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An overview on therapeutic potential and various applications of microbial collagenases

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ABSTRACT

Collagen is the most widely distributed class of proteins in the human body. Monomers of collagen are constantly being synthesized and degraded throughout the development of a healthy individual to adulthood. The collagenase subfamily found in human matrix (metalloproteinases), are capable of hydrolyzing native collagen under physiological conditions. Collagenases are produced by specific cells involved in repairs and remodelling processes and plays important role in connective tissue metabolism. Present article focus on the major sources, properties and therapeutic aspects of microbial collagenases in their relation with various diseases and its applications in medical and food industry. Collagenolytic enzymes are highly specific for collagen and have been the focus of much practical interest with respect to cosmetic, medical and food based applications. The most common uses of these enzymes appear to be in medicine as they have been used to treat burns and ulcers, to eliminate scar tissue and play an important role in the successful transplantation of specific organs.

Key Words: *Collagen, microbial collagenases, CCH, CCO, connective tissue metabolism, remodeling process, therapeutics, tenderizer.*

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INTRODUCTION

Collagens being the most abundant proteins in all higher organisms, a diverse spectrum of therapeutic and biotechnological applications exists for bacterial collagenase. Collagenases are generally defined as enzymes that are capable of degrading the polypeptide backbone of collagen [1]. Collagenase, specific enzymes for the collagen substrate, have been isolated and characterized from both microbial cells and animal tissues. Microbial collagenases are truly promising enzymes in relation to their extensive biological and industrial applications [2]. Microorganisms' *viz. Bacillus subtilis*, *B. licheniformis, B. tequilensis, Streptomyces* sp., *Thermoactinomyces*, screened from soil, water, earthworm and caviar are generally considered safe to produce collagenase [3]. Krepel and co-workers [4, 5] showed that a high proportion of *Peptostreptococcus magnus* strains isolated from diabetic foot ulcers were collagenase producers. Several *Bacteroides* species, particularly those of the *B. fragilis* group, are associated with necrotizing diseases such as bacterial synergistic gangrene, decubitus ulcers, and soft tissue infections of diabetic individuals. Different types of collagenolytic enzymes isolated from tissues of vertebrates, larvae of flies, microorganisms including bacteria, fungi, moulds and actinomycetes are listed in Table 1.

Bacterial collagenases are quite versatile, being capable of hydrolyzing both water-insoluble native collagens and water-soluble denatured collagens [37]. Microbial collagenases have been recovered from pathogenic microorganisms, principally *Clostridium histolyticum.* These collagenases split each polypeptide chain of collagen at multiple sites [38]. They are thought to function as an exotoxin, causing hydrolysis of collagen in the host cells and disrupting metabolism in connective tissues [39]. Controlled degradation of extracellular matrix is an essential event in a variety of physiological conditions

involving connective tissue remodeling such as uterine involution, ovulation, bone growth, embryonic growth and development, resorption, and wound healing [40]. In addition, excessive breakdown of connective tissue plays an important role in a number of pathological processes such as rheumatoid arthritis [41], atherosclerosis [42], pulmonary emphysema [43], tumor invasion and metastasis [44]. Collagenases have been divided into two types, on the basis of different physiological functions. Serine collagenases are probably involved in the production of hormones and pharmacologically-active peptides, as well as in various cellular functions. These functions include protein digestion, blood-clotting, fibrinolysis, complement activation and fertilization [1, 45]. The molecular weight range of the serine collagenases was 24-36 kDa [46]. On the other hand, metallocollagenases are zinc-containing enzymes that require calcium for stability [47]. In addition, these metallocollagenases have been involved in remodeling the extracellular matrix. Their molecular weights vary from 30 to 150 kDa. These enzymes have been widely studied from various mammalian tissues [48]; as well as from bacteria [45, 49] and snake venom [29]. However, the majority of connective tissue destruction is carried out by the matrix metalloproteinases [MMPs] a family of zinc-dependent enzymes that degrades all components of connective tissues [50-54]. Most MMPs are secreted as Zymogens, requiring proteolytic cleavage of the "pro" to be catalytic active [51-55]. Procollagenase is secreted from a variety of cell lines in culture, including fibroblasts [38] and umbilical vein endothelial cells [56], as a mixture of glycosylated 57 kDa and unglycosylated 52 kDa zymogens that are activated by removal of an amino-terminal propeptide to give the corresponding 48 and 42 kDa proteinases. Collagenases of the MMP family play major role in morphogenesis, tissue repair, and human diseases, but how they recognize and cleave the collagen triple helix is not fully understood [57].

The most extensive work has been carried out on the bacterial collagenases because they possess broad substrate specificities and degrade both native and denatured collagens [58]. Bacterial collagenases differ from vertebrate collagenases as they exhibit broader substrate specificity [58]; and unlike animal collagenases that split collagen in its native triple-helical conformation [59], bacterial collagenases can attack almost all collagen types and are able to make multiple cleavages within triple helical regions [60]. The microorganisms producing collagenase are mostly pathogenic in nature and presumly contribute to their pathogenicity by allowing organism to penetrate connective tissue barrier or involved in degradation of collagen under natural conditions [61, 62]. Among studied microorganisms, the non-pathogenic fungi *Penicillium aurantiogriseum* and *Rhizoctonia solani* stood out in volumetric and specific extracellular collagenase activity [63]. Thus, microbial collagen degrading enzymes have been sought and investigated using biochemical and molecular biology techniques. Collagenases, isolated from of variety of sources with different molecular properties have been described in Table 2.

Table 1: The major source of collagenase

Source	References	Source	References
Clostridium histolyticum	(6)	*Klebsiella pneumoniae*	(21)
Clostridium perfringens	(7)	*Penicillium chrysogenum*	(22)
Vibrio alginolyticus	(8)	*Aspergillus fumigatus*	(23)
Vibrio parahaemolyticus	(9)	*Aspergillus oryzae*	(24)
Porphyromonas gingivalis	(10)	*Trichophyton shoenleinii*	(25)
Escherichia coli	(11)	*Rhizoctonia solani*	(26)
Pseudomonas sp	(12)	*Scomber japonicas*	(27)
Bacillus alvei DC-1	(13)	*Tadpole tissue explant*	(28)
Bacillus licheniformis F11.4	(14)	*Snake venom*	(29)
Aeromonas sp	(15)	*Hypoderma Lineatum*	(30)
Bacillus tequilensis	(3)	*Penicillium sp.*	(31)
Bacillus subtilis ATCC 6633	(16)	*Streptomyces sp. Strain 3B*	(32)
Bacillus subtilis FS-2	(17)	*Aspergillus flavus*	(33)
Bacillus subtilis CN	(18)	*Streptomyces exfoliatus*	(34)
Bacillus cereus	(19)	*Candida albicans*	(35)
Bacillus pumilus Col-J	(20)	*Penicillium aurantiogriseum*	(36)

Table 2 Biochemical properties of some microbial collagenase

Sources	M_r (kDa)	Optimum pH	T°C	Inhibitors	References
Clostridium histolyticum	68-130	6.3-7.5	37	o-phenanthroline, EDTA, EGTA, Cysteine, histidine, DTT, 2-mercaptoethanol	(64)
Clostridium perfringens	120	7.0-7.5	42	---	(49)
Bacillus subtilis FS-2	125	9	50	EDTA, idoacetamide, Iodoacetic acid, Soyabean trypsin inhibitors	(17)
Bacillus pumilus Col-J	58.64	7.5	45	EDTA, EGTA and β-mercaptoethanol	(20)
Bacillus sp. Strain MO-1	210	7.1-9.3	60	EDTA and EGTA	(65)
*Bacillus licheniformis*F11.4	124-126	7.0	50	Fe^{2+}, Mg^{2+}, Mn^{2+}, Co^{2+}, EDTA and β-mercaptoetanol	(66)
Thermoactinomyces sp. 21 E	50	9.0-9.5	60	PMSF and DFP	(62)
Streptomyces exfoliates CFS 1068	14.5	7.0	30	PMSF	(34)
Streptomyces sp. Strain 3B	116.97	---	28	EDTA, 1,10-phenanthroline	(32)
Trichophyton schoenleinii	20	7.5	30	Cysteine and Urea	(25)
Rhopaloeides odorabile	116.25	7.5	25	---	(67)
Aspergillus oryzae	20	9-10	---	Di-isopropyl phosphofluoridate	(24)
Rhizoctonia solani	66	5.0	40	EDTA, Idoacetate and Arsenate	(26)
Scomberjaponicas	14.8	7.5	55	PMSF, TLCK, and soybean-trypsin inhibitor	(27)
Penicillium sp.	37	9.0	37	PMSF	(31)
Penicillium aurantiogriseum URM46.	39.16	8.0	45	---	(68)

Applications of collagenases in relation with various diseases

The role of collagenases in several human diseases includes either hyper production of collagenase or due to inadequate amounts of collagenase in the body. The dogma of these diseases includes understanding the involvement of collagenase and then developing the therapeutics for the treatment [69]. The collagenase has found wide spread applications that can be classified within two categories. Applications in which collagenolytic proteases are used directly and applications in which the reaction products [collagen/collagen peptides] produced by collagenolytic proteases are used. Collagenases have been emerged as potentially important agents in the degradation of necrotic tissue [70, 71] and used as important constituents of tissue culture media. The degradation of valuable hide used as raw material due to collagenase production by colonizing microorganisms is an important problem in the leather industry. Attempts have been made to develop eco-friendly procedures for raw hide processing by use of novel dehairing proteases from microbial sources [72, 73]. Microbial collagenases have the potential applications in food and nutrition sector such as meat tenderization, collagen peptides, hydrolysates, collagen extraction, by-products utilization and functional foods [2].

There is a wide range of industrial applications of collagenase including food industries [74], cosmetic industries [75] tannery and meat industries, [76, 77] but the most significant applications of collagenase is in the field of therapeutics. Collagenases have various non-invasive therapeutic applications such as treatment of Dupuytren's and Peyronie'sdisesase, burns, wound healing, intervertebral disc herniation, chronic total occlusions, glaucoma, cartilage repair, uterine fibroid, cellulite, keloid, nipple pain and degradation of human retained placenta [69, 78] and cancer gene therapy [79, 80]. The collagenases can mediate tumor invasion through several mechanisms, which include constitutive production of enzyme by the tumor cells, induction of collagenase production in the neighbouring stromal cells and interactions between tumor/ stromal cells to induce collagenase production by one or both cell types. Thus, elevated expression of the interstitial collagenases is associated with a poor prognosis in a variety of cancers, and therefore, these MMPs can serve as a marker of tumor progression [81].

Collagenase in treatment of lumbar disc herniation

Lumbar disc herniation is the most common illnesses occur in young adults in recent years and only surgical resection has been considered a common treatment. Investigations have shown that injection of enzymes such as collagenase can be an improvement in indications of disc herniation [82, 83]. In last decades, a variety of minimally invasive techniques have been used in clinical therapy of intervertebral disc, including percutaneous discectomy in combination with collagenase. This technique has been developed in China and widely been used [84]. The use of collagenase increases the contact area in nerve

root and soon the dissolution of collagen starts. The dissolved collagen gradually absorbed which further reduces the intradiscal pressure and relieves the pressure on the nerve root [85]. Wu et al. [86] assessed the therapeutic results of collagenase injection combined with oxygen and ozone for the treatment of lumbar disc herniation compared to the traditional surgery and concluded that it shows significant improvements in function and reductions in pain. This treatment can be considered as an option for the treatment of non-contained lumbar disc herniation instead of surgery. Recently, a study on the treatment of cervical intervertebral disc herniation was conducted via radiofrequency combined with low-dose collagenase injection into the disc interior via an anterior cervical approach which was found to be very effective and safe. The protrusion size was significantly decreased after the treatment [87].

Collagenase as debriding agent in wound healing

Debridement is the technique for removing dead necrotic or infected tissue from a wound that is an important health care issue. Collagenase enzymes have been experimentally investigated to increase the proliferation, angiogenesis, and the migration of dermal cells in the wound healing process [88]. Collagenase is well established enzyme preparation used for debridement. Its development as a debriding agent as well as for other applications came to a peak in the early 1970s [89]. The commercially available preparation of collagenase [Collagenase Santyl®, Smith and Nephew Inc., Largo, Florida] has been from *Clostridium histolyticum*. Collagenase can hydrolyze native collagen and thereby facilitate rapid debridement and healing of chronic wounds. The collagenase first degrades collagen into gelatin, upon which less specific enzymes then act. However, until collagenase cleaves collagen, no other enzyme is capable of breaking it down. Clostridial collagenase ointment [CCO], a type of enzymatic debridement, that potentially allows for epithelialisation while debriding. Pressure ulcers treated with CCO achieved higher rates of granulation and subsequent epithelialisation[90]. Wound bed preparation [WBP] is an established concept in chronic wound management. Facilitating maintenance debridement by collagenase can be used to complete wound closure [91]. Wound bed preparation optimization with collagenase enzymatic debridement offers opportunities to improve the management of chronic and difficult-to-heal wounds [92]. Enzymatic debridement by collagenase is used for diabetic foot ulcers and pressure ulcers in conjugation with antibiotics for burns yet there is risk of developing an adverse effect [93].

Collagenase in the treatment of Dupuytren disease

Dupuytren's contracture is fibroproliferative condition of the hand, characterized by the formation of nodules in the palm which progressively advance into fibrotic cords. Contracture of the cords produces deformities of the fingers [94]. This deformity results in considerable disability and can limit patient activities of daily living, manual activities, sporting hobbies and finally markedly reducing patients' quality of life and the prevalence of Duputyren's disease in the general population increases with age [95]. To restore the function fully, contractures have been treated by cutting the causative strands for nearly 200 years, ever since Baron Guillaume Dupuytren demonstrated his technique at the beginning of the nineteenth century. Surgery can be minimal [fasciotomy] or quite invasive [96]. It's the ongoing mainstay being surgical correction involving either release or excision of the affected palmar fascia though complications and recurrences are frequent [94, 95]. However, considerable attention has been paid for the non-operative treatment of Dupuytren disease. Injectible collagenase is more efficient than surgical fasciotomy and has milder side effects, which leads to higher patient satisfaction [97, 98]. Studies on the influence of CHCs on Dupuytren's cords and on Dupuytren's disease fibroblasts have demonstrated that clostridial collagenases can efficiently digest ECM of Dupuytren's disease cords without inducing significant cytotoxicity or structural damage to non-collagenous tissue elements [99, 100]. In the last decade translational research has introduced the non-surgical technique of enzymatic fasciotomy with collagenase injections and able to increase quality of life [96, 101]. This was proved to be successful with the use of injectable *C. histolyticum* collagenase [102-108]. Collagenase from *C. histolyticum*, lyses collagen and leads to disruption of contracted cords [109], was thought as new, minimally invasive, nonsurgical, investigational option for the treatment of advanced Dupuytren's disease. However, this treatment does not require anesthesia. Allergic reactions are extremely rare after treatment with *C. histolyticum* collagenase despite an immune response to CCH can manifest axillary pain and

lymphadenopathy [110]. MRI findings by Crivello et al. [111] suggest that there might be local chemical dissolution of the cord. In previous single-center studies, injectable collagenase reduced contractures of the metacarpophalangeal and proximal interphalangeal joints to 0 to 5 degrees of full extension in approximately two thirds of treated joints [112]. Recent studies shows that after the extension procedure by CCH, percentages of joint achieving a degree of contracture of 5° or less, or a relative contracture reduction of at least 50% were 64.9% and 90.1%, respectively; thus demonstrating CCH to be safe and effective [101]. CCH injection [Xiaflex] is a facile, potent, and well tolerated alternative to surgery. Although, recurrence rates after treatment with CCH are higher than surgery but if surgery is required after treatment with CCH, it is less complex than a primary procedure [110].

Collagenase in the treatment of peyronie's disease

Peyronie's disease is a chronic inflammation and progressive fibroblast proliferation of tunica albuginea accompanied by fibrous plaques/lesions in the penis. This deformity is characterized by abnormal curvature, pain with erection, penile shortening and erectile dysfunction in many cases [69]. Its occurrence is most frequent in men aged between 50 to 70 years; however its prevalence in men aged below 30 years is 3.2%, based on a survey [113] and affects 5% of men in the world [114]. Collagenase *Clostridium histolyticum* [CCH] was isolated in the mid-1900s and postulated as a potential pharmacologic strategy for breaking down the abnormal connective tissue plaques of Peyronie's disease. Intralesional collagenase is the first U.S. FDA approved drug for the treatment of Peyronie's disease. It works by breaking down the extra collagen in the penis that causes this disease [115]. A multicenter, double-blind, phase 3 randomized and placebo-controlled trial supports the efficacy and safety of intralesional CCH in the treatment of the subjective and objective aspects of significant Peyronie's disease in patients with curvature between 30° and 90° [116, 117]. Recent studies demonstrated that CCH treatment administered in multiple cycles led to significant benefit in both the psychological and physical aspects of Peyronie's disease. Although there are few adverse effects like penile pain, swelling and bruising after the treatment but resolves gradually indicating CCH to be safe, effective and valuable minimally invasive treatment against Peyronie's disease [118].

Collagenase and the pathology of rheumatoid arthritis

The involvement of the collagenases has been considered crucial in the destructive process [joint destruction, including the loss of both articular cartilage and bone]. The network of collagen fibers within articular cartilage provides the framework in which other macromolecules, such as the proteoglycans, are embedded. Together these fibers and molecules give cartilage its tensile strength, permitting it to resist loading compression and thereby allowing smooth articulation of the joints. The proteoglycans content is constantly turned over and chondrocytes replace the lost proteoglycans. However, damage to the collagen network was reported to be irreversible and leads to permanent joint damage [119]. The relationship between active collagenase and the pathology of rheumatoid arthritis is of particular interest. It has been suggested that the normally inhibited collagenase in articular structures may be activated thereby causing the characteristic tissue destruction in joints [120, 121]. Rheumatoid synovial fluid contains an activating material not found in joint fluid from patients with osteoarthritis [122, 123]. Harris et al. [122] reported that the collagenase inhibiting capacity of rheumatoid arthritis patients is reduced by one half. The collagenases are the only enzymes that are known to cleave collagen under physiological conditions and as result of this property they cause a key irreversible step in the destruction of cartilage [124]. Consequently, inhibition of these enzymes has been considered a therapeutic target for many years [125].

Collagenase in cardiac remodeling

Cardiac remodeling has been described as both an adaptive and a maladaptive process. The adaptive component enables the heart to maintain function in response to pressure or volume overloading in the acute phase of cardiac injury [126]. The myocardium consists of myocytes tethered and supported by connective tissue network composed largely of fibrillar collagen, synthesized and degraded by interstitial fibroblasts. Matrix metalloproteinases [MMPs] are traditionally known for degrading the extracellular matrix proteins. However, MMPs can also promote ECM production [and fibrosis] by regulating the activity of fibroblasts [127]. Myocardial collagenase is

thought to be an important proenzymepresent in the inactive form in the ventricle [128, 129]. Its activation after myocardial injury contributes to an increase in chamber dimension in response to the distending pressure that is thought to be a possible cause of myocyte slippage, and has been considered for chamber remodeling [130, 131].

Collagenase in emphysema

Histological studies have demonstrated that fibrils from type I and III collagen are widely distributed in the lung. They are present in the adventitia of pulmonary arteries, the interstitium of the bronchial tree, the interlobular septa, the bronchial lamina propria and the alveolar interstitium, where the pathological changes of emphysema are known to occur. There are a number of correlative studies which suggest that collagenase, a classical member of the metallomatrixproteinases family, may possibly be involved in several lung diseases [132, 133]. In addition, some studies suggest that collagen is degraded or damaged in pulmonary emphysema, for example, antibodies to collagen have been found in the serum of patients with emphysema [134]. The extracellular matrix is essential for the integrity of the lung and when disrupted can lead to the architectural changes seen in emphysema. The etiology of emphysema is believed to be due to an imbalance in the proteases and antiproteases within the lung. This expanded understanding of the pathophysiology of emphysema will lead to improved therapy in the treatment of the disease [135].

Collagenase as markers of tumor progression

Collagenase, acts as a valuable checkpoint in the migration of cancer cells from epithelial and endothelial membranes to distant parts in the body, leading to metastasis [136-138]. Higher levels of collagenase, an MMP, cause a more rapid unwinding of the collagen helix, leading to a rapid malignant transformation of the cells [139]. Collagenases have thus emerged as validated targets for synthetic MMP inhibitors for the treatment of cancer [140, 141]. These inhibitors have also shown promise as effective therapeutic molecules for the treatment of chronic obstructive pulmonary disease [COPD], where increased levels of MMPs cause changes in the alveolar tract, leading to decreased air flow [142, 143]. Collagenase-3 [MMP-13] is a member of the matrix metalloproteinase family of endopeptidases that is characterized by a potent degrading activity against a wide spectrum of substrates. This enzyme was first detected in breast carcinomas but it has also overexpressed in a variety of malignant tumors including head and neck carcinomas, chondrosarcomas, skin sarcomas and carcinomas of the female genital tract. Clinical studies have revealed that in all these tumors collagenase-3 expression was found to be associated with invasive and metastatic tumors. Analysis of the molecular mechanism underlying it's marked over expression in malignant tumor has allowed identifying different cytokines, growth factors and tumor promoters with ability to upregulate collagenase-3 expression in tumor cells, or in stromal fibroblasts surrounding epithelial tumor cells. The strategies designed to target this enzyme has been developed and mainly directed to prepare synthetic inhibitors with ability to selectively block the collagenase-3 proteolytic activity [144]. Genes that are up-regulated in stroma associated with tumor includes various ECM-related molecules, such as MMP-1 collagenase, which are expressed at high levels in invasive tumors [145]. Matrix Metalloproteinase 8 [Collagenase 2] expression by breast cancer cells is deleterious to long-term growth, but it induces the expression of Interleukins 6 and 8, factors that conventionally promote malignancy [146]. Activation of proMMP-1 is initiated by serine proteinase secreted by either fibroblast or tumor cells. Full activation of MMP-1 is obtained by stromelysin [MMP-3] secreted by fibroblast [53] and MMP-1 subsequently degrades collagen, leading to tumor invasion [81].

Matrix metalloproteinases [MMPs] have been known to be associated in cancer progression because of their ability to degrade the extracellular matrix. However, few members of this family have been identified as proteases with antitumor properties. Collagenase-2 [MMP-8] has been described to show a protective role in tumor and metastasis progression, but the molecular mechanisms underlying these effects are unknown. In this, Mmp8 expression causes a decrease in miR-21 levels that in turn leads to a reduction in tumor growth and lung metastasis formation by MDA-MB-231 [4175] breast cancer cells [147].

Collagenases in tissue dissociation

Clostridial collagenase has widely been used in biomedical research to dissociate tissues and isolate cells and as a therapeutic drug for the removal of necrotic wound tissues. Collagenase is

suitable for applications needing to avoid introduction of animal derived pathogens into bioprocessing. Researchers generally use highly purified collagenase preparations free of other proteolytic activities for collagen and biosynthetic studies [148]. However crude collagenase preparations combined with other enzymes such as elastase, trypsin or papain has been used for tissue dissociation [149]. There has been a strong interest to isolate intact cells from a broad variety of tissues for research purposes. If tissue dissociation is accomplished by perfusion of the intact organ with enzyme solution, then collagenase has always been considered as the enzyme of choice, either alone or in combination with other enzymes such as hyaluronidase or trypsin [150]. Collagenase has widely been used in medical industries and molecular biology experiments [151-153]. Collagenase has been successfully used for the isolation of cells from bone [154], cartilage, thyroid glands [155], ovarian and uterine tissues [156], skin, endothelial cells [157], neuronal cells [158], and others. Collagenases are used in enzymatic debridement, non-surgical removal of debris from wounds and removal of dandruff [159]. Bacterial collagenase portal perfusion can efficiently delay liver cirrhosis development and hasten the regression of well-established liver cirrhosis in rabbits [160]. Recent results indicate that a high proportion of class 1 collagenase from *C. histolyticum* [116 kDa] was found to be critical for successful human islet isolation [161], whereas the other forms of collagenase have been successfully used in hepatocyte and adipose stem cell isolation.

Collagenase in control of plant pathogens

The collagenase plays important role in the control of plant pathogens [162]. Cuticles degradation of nematodes *Caenorhabditis elegans*[163] and *Panagrellus silusiae* [164], and animal parasitic Ascaris species [165], by the bacteria have been extensively characterized. The entire surface of plant parasitic nematodes is covered by a multilayered cuticle. Cuticle also lines the mouth, buccal capsule and oesophagus, as well as the rectum and anus [166]. The major structural components of these cuticles are collagens [167]. Hence cuticle degradation could be an effective way of controlling preparasitic and parasitic forms of root knot nematodes. A procedure to extract viral RNA from nematodes must take into account that soil-inhabiting nematodes have evolved to survive extremes in soil structure, moisture, temperature and salinity. This has been accomplished by a complex, tough cuticle that serves as a barrier to the environment and as a hydrostatic skeleton, against which muscles contract. This cuticle also covers the stylet and stylet extension of the nematodes, and lines the digestive tract. The outermost layer of the cuticle is made primarily of collagen, a cross-linked protein secreted by cells of the inner layers of the cuticle [168]. Thus, to detect plant viruses carried internally by these nematodes the nematode cuticle must be disrupted by rather severe means. Researchers have disrupted nematode cuticles mechanically by micro-dissection [169], or by vortexing in the presence of phenol and glass beads [170]. These methods were evaluated for the detection of *Tomato ringspot virus* [ToRSV] in *Xiphinema americanum*, transmitted to healthy plants by viruliferous nematodes in the soil. None of these methods were found to be suitable for detection of ToRSV in *X. americanum*. The commercial collagenase, preparation was evaluated as means to more effectively and efficiently disrupt the cuticle of nematodes and to improve the extraction of viral RNA from nematodes [171].

Collagenase in food industry and nutrition

Food processing by enzymatic hydrolysis using biological agents is well known approach which increases nutritional and functional properties of food [172]. Toughness of red meat is due to collagen and meat tenderness can be achieved with collagen digestion by microbial collagenases [77]. Collagenolytic enzymes has widely been used in meat industry as it can tenderize meat by digesting collagen and generate taste and flavor in meat products [173]. Attributes of a good meat tenderizer should be high specific activity at room temperature and gets deactivated during cooking process [74]. Application of collagenase on meats enhances juiciness, decrease its bitterness that is associated with meats treated with traditional proteases. The demand for scaleless fish fillets of species such as salmon, perch, ocean bream, silver carp, and tuna has resulted in the development of a new enzymatic method for the removal of scales from fish with the help of collagenases. Collagenase preparation from crab hepatopancreas has been used for deskinning of squid [174]. Bacterial collagenases holds the potential as significant component for bioactive functional ingredients [functional foods], for peptides preparation [collagen peptides] which provides health benefits [2].

Other applications of collagenases

Collagenases can also be used in blood clot removal [175]. Extensive researches in the field of bioengineering were carried out on enzymatic hydrolysis of leather wastes predominantly containing the collagen [13, 49]. This hydrolysis was carried out by collagenases which are enzymes that can hydrolyze both native and denatured collagens. Tannery wastes can also be treated biologically by collagenase producing microorganisms and the enzymatic hydrolysis could be a safe option of recycling these organic materials [176].

Applications of collagen and collagen peptides: The reaction products of collagenases

Collagen is the most distributed class of proteins in the human body. The use of collagen-based biomaterials in the field of tissue engineering applications has been intensively growing over the past decades. Various cross-linking approaches were explored and differential combinations with other biopolymers were analyzed in order to enhance tissue function. Collagen possesses a major advantage in being biodegradable, biocompatible, easily available and highly versatile. After all, collagen is a protein, it remains difficult to sterilize without alterations to its structure [177]. Collagen beholds both biomedical and industrial applications. It is a common constituent of soaps, shampoos, facial creams, body lotions and other cosmetics and of food grade gelatin. In medicine, collagen has been used in cardiovascular surgery, plastic surgery, orthopedics, urology, neurology and ophthalmology. The major medical application of collagen has been as catgut suture which has been derived from intestinal collagen and promotes wound healing. The alluring aspect of collagen as a biomaterial relies extremely on the fact that it is a natural material of low immunogenicity [178].

Collagenolytic enzymes are used in several industries [179], to hydrolyse proteins, including native collagen and gelatin. Collagen is produced in large quantities as a by-product in the livestock industry, and it has been shown that peptides derived from collagen possess physiological activities that are useful in food and medical products [180, 181]. Furthermore, collagen, collagen peptides, and other proteins could be used as effective skin moisturizers, and they are advantageous as moisturizers or stabilizers in cosmetics and biological products [8]. Oral administration of collagen peptides prevents osteoporosis, protects against gastric ulceration, relaxes hypertension, and stimulates skin metabolism [180, 182]. The collagen present in the dermis of the skin is a fibrous protein that fills the gaps between cells and helps maintain tissue flexibility. The effective increase in the skin collagen has considered an important goal for cosmetic research. Recent research has shown that soybean peptide has anti-fatigue activity, antioxidant activity, and the ability to increase type I collagen, while this collagen peptide has the ability to enhance corneal moisture content and viscoelasticity, and also increase the levels of hyaluronic acid synthesizing enzymes in human skin [183].

CONCLUSION

Collagenases have assumed increasing importance every day, having been implicated in angiogenesis, wound repair, inflammation, ageing etc. apart from the various disease conditions described. Collagenases were used to anchor signalling molecules to the collagen containing tissues, presenting a great potential for targeting drug delivery of anti-arthritic and anti-cancer agents. The use of recombinant collagenase preparations also needs to be explored for the development of effective treatment strategies. The use of standard chemotherapeutics together with newer compounds with novel modes of action has emerged as a treatment modality. The developments of therapies are the need of time that totally abandons the traditional treatments in favour of a more gene specific approach. Further by understanding the roles of collagenase in tumor invasion, researchers may develop drugs that could be effective at various stages of tumor growth and progression. This concept might come closer to become a reality, once the knowledge of molecular mechanisms regulating the expression of the MMPs will completely unfold.

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