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Microbial Keratinases: Then and Now

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Abstract:- Keratinase are special proteases which have the ability to degrade recalcitrant, proteinase K resistant proteins. They are elaborated by compendium of micro-organisms that include bacteria, actinomycetes and fungi. The keratinase research can be divided into three phases. The first phase from was completely dominated by Ker A involving work on its characterization, production, expression and application. The establishment of Ker A as a biotechnologically important enzyme gave way to the second phase of research wherein scientists started exploring the diversity of keratinolytic micro-organisms and understanding its role in keratin degradation. The third phase came with the revealation of its ability to degrade recalcitrant prion protein. Since then, there have been increasing number of reports on keratinases and their characterization. The present article elaborates the journey of keratinase research starting from its establishment as a special protease to the present-day advancements.

Keywords:- *Keratinase, Protease, Sustainability, Feather Degraation, Prion Degradation, Ungual Enhancer.*

I. INTRODUCTION

Keratinase research has witnessed tremendous advancement in terms of attaining a special status amongst proteases three decades ago to currently capturing significant position in the global protease market. This upward trajectory of keratinase research is majorly attributed to their remarkable ability to attack 'hard-to-degrade' proteins, their robustness and potential towards various green technological process that promote environmental sustainability and innovative biotechnological product development.

Keratin degradation was an age-old phenomenon associated with dermatomycosis and certain +-fungi such as *Aspergillus*, *Ctenomyces* and *Streptomyces* were recognised as keratinase producers. So to say, it was mainly a domain of medical mycologists. However, its environmental and biotechnological importance came to light with the first report on the isolation and characterization of a feather degrading bacterium *Bacillus licheniformis* PWD-1 by Shih *et al.* [1]. Shih and his co-workers at that time focussed mainly on feather recycling and feather meal production. They established Ker A, keratinase from *Bacillus licheniformis* PWD-1 as a potential keratinase. Researchers started recognizing it as a unique class of proteases which stands apart from conventional proteases in terms of its substrate specificity and ability to cleave recalcitrant, proteinase-K resistant proteins. Keratinase attained unique EC number amongst proteases such as EC 3.4.99. Further, Ker A was extensively characterized including its sequence and expression in various heterologous hosts [2,3]. A number of papers and patents were published on Ker A [4, 5]. However, during the first decade keratinases did not perceive much attention. Gradually, with the reports of Shih *et al*, it was established that Ker A is a variant of subtilisin Carlsberg and hence researchers started exploring other subtilisins for their keratinolytic potential [6]. Efforts were now made towards understanding the mechanism of keratin degradation wherein the role of cell redox, disulfide reductase and substrate specificity was put forth [7,8,9]. At the same time, various genetic engineering techniques were also being employed to enhance the production of KerA. Production optimisation by chromosomal intergration of the Ker A gene and by forming asporogenic *B. licheniformis* strains was also documented [10, 11].

II. APPLICATIONS OF KERATINASE

Keratinase research gained pace with the exemplification of diverse applications of this enzyme which revolutionized the prospects presented by conventional proteolytic enzymes. First and most important of these applications was its role in valorization of keratinous waste. Environment protection laws significantly focus on efficient management of recalcitrant wastes and recycling it to essential products [12]. Poultry processing plants, slaughterhouses and leather industries generate large quantities of one such recalcitrant waste ie. keratinous waste which is hard to degrade owing to its structural stability and disulfide cross-linkages present in keratin. Keratinases play a pivotal role in degrading this waste and converting it into various useful products such as protein rich feed, organic fertilizers, microbial growth media, bioenergy feed stocks and industrially important enzymes [13, 14]. Thus, keratinases support both environmental sustainability and bio-economy. Application arena of microbial keratinases was further extended to other industries such as leather industry where they play an important role in dehairing of hides, bating and tanning process; detergent industries where they

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are essential part of detergent formulations to remove tough stains; cosmetic industry where there are part of personal skin and hair care products. Keratinases play an important role by efficiently enhancing the drug permeabilization. In the pharmaceutical sector keratinases are also part of formulations used for elimination of keratin in acne opsoriasis, elimination of human callus and degradation of keratinized skins [15,16,17,18]. One of the latest applications of microbial keratinase is in the pharmaceutical industry where they are used as ungual enhancers to deal with the persistent problem of nail infections. Drug permeabilization through the nail plate is a major problem for most of the topical drugs of onychomycosis which leads to recurrence of the disease.

III. KERATINASE AS GREEN SOLUTION TO PRION DECONTAMINATION

The breakthrough discovery that intensified keratinase research was the prion degrading potential of keratinases. The prion degrading potential of Ker A was reported at the same time when the Mad cow disease was in news [19]. Transmissible spongiform encephalitis (TSE) caused by prion is a major threat to human health as till date there is no treatment or vaccine for this deadly neurodegenerative disease. The iatrogenic transmission of vCJD (variant Creutzfeldt-Jakob disease) can occur during treatment using contaminated medical devices or through transplantation of contaminated tissue. Forensic pathologists and autopsy surgeons are also at a serious occupational risk of prion diseases as the postmortem and mortuary rooms are another potential source of infection. Controlling TSE has become a major challenge for the public health organizations. Processes are required to reduce the prion load in the environment including decontamination of carcasses of infected animals and hospital instruments. However, the major bottleneck in prion decontamination is their high level of resistance to the conventional chemical and physical procedures [20]. The existing decontamination methods involve the use of hazardous chemicals. Their use has numerous drawbacks including concerns about worker safety, environmental impact and risk of damaging medical instruments. In this respect, keratinase-based methods emerged as potential green solution to curb this serious problem of prion decontamination. The structure of prion is highly homologous to the β-pleated structure of keratin [19]. Thus, it was hypothesized that keratinases which degrade the β-keratin can accomplish degradation of these structural proteins. Several keratinases esp. from *Bacillus* sp. and *Streptomyces* sp. were documented to effectively degrade prion [19, 21]. Since then, keratinase are being looked upon as potential green solution to curb the existing prion havoc.

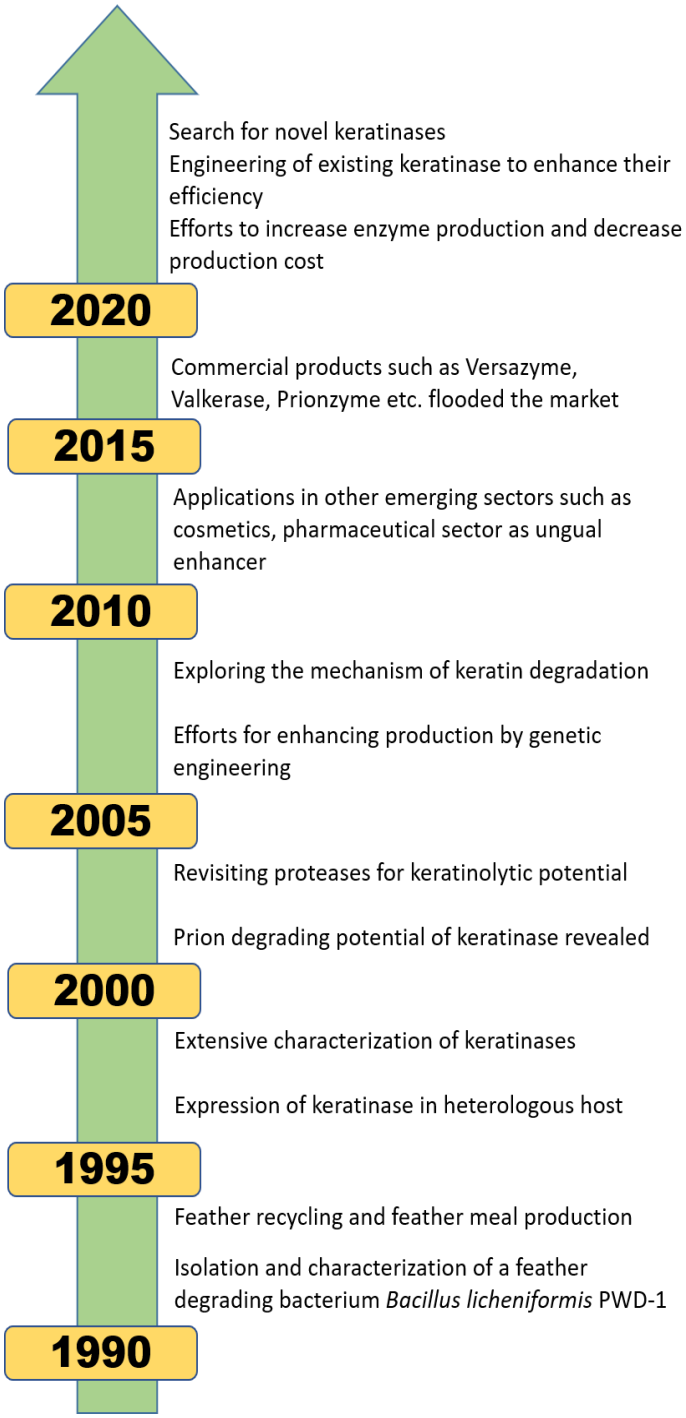

Fig. 1: Milestones of keratinase research

IV. COMMERCIAL STATUS

Realizing the potential and widespread applicability of keratinases various commercial products were launched in the market. Versazyme(R) was the first commercial keratinase product marketed for use in poultry feed by BioResource International, Inc. (BRI). It claims to have beneficial effects on the body weight of broilers alongwith better feed conversion rates. It reduces feed cost without compromising on animal productivity. Valkerase(R) is also a product of BRI which claims of improving the nutritional value of poultry feather meal in an economical and ecological way. Next, Prionzyme™ was launched by Gencor International in

partnership with the Health Protection Agency, United Kingdom for effective removal of prion from medical and dental instruments. It is the first enzyme-based decontamination technology in the market that demonstrated significant removal of prions. It canbe effectively used for disinfection of surgical instruments used in high risk surgeries where risk if vCJD is possible. It is safe to use and eco-friendly. Currently the global market is flooded with many more commercial keratinase based products such as PURE100 marketed by Zurko bioresearch alongwith Proteos biotech for its biomedical, pharmaceutical and cosmetic applications; Keratoclean senstive PB by PROTEOS Biotech for skin care products; CIBENZA® DP100 by Novus International, Inc for poultry and swine feed ingredient digestion; FEED-0001 and NATE-0853 by Creative Enzymes®.

V. CONCLUSION AND FUTURE PROSPECTS

Overall, the thrust area of research in microbial keratinases has been isolation of keratinase producers, keratinase characterization, production optimization, keratinous waste degradation, keratinase gene cloning and expression, feather recycling, upgradation of keratinases for applications in various sectors and commercialization of keratinase-based products. India has ranked first amongst the top twenty most productive countries on keratinase research with 25.99% of total research outputs published in the last two decades (Nnolim and Nwodo, 2021). However, despite the advancements in research, the most exploited keratinase is still from *Bacillus licheniformis*. Most of the commercial products are based on its keratinase. Bottleneck in commercialization of keratinases has also been their low production levels. Keratinases need to be produced in sufficient amounts to meet the increasing market demand. To meet these issues the main focus of keratinase research in the current times is to increase enzyme production, decrease production cost, enhance enzyme characters in addition to enzyme stability.

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