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Unlocking the Power of Fibrinolytic Enzymes in Traditional Indian Fermented Foods: A Gateway to Natural Antithrombotic Agents

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Abstract

Cardiovascular diseases (CVDs) persist to be the leading cause of death worldwide, mostly driven by thrombus formation brought on by fibrin buildup in blood vessels. Despite the fact that current thrombolytic medications like urokinase, streptokinase, and recombinant tissue plasminogen activator (tPA) efficiently activate plasminogen and dissolve fibrin, their short plasma half-life, high cost, bleeding complications, and immunogenicity limit their clinical use. These restrictions have accelerated the hunt for more affordable, safe, and fibrin-specific substitutes. Because of their long history of dietary safety, high substrate specificity, and reduced immunogenic potential, microbial fibrinolytic enzymes derived from fermented foods have become promising candidates. Fermented foods enriched with fibrinolytic enzymes not only aid in thrombus degradation but may also support gut health, reduce inflammation, and improve cardiovascular wellness. Indian fermented foods enhanced with a variety of *Bacillus species* are an understudied but incredibly potent source of fibrinolytic enzymes with significant therapeutic potential. This review highlights the significance of fibrinolytic enzymes in thrombolytic therapy while summarizing their types, mechanisms, and biochemical characteristics. There is increasing scientific evidence that the enzymes extracted from traditional Indian fermented foods have thrombolytic potential. This review highlights the need for systematic research and clinical evaluation of food-derived microbial fibrinolytic enzymes as next-generation biotherapeutics for the safe, efficient, and cost-effective treatment of cardiovascular diseases.

Keywords: Fibrinolytic Enzymes, Fermented Food, Anticoagulant Activity, Natural Thrombolytics, Health-promoting Enzymes

Introduction

Cardiovascular illnesses, which include high blood pressure, ischemic heart disease, and myocardial infarction, are the leading causes of death worldwide. Usually, fibrin buildup in blood vessels causes thrombosis **(**Fig 1**),** which results in myocardial infarction and other cardiovascular disorders. Plasmin lyses the blood clot fibrin in vivo after being triggered from plasminogen by tissue plasminogen activator (tPA) (1,2). More than half a billion people in the world continue to suffer from cardiovascular diseases, with 20.5 million deaths in 2021, almost one-third of the deaths in the world and more than the predicted

deaths of 121 million from CVD. Moreover, the global burden has increased because of a positive correlation between the incidence of cardiovascular damage and higher COVID-19 mortality (3,4).

Atherosclerotic plaque develops and spreads as a result of arterial thrombosis, which is brought on by the accumulation of platelets, fibrin, and thrombin in the arteries (1,5). This can lead to myocardial infarction. One way to treat the thrombus is to either remove it or stop it from growing. Thrombolysis is the breakdown of thrombi, while fibrinolysis is the breakdown of just the fibrin mesh around the blood clot (6-8). Patients with CVDs treated by thrombotic drug available in the market but their short half-life and poor affinity for fibrin, they need to be injected frequently to be successful. They also have various side effects, including as allergic reactions and internal haemorrhages (9,10). Numerous relevant investigations on enzymes derived from food-grade microbes have demonstrated their safety as thrombolytic drugs in recent decades (11).

Strong thrombolytic medicines called fibrinolytic enzymes are used to treat and prevent heart diseases. They are categorized into two groups depending on how they work: plasminogen activators, which break down fibrin by converting plasminogen into active plasmin, and second is plasmin-like proteins, which break down fibrin directly (12). Fibrinolytic enzymes are isolated from plants, bacteria, fungi, earthworm, algae, but they face side effects like internal bleeding, hypertension, allergic reaction. So, it prompting ongoing search for novel source having a diminished immunogenicity potential, reduced cost and health benefit. In addition to their thrombolytic action, several food-derived fibrinolytic enzymes also exhibit anti-inflammatory, antioxidant, and cardioprotective effects, making them promising candidates for preventive cardiovascular healthcare.

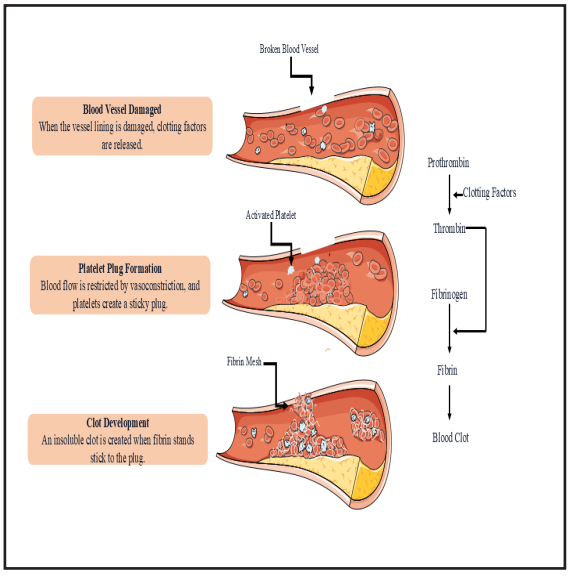

Fig 1. A schematic depiction of the coagulation pathway demonstrating how clot formation protects against blood loss following vascular damage.

However, there are a number of advantages in utilizing food sources, particularly fermented foods, over those presently in use. Food-derived microbial fibrinolytic enzymes are gaining more interest due to their high substrate specificity and reduced immunogenicity. The main focus at present is on research into the traditional fermented food of India as sources of fibrinolytic enzymes due to their long history of safe ingestion and high microbial biodiversity. This study examines the enzymatic properties and potential health benefits of fibrinolytic enzymes in fermented Indian foods-a characteristic of Indian cuisine. These are not only palatable dishes but also carry with them bioactive molecules like the fibrinolytic enzymes which may prevent and cure heart disease. Microbial fibrinolytic enzymes sourced from food have received greater medical attention lately (13). Many fermented foods from south India and Gujarati cuisine can actually inhibit the formation of blood clots very well. Several strong fibrinolytic enzymes were identified and isolated from fermented food items and it was

isolated from several microbes belonged to the genus *Bacillus* (14-16). Bacteria isolated from fermented foods have been shown to be rich in fibrinolytic enzymes, which are highly effective both *in vitro* and *in vivo* fibrinolysis. Since fermented foods already form a part of regular diets, the fibrinolytic enzymes herein may provide a safe, affordable, and accessible functional-food–based strategy for improving cardiovascular health at the population level (17-20).

Fibrinolytic enzymes

Fibrinolytic enzymes belong to the group of proteolytic enzymes, which catalyse the disintegration of fibrin-the main building block of blood clots. Fibrinolytic enzyme either degrades the fibrin into fibrin degradation products or converts the inactive circulating plasminogen into active plasmin in order to dissolve a fibrin clot and return the vascular architecture to normal. (10). The proteolytic enzyme plasmin lyses fibrin. The body's fibrinolytic and coagulation systems are intimately connected and reliant on one another. The body's fibrinolytic system is also spontaneously triggered after a coagulation response takes place, lowering fibrinogen levels through negative feedback and preventing excessive fibrin agglomeration. Two distinct processes can lead to fibrinolysis **(**Fig 2**):** (1) direct fibrinolysis brought on by plasmin-like enzymes, and (2) indirect fibrinolysis regulated by plasminogen activation to plasmin. They can either directly degrade pre-existing insoluble fibrin into smaller pieces called fibrin degradation products through direct fibrinolysis-like nattokinase and lumbrokinase, which restores normal vascular function, or indirectly promote the lysis of fibrin by activating circulating plasminogen and converting it into active plasmin that acts on the fibrin, a process known as indirect fibrinolysis.

Moreover, α2-antiplasmin and α2-macrophulin are inhibitors that halt fibrinolysis, whereas t-PA and uPA are activators of plasminogen. Together, they regulate the fibrinolytic system, preventing excessive breakdown of fibrin and potential bleeding. Plasminogen activators enhance the conversion of plasminogen to plasmin, facilitating the dissolution of fibrin clots.

Enzymes related to fibrinolytic action are classified into metalloproteases, Metallo proteases, and Serine- Metalloproteases, playing a vital role in preventing thrombotic disorders like myocardial infarction and stroke. Fibrinolytic enzymes, which facilitate the breakdown of harmful blood clots, are notably produced by *Bacillus* species found in fermented foods. Various methods for their identification and measurement, along with biotechnological advancements for enhanced production, have been documented. A summary table outlines key Bacillus species and their corresponding fibrinolytic enzymes (Health-promoting Enzymes) derived from fermented foods (21).

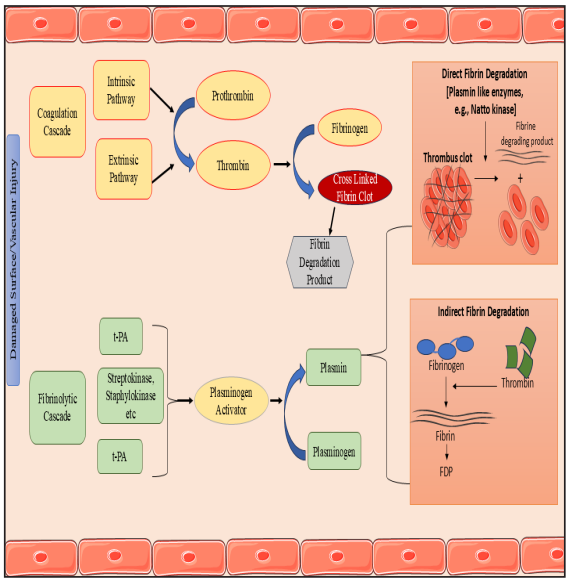

Fig 2. Schematic illustration of the fibrinolytic system: NK and lumbrokinase directly affect fibrin clots; PAI-1 and PAI-2 regulate plasminogen activation, whereas α2-antiplasmin and α2-macroglobulin inhibit plasmin. Physiological activators (t-PA and urokinase) convert plasminogen into plasmin, which hydrolyzes fibrin and forms fibrin degradation products.

Denis P.S. reported the first instance of a blood clot dissolving on its own in 1838 ("Sang de L'homme," by Denis P.S."). After that, **the** French chemist Albert Dastre first described the phenomena as "Fibrinolyse dans le sang" in 1893, coining the term "fibrinolysis" in connection. In the 1900s, the key components of fibrinolysis as we know it nowadays were identified and described. Moreover, thrombolysis was initially utilized to treat myocardial infarction patients; however, towards the mid-1990s, tPA was approved for the specific purpose of treating patients with acute ischemic stroke, subject to certain restrictions (22). Another group discovered in 1903 that the presence of chloroform could increase the fibrinolytic activity in plasma. However, when plasma was added back to the sample, the activity decreased, indicating that plasma also contained an inhibitor to fibrinolysis, according to Delezenne and Pozerski. In the 1930s, exogenous plasminogen activators were discovered and the fibrinolytic agent - known as "fibrinolysin" - produced by hemolytic streptococci was reported in 1933, which was a big find. Haskell Milstone later discovered in 1940 that fibrinolysin could not degrade fibrin on its own, but it could when it was mixed with plasma. Thus, it became clear that there was a "plasma zymogen," which Christensen and MacCloud first called "pro-fibrinolysis" until renaming it "plasminogen" in 1945 (23-25). Clinical development of tPA and uPA began in the 1950s, but they gained popularity in the 1980s when large-scale protein manufacturing was made possible by recombinant DNA technology. Streptokinase, a fibrinolytic enzyme derived from hemolytic *Streptococci*, underwent its first human trials in 1958 (26). The 2000s saw significant advancements in targeted fibrinolytic therapy.

Fibrin-degrading enzymes have been derived from a wide range of bacteria. For example, *Streptococcus hemolyticus* produces streptokinase (Banerjee et al., 2004), *Bacillus* subtilis produces nattokinase (27), *Bacillus* sp. produces bafibrinase, and *Serratia sp*. also joins this list (21). In addition, the fibrinolytic enzymes derived from *Bacillus sp.* isolated from traditional fermented foods have already been used to develop potent food additives and pharmaceuticals that treat or delay the onset of thrombosis and disease-related conditions (28,29).

Peng et al. documented that the microbial fibrinolytic enzyme subtilisin DFE from *Bacillus subtilis* demonstrated low production expenses and high specificity. Further, there were reports of a relatively high yield in mass culture and that it has been possible to improve it further through genetic engineering (30,31). Since the enzyme is also quite similar in properties to serine protease, the results indicate that FP84 may be a novel serine metalloprotease with applicational potential in thrombolytic therapy (32). Furthermore, detailed studies on specific enzymes, such as subtilisin DFE, provide insights into the unique properties and possible applications of food-derived microbial fibrinolytic enzymes. However, the microbial and non-microbial fibrinolytic enzymes isolated from sources other than foods demonstrated a lack of selectivity for fibrin. Furthermore, it has been established that most enzymes investigated from sources other than foods, in addition to causing fibrinolysis, also result in fibrinogenesis, thereby severely compromising appropriate hemostasis (33). For this reason, the unusual sources have serious drawbacks and thus far have not delivered what was expected from them.

Categories of fibrin-degrading enzymes

Fibrinolytic enzymes are members of the family of protease enzymes. These enzymes cleave α-peptide and isopeptide bonds, hence allowing for proteolysis. They thus make up the largest enzyme family (34). Based on the metal ion or amino acid that makes their active site, proteases have been grouped into six classes: serine, cysteine, and threonine peptidases use covalent catalysis; aspartate, glutamate, and

metallopeptidases use acid-base catalysis. On the basis of their mode of action, fibrinolytic enzymes have been further subclassified into serine proteases, metalloproteases, and serine metalloproteases (35,36).

Serine -based proteolytic enzymes

Serine proteases are a major group of endopeptidases, comprising over one-third of known proteolytic enzymes, which hydrolyse peptide bonds in proteins using serine as the nucleophile (37,38). The research into serine proteases' fibrinolytic capabilities highlights a risk of non-specific clot lysis, which could compromise the blood-brain barrier. Currently, only two indirect serine proteases, tPA and uPA, are FDA-approved for fibrinolytic therapy. Recent studies have identified fibrinolytic enzymes from various organisms, including *Brachybacterium paraconglomeratum, microalga Dunaliella tertiolecta, Pseuderanthemum latifolium, Euphausia superba, Marine actinomycete Actinoalloteichus caeruleus, Agrocybe aegerita, and Streptomyces parvulus* (39,40).

Metal-dependent proteases

Metalloproteases are a diverse group of proteolytic enzymes that include both exopeptidases and endopeptidases. Their active sites typically contain one or two divalent metal ions coordinated by three amino acid sidechains, with a water molecule serving as a fourth ligand, which is critical for the hydrolysis of peptide bonds. Key metal ligands in metalloproteases include histidine (His), glutamic acid (Glu), aspartic acid (Asp), and lysine (Lys) (41,42). The arrangement of metalloprotease catalytic site ligands is His > Glu > Asp > Lys. While most enzymes in this class require one divalent metal ion for catalysis, some require two. Their activity depends on various divalent metal ions, including Hg2+, K+, Ca2+, Mg2+, Zn2+, and Co2+. EDTA inhibits their activity, and only a few, such as CMase, PoFE, FVP-I, AMMP, BKII, and TSMEP I have been purified, despite extensive studies on the effects of metal ions on crude extracts (21, 43-47).

Hybrid serine–metal proteases

Serine metalloproteases, a unique group of fibrinolytic enzymes, possess characteristics of both serine and metalloproteases, enabling proteolysis via dual catalytic mechanisms (48). They play crucial roles in blood coagulation, extracellular matrix remodeling, fibrinolysis, and immune responses in various conditions. Recent discoveries have expanded this group to include newly characterized fibrinolytic proteases, including BSFE1 from marine *Bacillus sp.* S-3685 and a potent serine-type thrombolytic enzyme from *Brachybacterium paraconglomeratum* reported in 2024–2025. These add to the earlier examples described in the literature, such as M179, CFR15, AprE176, and velefibrinase from Marine *Bacillus velezensis* Z01, most of which are inhibited by PMSF and EDTA (49). The respective fibrinolytic enzymes from *Serratia marcescens subsp. sakuensis* and *Bacillus licheniformis* HJ4-fermented seafood Hwangseokae jeotgal also reflect the broadening microbial source base. Together, these provide evidence for the wide ecological range and therapeutic potential of serine-metalloprotease type fibrinolytic enzymes (50,51).

Thrombolytic therapeutics

Primary generation thrombolytic agents

Urokinase and streptokinase, first-generation thrombolytic medications, act as non-selective thrombolytics by converting plasminogen into plasmin and directly lysing fibrin, disrupting fibrin clot formation. They are applied in treating conditions such as myocardial infarction and deep vein thrombosis (DVT) by dissolving blood clots and restoring blood flow to prevent tissue damage (52). Recent studies suggest that truncated or mutated streptokinase can reduce immunogenicity while maintaining its clot-lysing function, and lipid modification of recombinant streptokinase aims to improve its stability and biological activity, addressing its short half-life.

Urokinase

Urokinase, a fibrinolytic enzyme produced by renal epithelial cells and macrophages, was discovered in human urine by MacFarlane and Pilling (1947) and named by Sobel et al. in 1952. Initially inactive as pro-urokinase (P), it is a glycosylated zymogen consisting of 411 amino acids (53) and three domains: a growth factor domain, a kringle domain, and a serine protease domain. Pro-urokinase undergoes two proteolytic cleavages after secretion, primarily by plasmin, producing an active 33 kDa form of uPA and an inactive amino-terminal fragment. The binding affinities of pro-uPA and its derivatives to the uPAR receptor are similar (54,55). Urokinase was first isolated from human urine and is now produced by recombinant DNA and tissue culture. It is FDA-approved for the treatment of cardiovascular diseases due to its plasminogen activation action (56,57).

Streptokinase

The development of streptokinase (SK, EC 3.4.99.22) by Dr. William Smith Tillet in 1933 marked the beginning of thrombolytic therapy for cardiovascular conditions (52), particularly for acute myocardial infarction that applied in 1958. It is a non-fibrin-specific extracellular enzyme which has a molecular weight of 47 kDa and is made up of 414 amino acids (58). It indirectly activates the circulatory plasminogen (59) and shares three domains with urokinase. Although it lacks plasmin activity, it has plasmin activity when binds to plasminogen in a 1:1 ratio (60). Jackson and Tang were the first to identify the entire amino acid sequence of streptokinase, which consists of three domains connected by flexible loops (1982). The enzyme indirectly transforms plasminogen into active plasmin by forming a 1:1 streptokinase-plasmin complex through a series of protein interactions (61). This complex breaks the arginine-valine bond in plasminogen, which results in the production of plasmin, a proteolytic enzyme that breaks down thrombus matrix and facilitates the removal of blood clots and arterial blockages, two major causes of myocardial infarction and heart attacks (62).

Intermediate generation thrombolytic agents

Second-generation thrombolytic medications were designed to increase selectivity and specificity for fibrin-rich thrombi in order to reduce the bleeding problems associated with previous treatments that led to non-specific fibrin breakdown. These agents, which include tenecteplase, alteplase, and reteplase, increase the efficacy of treatment for thrombotic conditions like acute ischemic stroke and pulmonary embolism by activating plasminogen attached to fibrin in blood clots.

Saruplase

A naturally occurring prodrug called sarulplase is converted by plasmin into its active form, low-molecular-weight double-stranded urokinase, in vivo (63). This recombinant single-chain urokinase-plasminogen activator is known as r-scu-PA. It can also activate plasminogen directly (64). Saruplase has a brief half-life of 7 to 8 minutes. It decreases systemic plasmin, which in turn decreases fibrinogen and α2-antiplasmin levels and increases fibrinogen degradation products. Despite having a weaker ability to bind fibrin than streptokinase, it has a stronger fibrinolytic activity than alteplase (65).

Recombinant tissue plasminogen activator (r-tPA)

A serine protease (tPA) is a 70 kDa and 527 amino acids,thrombolytic protein specific to fibrin (66), with two types of tissue plasminogen activators: single chain form (sct-PA) and two chain form (tct-PA). It has four domains: fibronectin type I finger (F), two kringle domains, an epidermal growth factor domain, and active site residues (67). The PA activity of t-PA is inhibited by plasminogen activator inhibitor-1 (PAI-1) (68). The inhibition by PAI-1 was linked to the amino acids at 296-299 in t-PA (69). When fibrin is not present, t-PA is a poor enzyme for

plasminogen activation. However, it speeds up the plasminogen activation rate when fibrin is present.

Anistreplas

Anistreplase, also known as anisoylated plasminogen streptokinase activator complex, is a distinct kind of streptokinase (70,71). Upon spontaneous deacylation, this complex, which consists of human plasminogen and acylated streptokinase conjugated at an equal molecular concentration, converts subsequent plasminogen into plasmin. Anistreplase deacylation results in the release of the p-anisoyl group (68). This activator complex is a fibrin-non-specific binding thrombolytic medication that shares streptokinase's adverse effect profile but offers the advantage of single-bolus administration (72). The total TIMI grade 2 and TIMI grade 3 flow rates were between 50 and 60 percent following 90 minutes of anistreplase administration. The ISIS-3 study reported a 0.6% cerebral hemorrhage and a 10.5% death rate at 35 days after a single anistreplase injection (73).

Latest generation thrombolytic drugs

These are modified second-generation thrombolytics that are intended to be more convenient, have a longer half-life, a lower risk of bleeding, and an even greater affinity for fibrin than previous generations. Tenecteplase and *Satphylokinase* are two examples. Because they can be administered as a single bolus injection, they are easier to administer and are linked to even lower rates of bleeding complications. Third-generation medications are even more likely than second-generation medications to clot-bound plasminogen rather than plasma plasminogen.

Tenecteplase (TNK)

The bindings to PAI-1 of tenecteplase (TNK-tPA), a mutant rt-PA with three sites where the protein half-life is increased by up to five or six times, are 80 times lower than those of alteplase (74). Tenecteplase is also bound to fibrin, just like alteplase (75). However, compared to alteplase, its specificity is fifteen times higher. Its effective dosage as a single bolus is 0.25 mg/kg. The ASSENT-1 clinical trial proved tenecteplase's efficacy and safety (76). However, in the ASSENT-2 clinical study in patients with AMI, this medication was associated with allergic reactions and bleeding (77).

Staphylokinase (SAK)

Among other side effects, classic thrombolytic drugs may cause bleeding, vessel re-occlusion, or immune reactions. Recent studies confirm the potential of Staphylokinase as a third-generation, fibrin-specific thrombolytic agent. It is a small protein (~15.5 kDa, ~136 amino acids) that preferentially activates plasminogen at clot sites, with minimal systemic fibrinogen degradation. A non-immunogenic variant of SAK has shown equal efficacy compared to conventional treatments but with a lower hemorrhagic risk and single-bolus administration in clinical trials (77-81).

Staphylokinase, also known as staphylococcal fibrinolysin or Müller's factor, converts plasminogen to plasmin for thrombolytic activity and forms a stoichiometric complex with plasmin or plasminogen to disintegrate blood clots. However, staphylokinase produced by *S. aureus* elicits a strong immune response when administered intravenously, and producing it in high yields presents challenges. Researchers are addressing these issues through protein engineering and recombinant DNA technology to develop modified recombinant staphylokinase molecules, aiming to enhance its fibrinolytic action. The efficacy of third-generation recombinant thrombolytic medications in improving fibrin selectivity and reducing bleeding risks requires validation through further clinical trials (82,83). Recent updates report the development of next-generation low-immunogenic SAK variants, including glyco-engineered and PEG-modified forms, which demonstrate significantly reduced antibody response and improved clot-lysis efficiency

in preclinical models, hence showing strong potential for safer clinical use in the future. The current staphylokinase research shows quite remarkable improvement. A 2025 study reported a 2.2-fold increase in the production of SAK and confirmed ~42% human clot lysis in vitro. A 2024 clinical trial showed that this non-immunogenic recombinant SAK variant is as effective as alteplase but with fewer bleeding risks. Engineered SAK mutants now show considerably higher plasmin affinity and better fibrin selectivity, while in preclinical models, modified forms are confirmed to retain strong thrombolytic activity with reduced immunogenic side effects (84).

Potential for discovering new fibrinolytic enzymes in understudied Indian fermented foods

Fibrinolytic enzymes can be derived from a number of sources, including both food and non-food sources, as well as microbes isolated from these sources. Food sources of fibrinolytic enzymes include fermented and non-fermented foods, while non-food sources include microbes isolated from soil and marine environments, fungi, animals, etc. Although non-food sources of fibrinolytic enzymes are generally known to be potent, food sources have the added advantage of being readily available, affordable, and safer for oral consumption, as well as having potential health benefits beyond their fibrinolytic activity. For instance, fibrinolytic enzymes like papain (85) and bromelain (86) from papaya and pineapple, respectively, have been found to have anti-inflammatory and digestive properties, while nattokinase is linked to cardiovascular health benefits.

Dietary sources containing fibrinolytic enzymes

Many fibrinolytic enzymes from natural sources, such as bacteria, earthworms, vampire bats, and snake venom, have been discovered in the last few decades. Due to their abundance, low cost of production, and potential for genetic engineering, microbial fibrinolytic enzymes—particularly those derived from traditional fermented foods—are promising sources of thrombolytic agents (87). The main functional element that breaks down thrombus during food fermentation is fibrinolytic enzymes, which are produced by microbial strains. Although there are many different types of fermented foods, fermented soy products like Korean Cheonggukjang, Douchi, Natto, and Pigeon Pea are the most popular (88) (20). Because of their high nutritional content and demonstrated capacity to break down fibrin, they can be used to both prevent and treat cardiovascular issues. These fibrinolytic enzymes have been the subject of a growing body of research on cardiovascular diseases in recent years (89). Food sources have several advantages, such as accessibility, affordability, oral safety, and possible health benefits in addition to their fibrinolytic action (90). Papain from papayas and bromelain from pineapples are two examples of fibrinolytic enzymes that have been shown to have anti-inflammatory and digestive qualities, respectively, while nattokinase is associated with benefits for cardiovascular health (27). Furthermore, consuming complete meals that contain fibrinolytic enzymes instead of relying solely on medications or supplements might offer a more varied and all-encompassing method of obtaining these enzymes (91,92).

Functional microorganisms alter the chemical components of raw materials from plant and animal sources during food fermentation, increasing the bioavailability of nutrients, enhancing the food's sensory quality, adding bio preservative effects and improving food safety, breaking down toxic and anti-nutritive components, producing antioxidant and antimicrobial compounds, boosting probiotic functions, and fortifying with certain health-promoting bioactive compounds (93,94). When selecting the starter culture or cultures to use in the production of functional foods, probiotics, antimicrobial, antioxidant (95),

peptide production, fibrinolytic activity, poly-glutamic acid (96), degradation of antinutritive compounds (97), and other functional traits of microorganisms in fermented foods may be important considerations.

Fermented food-based sources of fibrinolytic enzymes

Fermented foods have long been recognized as an excellent source of bioactive compounds with several health benefits. In particular, fermented foods have shown promise as sources of fibrinolytic enzymes and their producers, which are critical for disintegrating blood clots. Numerous studies have examined the fibrinolytic action of fermented foods. These enzymes have demonstrated fibrinolytic properties in vitro and in animal models, suggesting potential therapeutic applications.

In 1987, natto, a traditional fermented soybean dish from Japan, contained nattokinase, a fibrinolytic enzyme linked to cardiovascular health. Despite its benefits, it is sold as a dietary supplement and is not FDA-approved (71,98,89). Other Japanese fermented foods like funazushi and shiokara also contain fibrinolytic enzymes, but further research is needed to determine their efficacy and safety. Korean fermented foods like chungkook-jung and jeotgal contain similar enzymes from various *Bacillus* strains, with *B. licheniformis* HJ4 from jeotgal showing notable activity (99). Fibrinolytic properties are also present in kimchi. When *Bacillus subtilis* DC27's enzyme DFE27 is used in Chinese cooking, douchi demonstrates thrombolytic potential (100,101).

Traditional Indian fermented products

Fermented foods have long been integral to the diet of the Indian subcontinent, enriching its culinary tradition and possibly providing various health benefits. Research on fermented soy-based foods and fish products from Northeast India showed that their fibrinolytic activity is attributed to microorganisms (102). Traditional Indian fermented products have long been part of the diet, offering potential health benefits. Studies show that fermented soy-based foods, hawaijar from Manipur, and fish products from Northeast India exhibit notable fibrinolytic activity, largely from *Bacillus species* producing nattokinase or similar enzymes. Recent trends (2023–2025) in India (Table 1) focus on exploring underutilized fermented foods such as kanji (fermented carrot/beetroot drink), panta ilish (fermented rice), bamboo shoot-based pickles, idli/dosa batter, and dhokla batter to discover novel fibrinolytic enzymes using metagenomic and high-throughput screening methods (87).

Table.1 Fibrinolytic enzymes from Indian fermented foods and emerging exploration of underutilized sources.

Fermented Food	Region / State	Source of Fibrinolytic Activity	Enzyme Name	Molecular Weight (kDa)	Reference / Link
Hawaijar	Manipur	*Bacillus spp.*	Nattokinase-like enzyme	~27	PMID: 37316061 (2023)
Fermented Soy Products (e.g., kinema)	Northeast India	*Bacillus spp.*	Nattokinase / subtilisin	27–30	Tamang, J. P. (2023)
Fermented Fish Products	Northeast India	Endogenous enzymes	Fish-derived fibrinolytic enzyme	18–20	Thangjam S. (2014)
Kanji (fermented carrot/beetroot drink)	Pan India	*Bacillus* / LAB	Potential nattokinase-like enzyme	Not reported / estimated 25–30	PMID: 34393474 (2021)
Panta Ilish (fermented rice)	West Bengal / Odisha	Potential *Bacillus spp.*	Potential fibrinolytic enzyme	Not reported	PMID: 25003130 (2014)
Bamboo Shoot Pickles	Northeast India	Potential *Bacillus spp.*	Potential subtilisin-like enzyme	Not reported	Tropical Life (2022)
Idli / Dosa Batter	South India	*Bacillus spp*. / LAB	Subtilisin-like protease	28–32	Springer (2023)
Dhokla Batter	Gujarat	*Bacillus spp.*	Nattokinase-like enzyme	~27	PMID: 29892105 (2018)

Biotechnological utilization of fibrinolytic enzymes

Fibrin can be broken down by most of the proteases secreted by the *Bacillus species*. Strong fibrinolytic enzymes have been discovered in various *Bacillus species* (Xiao et al., 2004) and E. coli of purification, higher yield, and for possible lactic acid bacterial system starting cultures (102). These fibrinolytic enzymes can also be encapsulated in nano-capsules for increased stability and easier oral applications (103). NKCP was encapsulated in Shellac with an enzyme activity retention of about 60% after encapsulation. Shellac particles had a low acid permeability (104). Alginate microparticles were made in order to assess the effect of Korean fermented soybean paste on fibrinolytic enzymes. Using chickpeas as a substrate, extracted and precipitated nattokinase from *B. subtilis* LSSE-22 using ethanol. To increase stability at acidic pH, the nattokinase was encapsulated in a methacrylic acid–thylacrylate copolymer (105). Numerous fibrinolytic enzymes from the genus *Bacillus* have been discovered; these enzymes differ in molecular weight and substrate specificity and have the potential to become inexpensive, oral, direct-acting thrombolytic medications (106). Oral NK has been produced commercially since it was demonstrated to be safe and effective in canine, and human trials. Among them are NSK-SDTM, Cardiokinase, Natto-K, Nattokinase, Orokinase, Nattozyme, Best-Nattokinase, Nattokinase-plus, Serracor-NK, and Nattobiotic. Numerous peptidases and proteases are encoded by genes found in the Bacillus subtilis168 genome sequence. Thus, analyzing and finding new fibrinolytic enzymes may be made easier by comparing the genomes of related bacteria. The N-terminal sequence of fibrinolytic enzymes contains a number of conserved amino acid sequences. However, significant changes are observed in their traits (107).

Future Directions

Only a few Asian countries have conducted extensive research on microbial fibrinolytic enzymes isolated from fermented foods, and although studies published since 2024 show a slight expansion into countries like Indonesia and Italy, the rich diversity of fermented foods worldwide remains largely unexplored. Despite the long history of fermented foods in India, such as dosa batter, idli batter, dahi, kanji, jalebi ferment, and other regional rice and millet-based ferments, their fibrinolytic potential has not been fully investigated when compared to Chinese, Japanese, or Korean products. Traditional meals from Latin America, the Middle East, Africa, Europe, and other regions must also be carefully examined because their fibrinolytic enzymes might be far different from those present in Asian foods. A more diversified approach is necessary to attain this unrealized potential. Besides studying novel fermented food sources, detailed enzymatic characterization along with bioinformatic analysis by sequencing of the extracted enzymes should be performed. Furthermore, direct screening of food matrices for fibrinolytic enzymes by employing omics-based approaches such as metagenomics and metatranscriptomics can lead to the discovery of distinct enzymes of high therapeutic value, including those produced by non-culturable microorganisms or even of non-microbial origin. Integrating traditional knowledge with modern advanced technologies such as molecular modeling, enzyme engineering, and AI-based sequence prediction will accelerate new enzyme discovery at a much faster pace. This kind of comprehensive approach will identify many new fibrinolytic enzymes with food, nutraceutical, and pharmaceutical applications.

Conclusion

Fibrinolytic enzymes have come a long way from early nonspecific thrombolytics to highly engineered fibrin-targeted biotherapeutics. However, issues such as immunogenicity, re-occlusion, and systemic bleeding still restrict the clinical efficacy of available drugs. The substantial therapeutic potential of fibrinolytic enzymes sourced from traditional fermented foods, especially of Indian

origin, has been demonstrated through an expanding body of research on these enzymes. Affordable production and GRAS-level dietary safety, along with favorable biochemical characteristics, high thrombolytic efficiency, and reduced side-effect profiles continue to favor these enzymes. The yield, stability, and fibrin specificity of food-derived enzymes can now be improved thanks to developments in microbial biotechnology, protein engineering, and fermentation optimization. However, thorough investigations that include structural characterization, mechanistic clarification, in vivo validation, and ultimately clinical translation are crucial. Overall fermented foods are an important and mainly unexplored source of new fibrinolytic enzymes that can act as more accessible and safe substitutes for current thrombolytics, assisting future advancements in the management and prevention of cardiovascular diseases.

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Conflict of interest

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Data availability

Not applicable

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