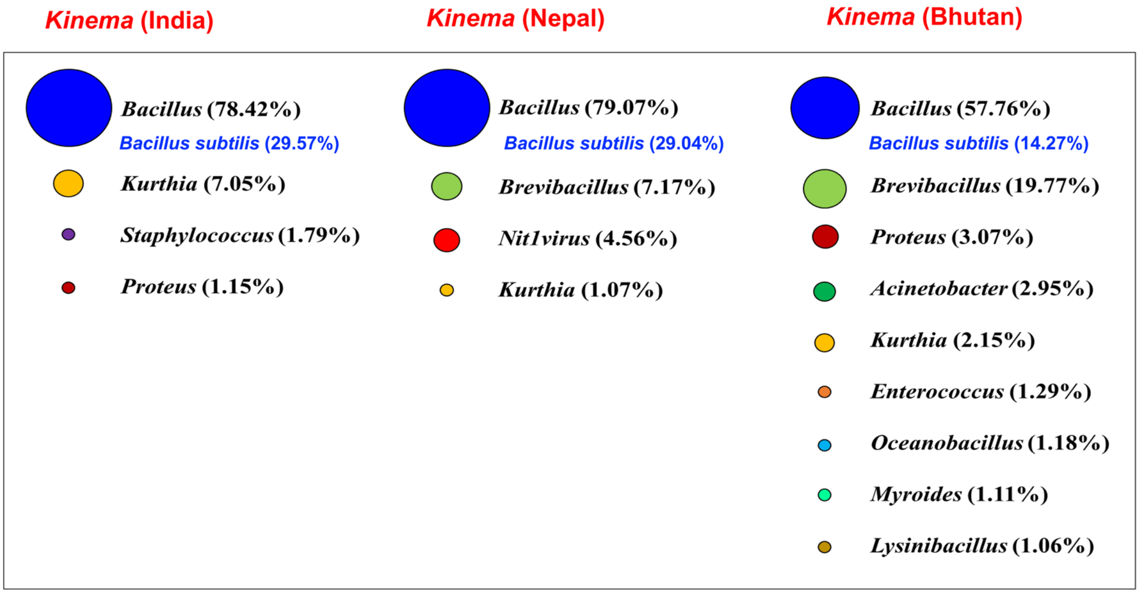

Fig. 4 *Bacillus* is the most common genus found in the *kinema* metagenome of North East India, Nepal, and Bhutan, with other bacterial genera also present in abundance (> 1%) [29]

vast microbial communities, due to enhanced taxonomic resolution at low-abundance genera or species. We recently discovered a new strain of *Bacillus subtilis* Tamang in *kinema* samples from Darjeeling Hills, India. This strain produces high levels of poly-γ-glutamic acid (γ-PGA), has a high molecular weight, can solubilize iron, and contains multiple biomarkers and genes without any antimicrobial resistance (AMR) genes or virulence factors [31]. The dominance of *Bacillus* in *kinema* is attributed to its proteolytic activity, which leads to the product's alkalinity during fermentation [32]. The diversity of *Bacillus* species, both cultivable and uncultivable, in *kinema* supports their coexistence and competition for survival. Myoviridae and *Bacillus* phage phiNIT1 were prevalent viral families and phages in *kinema*, potentially inhibiting the growth of pathogenic bacteria for food safety. Among eukaryotes, there were 17 species of moulds and yeasts each, and 22 species classified as other microbial eukaryotes. Although few archaeal species were found in kinema, their impact on health and disease is not yet understood. The microbial compositions in *kinema* from India, Nepal, and Bhutan are largely similar due to shared sensory characteristics.

However, the presence of unique genera in each country's samples suggests distinctiveness possibly influenced by various factors like the use of different soybean cultivar varieties, equipment, wrapping materials, seasonal variations, geographical locations, and discrepancies in traditional preparation methods practiced by locals. Multivariate analysis comparing the microbial communities of *kinema* with *grep-chhurpi, peha, peron namsing,* and *peruñyaan* from Arunachal Pradesh in India [33], as well as *pe poke* from Myanmar [34], *thua nao* from Thailand [35], *cheonggukjang* from Korea [36], and *sieng* from Cambodia [37] revealed differences in microbiome composition within the same major cluster (Fig. 5). The research on microbial communities in similar Asian sticky fermented soybean foods indicates that *B. subtilis* is a common bacterium across all products. The presence and interaction of different types of bacteria, mainly *Bacillus* spp., along with some eukaryotes like yeasts and moulds, viruses such as bacteriophages, and a few archaea in the natural fermentation process of *kinema* show the untapped variety of food-related microorganisms in the challenging weather conditions and mountainous landscapes of the Himalayas. This is why *kinema* could be seen as a distinctive fermented soybean food from the Himalayas that can be compared to other fermented foods.

Probiotics

In the spontaneous fermentation process of *kinema*, along with the predominant *Bacillus* spp. a few lactic acid bacteria (LAB) also appeared. Among the LAB found were *Pediococcus acidilactici* and *Enterococcus faecium*, which demonstrated potential probiotic properties [38]. These LAB showed high survivability in acidic and bile salt conditions, as well as adhesion, auto-aggregation,

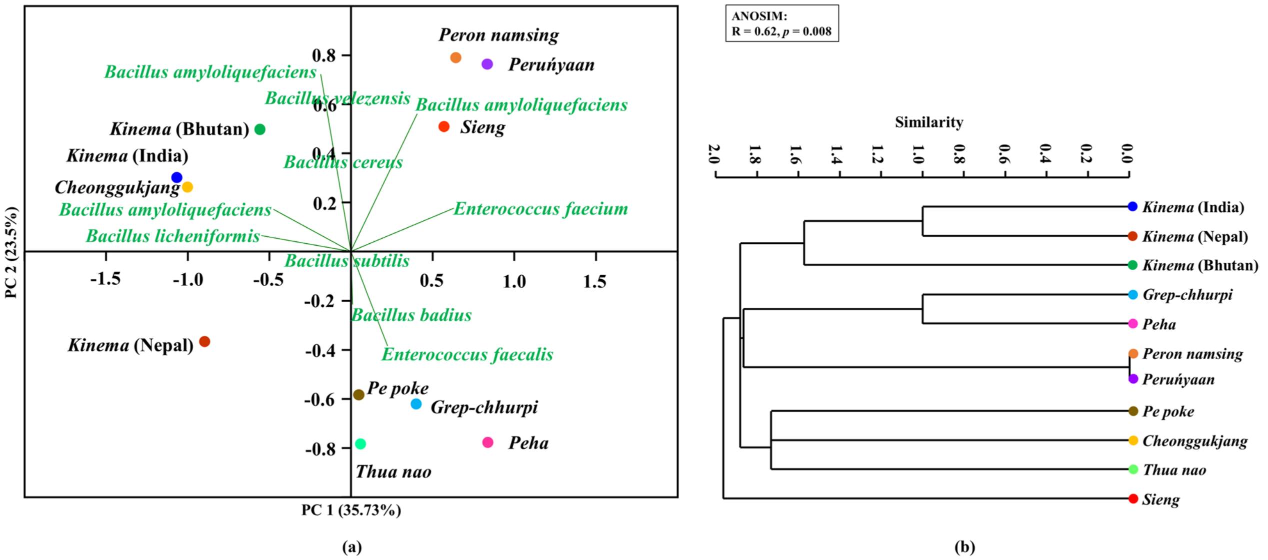

Fig. 5 Microbial community structures in sticky fermented soybean foods from the Eastern Himalayas (*kinema, grep-chhurpi, peha, peron namsing*, and *peruñyaan*) were compared with similar sticky fermented foods from Asia (*pe poke* of Myanmar, *cheonggukjang* of Korea, *thua nao* of Thailand, and *sieng* of Cambodia) through principal component analysis (PCA) biplot and Unweighted Pair Group Method with Arithmetic Mean (UPGMA) hierarchical clustering analysis. The multivariate analysis indicated significant differences between the groups (ANOSIM: $R = 0.62$, $p = 0.008$) and emphasized the similarities of *Bacillus subtilis* [29, 33–37]

and co-aggregation abilities. Analysis of the genome of *Lactiplantibacillus plantarum* DKP1, isolated from *kinema*, revealed a total of 3277 protein-encoding genes, 89 RNA genes (69 tRNA, 16S rRNA, 4 ncRNA), and 23 plantaricin encoding loci, spanning 20.5 kb, for antimicrobial compound production [39]. The whole-genome sequence of *Lactiplantibacillus plantarum* DKP1 from *kinema* also indicated the presence of biomarkers for numerous potential probiotic genes [39]. The genome of the *B. subtilis* Tamang strain, which was isolated from *kinema*, contains a total of 132 carbohydrate-active enzymes (CAZy), indicating its prebiotic features [31].

γ-PGA

The presence of sticky threads-like materials on fermenting soybeans is widely accepted as a key indicator of high sensory quality for *kinema*. These sticky threads are actually poly-γ-glutamic acid (γ-PGA), a chemical produced by *Bacillus* spp. in various Asian fermented soybean foods [40]. γ-PGA is a polymer made up of D- and L-glutamic acid units connected by γ-amide linkages [31]. It offers several health benefits to consumers [41]. Bacteria like *B. subtilis, B. licheniformis,* and *B. sonorensis*, known for producing γ-PGA, have been found in Indian *kinema* samples [25]. On the other hand, *B. subtilis* subsp. *stercoris* and *B. licheniformis* have been identified in *kinema* samples from Nepal [42]. It is important to note that only *Bacillus* species are capable of producing PGA, whereas lactic acid bacteria do not produce PGA in *kinema* [25].

The γ-PGA synthesized by the *Bacillus subtilis* Tamang strain has a molecular weight of 660 kDa and contains carboxyl, hydroxyl, carbonyl, and amide groups, along with an α-helical structure [31]. This particular γ-PGA has exhibited the capacity to dissolve iron, protect ferritin in Caco-2 cells, and display antibacterial qualities. The genome analysis of *B. subtilis* Tamang revealed the presence of genes linked to the production of the natural biopolymer γ-PGA, facilitated by the γ-PGA synthase complex (capBCA). The regions of the capsular γ-PGA synthase complex were identified, including *capA, capB*, and *capC*, as well as the *gltAB* operon associated with glutamic acid biosynthesis, a precursor for γ-PGA generation. The orientation of the capBCA complex was found to be consistent in *B. subtilis* Tamang and *B. subtilis* subsp. *natto* VK161, while other *Bacillus* strains exhibited a different gene orientation in their genomes [31].

Flavour

Flavour is a crucial aspect of food that affects how consumers perceive it as a palatable product. The tastes of salty, sweet, sour, bitter, and *umami* play a significant role in shaping consumers' overall sensory experience with food [43]. *Umami*, a term of Japanese origin, refers to the flavour resulting from the breakdown of proteins in fermented foods [44]. It consists of glutamate, 5′-inosinate, and 5′-guanylate [45]. *Kinema*, popular among the Himalayan people, is abundant in glutamic acid, an amino acid that contributes to its *umami* taste preference (Tamang and Kharnaoir unpublished). In addition to glutamic acid, *kinema* contains several other compounds that either enhance its aroma or serve as precursors to flavour development. A total of 15 flavour compounds have been identified in *kinema*, including glutamic acid, citric acid, cinnamic acid, carveol, punicate, farnesylacetone, jasmonic acid, acetophenone, 4-aminobutanal, indole, methyl jasmonate, methyl salicylate, salicylic acid, benzoic acid, and sclareol. Analysis of the metagenome of *B. subtilis* in *kinema* has revealed the presence of genes responsible for producing various enzymes and secondary metabolites associated with its flavour profile and potential health benefits [29]. Besides the *umami*-encoding genes, *B. subtilis* Tamang's genome also includes genes that produce methionine, tryptophan, branched-chain amino acids, and acetoin [31]. These genes are thought to play a role in flavour development.

In Eastern Himalaya, *umami*-flavoured *kinema* is traditionally enjoyed by Mongoloid communities belonging to the Tibeto-Burman ethnic group. It is likely that non-Mongoloid groups have not embraced the strong *umami* taste of these food items in their diet. The dietary preferences of non-Mongoloid communities in the Central and Western Himalayas, as well as other regions of India and Nepal, lean towards non-umami flavoured foods. This choice may explain why they do not consume the intense umami-flavoured *kinema* and other similar fermented soybean products from North East India.

Nutritional value and health benefits of *kinema*

Kinema is a cost-effective, plant protein-rich food commonly included in local diets. Its nutritional composition per 100 g includes: 47.7% protein, 17.0% fat, 28.1% carbohydrates, 454 kcal of energy, 42,618.0 mg of total amino acids, 5129.0 mg of free amino acids, 7.2% ash, 432.0 mg of calcium, 27.7 mg of sodium, 17.7 mg of iron, 5.4 mg of manganese, and 4.5 mg of zinc [18, 46]. Throughout the fermentation process of *kinema,* there is an increase in total amino acids, free amino acids, and mineral contents [23, 47]. *Bacillus subtilis*, during fermentation, produces proteolytic enzymes that transform proteins into peptides and amino acids, thereby improving digestibility [47]. Additionally, *kinema* contains various vitamins such as biotin (Vitamin B7), nicotinamide (Vitamin B3), pyridoxamine (Vitamin B6), pyridoxine (Vitamin B6), (R)-pantothenate (Vitamin B5), riboflavin (Vitamin B2), menaquinone (Vitamin K), and cobalamin (Vitamin B12) [29, 31].

Kinema offers various health benefits to consumers [48] due to its high content of Group B saponins [49], vitamins mostly B12, bioactive compounds, and immunomodulators [29]. These benefits include antioxidant, anti-inflammatory, antiobesity, and antidiabetic effects, as well as the production of flavone and isoflavone [50]. The *B. subtilis* strain found in *kinema* exhibits high fibrinolytic-enzyme activity and antithrombotic properties [50]. Furthermore, *kinema* demonstrates strong antioxidant activity [51, 52] myeloperoxidase inhibition [53], and the production of biopeptides for overall health advantages [54, 55].

Metabolome of *kinema*

A study of *kinema* samples collected from various regions of the Eastern Himalaya found a total of 361 metabolites through liquid chromatography-mass spectrometry (LC–MS) analysis [29]. These metabolites include both targeted (primary) and untargeted (secondary) compounds. The untargeted metabolites in the *kinema* samples exhibit potential immunomodulators such as daidzein, chrysin, chrysophanol, genistein, genistin, swainsonine, serotonin; bioactive compounds like melatonin, (R)-lipoate, harmalol; along with other bioactive compounds like soyasaponin and various vitamins including B-complex [29]. Serotonin, known as a neurotransmitter [56], can influence signalling pathways either intracellularly through serotonylation or extracellularly via membrane receptors [57]. Swainsonine plays a role in the synthesis of tropane, piperidine, and pyridine alkaloids, offering various health benefits [58]. Additionally, unsaturated fatty acids (UFAs) such as oleate, linoleate, and linolenate have been identified in *kinema* [29], contributing to responses to both biotic and abiotic stresses [59]. The association between *Bacillus* species and the abundance of primary and secondary metabolites indicates that *Bacillus* spp. play a significant role in the production of various metabolites in *kinema*, offering health benefits and therapeutic potential [29]. *Kinema* contains a high amount of secondary metabolites, and the *B. subtilis* Tamang strain has potential genes that benefit the health and well-being of consumers (Fig. 6).

Transcriptome of *B. subtilis* Tamang strain

The *Bacillus subtilis* Tamang strain library [31] was created by depleting ribosomal RNA with Illumina NovaSeq. Within the Tamang strain transcripts, the genes gadB and gabP are involved in Gamma-aminobutyric acid (GABA) production, a crucial amino acid serving as the main inhibitory neurotransmitter for the central nervous system (Tamang et al. unpublished). Additionally, genes *btuF* and *btuD* play a role in Vitamin B12 synthesis, while genes *htrA, htrB,* and *isp* contribute to fibrinolytic activity. The gltA gene was observed to be downregulated for PGA production (Tamang et al. unpublished).

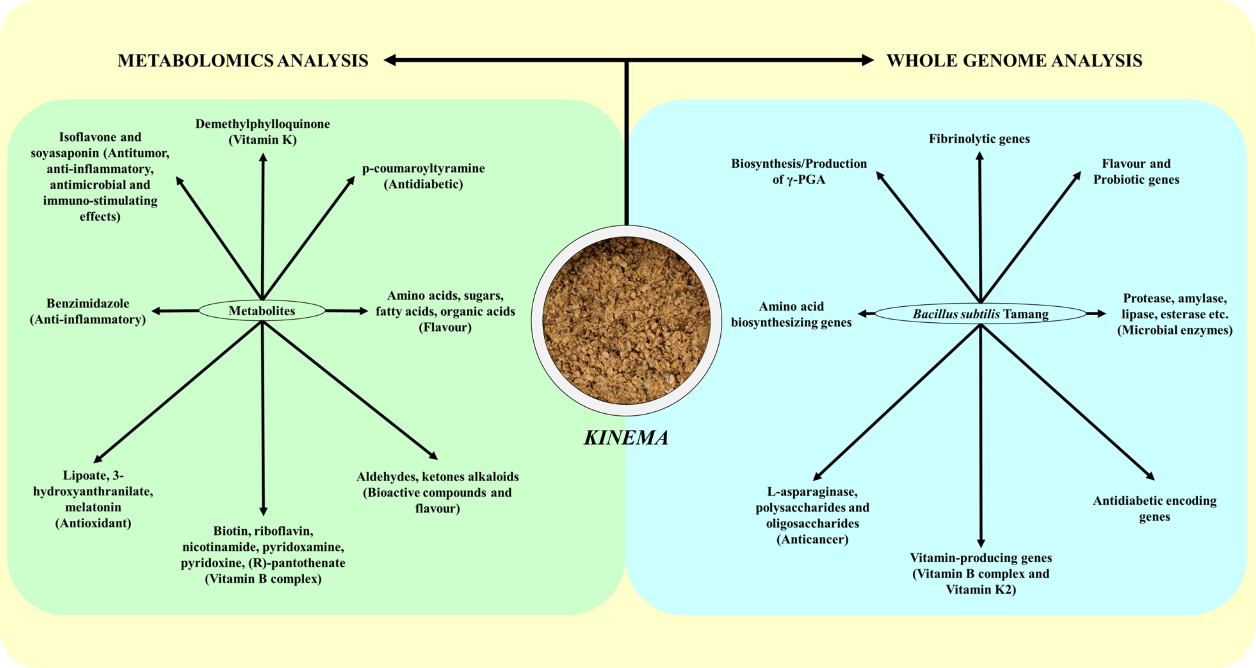

Fig. 6 The metabolome of *kinema* shows metabolites and bioactive compounds linked to its health benefits, as revealed [29]. The whole-genome sequence of *B. subtilis* Tamang strain, derived from *kinema*, contains genes that serve as biomarkers for promoting health and well-being [31]

Safety considerations of the *B. subtilis* Tamang strain and *kinema*

Safety assessments for the *B. subtilis* Tamang strain involved conducting in vitro safety assessment tests, as well as in vivo animal toxicity tests through acute and sub-chronic toxicity studies. The blood biochemistry and histopathology report showed that the Tamang strain did not cause any toxicity (Tamang et al. unpublished). *Kinema* made using the *B. subtilis* Tamang strain was given to mice and assessed for acute and sub-chronic toxicity. No mice showed any signs of toxicity or died. Therefore, both the *B. subtilis* Tamang strain and its fermented product *kinema* are safe to consume (Tamang et al. unpublished).

Kinema production by pulverised *B. subtilis* Tamang strain

Soybean seeds were chosen, cleaned, and soaked for 8 h. The soaked seeds were boiled for 1 h and 15 min and then drained. The soybeans were lightly crushed with a sterile mortar and pestle before being transferred to a clean container. A 1% pulverized starter culture containing *B. subtilis* Tamang strain was added, and the mixture was left to ferment at 40 °C for 22 h. The resulting *kinema* had a sticky texture. A total of 85 participants, 43 males and 42 females, were involved in the study. According to the average ratings, *kinema* made with *B. subtilis* Tamang strain was preferred in terms of flavour, texture, colour, stickiness, and overall likability (Tamang et al. unpublished). A hazard analysis critical control points (HACCP) system was implemented to identify and mitigate specific hazards in both traditional and laboratory-prepared kinema to ensure product safety. An HACCP model was suggested to lower pathogen levels in *kinema* production [60].

Challenging to accept *kinema* beyond the Himalayas?

Consumers choose foods based on their flavour, aroma taste, texture, colour, and other sensory characteristics. Many people tend to avoid strongly flavoured foods because they are less accepted and have a lower tolerance for intense flavour [61]. However, some enjoy strongly flavoured foods. Asian sticky fermented foods like *kinema, cheonggukjang, natto, thau nao, sieng, pe poke*, and others are rich in *umami* flavour and have a sticky appearance, making them popular among local consumers familiar with such products. Consumers who are not accustomed to traditional products may dislike the slimy texture of fermented soybean foods with *umami* flavour, sometimes describing it as having a 'foul smell'. The preference for sticky fermented soybean food is affected by genetic and cultural factors, regional influences, and the history of soy consumption. Americans, Europeans, Australians, individuals from the Indian subcontinent, West and Middle-East Asia have not adopted traditional fermented soybean foods, unlike Africans who have a history of consuming similar fermented legumes [62]. *Cheonggukjang* [63] and *natto* [64] have been clinically tested and claimed as healthy foods with therapeutic uses [65, 66]. However, their acceptance outside their place of origin is limited due to their *umami* flavour and slimy appearance. This presents a significant challenge in promoting these highly regarded fermented soybean foods globally. On the other hand, Asian fungal fermented soybean products like *tempeh, soy sauce,* and *miso* are widely embraced worldwide, particularly by younger generations. These factors hinder the global expansion of sticky and *umami*-flavoured soy foods like *kinema*. Instead of promoting the entire product, the bioactive components or by-products of these fermented foods, along with safe starter cultures suitable for functional food production (such as clinically proven *Bacillus subtilis* strains), could be utilized to create food supplements like PGA, nattokinase, Vitamin B12, and Vitamin K extracted from *kinema*.

Conclusion

The food heritage of the Himalayan people is unparalleled and distinctive, with local varieties of soybeans being traditionally used to create a flavourful and delicious fermented product called *kinema* in the Eastern Himalayan regions of India, Nepal, and Bhutan. *Kinema* is typically made through natural fermentation, primarily by predominant *Bacillus* species in various parts of the Eastern Himalayas, following a generally similar preparation process. Traditionally, soybeans are soaked and boiled until soft, then wrapped in leaves such as banana, fig, or ferns, and placed in a bamboo basket. *Kinema* is a valuable source of proteins, nutrients, biopeptides, vitamins, immunomodulators, and bioactive compounds. It offers health benefits and therapeutic uses, especially for individuals with limited consumption of meat and dairy products. Fermented soybean foods have garnered global consumer interest for their physiological and health benefits. Finding a new strain of *Bacillus subtilis* Tamang in *kinema* that can produce high molecular weight γ-PGA and contains multiple biomarkers or signature genes for biological functions is a significant advancement in the study of a traditional Himalayan fermented food. Globalizing and promoting *kinema* beyond its place of origin continues to be a challenge, mainly because of its unique taste and slimy texture, despite its known health benefits. Yet, *kinema* holds a significant cultural and historical importance as one of the oldest fermented soybean foods, symbolizing the culinary heritage of the ethnic Himalayan communities.

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JPT wrote the manuscript, prepared the literature overview, and interpreted data. JPT conceived the study, participated in data interpretation, participated in coordination of the study, and performed critical revisions of the manuscript.

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