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Review

Improved microbial biosynthesis strategies and multifarious applications of the natural biopolymer epsilon-poly-L-lysine

Ajay Kumar Pandey, Awanish Kumar*

Department of Biotechnology, National Institute of Technology, Raipur, India

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ABSTRACT

Epsilon-poly-L-lysine (ε-PL) is a water-soluble, thermostable, biodegradable, and cationic homo-biopolymer with no toxicity to human health and the environment. It exhibits a characteristic peptide bond between the α-carboxyl groups and ε-amino groups of 25–35 L-lysine residues. It has a wide antimicrobial spectrum. Microorganisms such as bacteria and fungi do not easily develop resistance to it. ε-PL is polycationic in nature; therefore, it interferes with their cell membranes by ionic adsorption, leading to physiological damage to the microbial cell. ε-PL producers commonly possess a membrane-bound ε-PL-degrading aminopeptidase, which has high enzyme activity at pH above 4.0 and may play a role in self-protection. Because ε-PL is safe for human consumption and is biodegradable, therefore it has been used in many novel applications in the fields of food, medicine, environment, agriculture, and electronics in past decades. This review article addresses the occurrence, microbial synthesis, physiochemical properties, production enhancement, biodegradation, and potential applications of ε-PL.

Contents

* Corresponding author at: Department of Biotechnology, National Institute of Technology, Raipur 492010, Chhattisgarh, India. Tel.: +91 8871830586..
E-mail addresses: drawanishkr@gmail.com, awanik.bt@nitrr.ac.in (A. Kumar).

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1. Introduction

Biopolymers are abundantly present in living matter. Microorganisms are natural sources of natural biopolymers such as polysaccharides, polypeptides, polynucleotides, polyesters, and polyphosphates. Natural homo-biopolymers, such as γ-polyglutamic acid (γ-PGA) and ε-poly-L-lysine (ε-PL) uses a single type of amino acid as their building blocks [1]. ε-PL consists of 25–35 L-lysine residues with linkages between α-carboxyl groups and ε-amino groups (Fig. 1). It is produced by many *Streptomyces albulus* species and by some other microorganism [2,3]. The molecular size of ε-PL is a key factor for its antimicrobial activity. ε-PL molecules with more than nine L-lysine residues show considerable potential to inhibit the growth of microorganism, molecules with eight L-lysine residues exhibit negligible antimicrobial activity [4]. ε-PL exhibits more activity than chemically synthesized α-poly-L-lysine, which is a considerably longer chain of L-lysine residues (~50 residues) with linkages between the α-carboxyl and α-amino groups [3–5]. ε-PL is active against both Gram-negative and Gram-positive bacteria such as *Escherichia coli*, *Pseudomonas aeruginosa*, *Serratia marcescens*, *Staphylococcus aureus*, and fungi such as *Candida albicans* [5].

This biopolymer has various advantages over other existing forms of lysine because it is biodegradable, water-soluble in nature and has various functions and properties (Table 1). Because this homo-biopolymer has shown antimicrobial activity against many microorganisms and is practically non-toxic in acute, sub-chronic, chronic animal studies, and is non-mutagenic in bacterial reversion assays, it is widely used as a natural food preservative in many countries, including Japan, Korea, and the United States [6,7]. ε-PL is a slightly bitter in taste, hygroscopic in nature, light yellow natural biopolymer. It is highly water soluble, slightly soluble in ethanol and insoluble in organic solvents such as ethyl acetate, ether, *etc*. It is highly thermo stable up to 120 °C. Its activity is not affected by pH, and the isoelectric point of ε-PL with 25–35 residues is approximately 9.0 [7]. It is a cationic-polymer and can be completely digested by the body. In water, ε-PL contains positively charged hydrophilic amino groups and is electro statically adsorbed to the cell surface of the bacteria, which causes the stripping of the outer membrane [8]. This eventually leads to abnormal distribution of the cytoplasm, damaging the bacterial cell. ε-PL holds potential not only as an antimicrobial agent but also in bioremediation for the removal or neutralization of pollutants from a contaminated site. The properties of ε-PL make it an ideal molecule for multifarious uses. This review focuses on recent advances in regard to the occurrence, biosynthesis, biodegradation, and production enrichment strategies, potential industrial, medical, and other applications of ε-PL.

Table 1
Various properties of epsilon-poly-L-lysine (ε-PL).

S. No.	Properties	References
1.	Anti-microbial	[3]
2.	Antiphase action	[3]
3.	Emulsifying agent	[27,32,33]
4.	Dietary agent	[34]
5.	Endotoxin-selective removal	[40]
6.	Biosensor development	[42–44]
7.	Antiobesity action due to inhibition of pancreatic lipase	[34]
8.	Practically non-toxic in acute, subchronic and chronic feeding studies in rats	[6]
9.	Non-mutagenic in bacterial reversion assays	[7]
10.	Natural food preservative in many countries	[7,10]
11.	Bacteriostatic sausage casing	[51]
12.	ε-Poly-L-lysine-graft-methacrylamide (EPL-MA) hydrogel	[5]
13.	Aldehyde dextran-ε-PL hydrogel	[47,48]

2. Microbial biosynthesis of ε-PL

ε-PL is naturally secreted by various *Streptomyces* sp. and some filamentous fungi. *Streptomyces* sp., which has a characteristically complex secondary metabolism, has been found to be a major producer [1,2]. ε-PL was discovered as a Dragendorff-positive substance produced by the filamentous actinomycete *S. albulus* NBRC14147 [9]. In the first fermentation study with wild-type *S. albulus* in basal medium, the yield of ε-PL was up to 4–5 g/l under acidic conditions by resting cells using glucose and $(NH_4)_2SO_4$ as substrates [10]. At a pH range of 5–6, when incubated with cultivated cells, ε-PL was rapidly degraded, and the yield of ε-PL in the culture medium was only 0.5 g/l after 48 h at 30 °C. Therefore, above pH 5, *S. albulus* produced an ε-PL-degrading enzyme. In another study with *S. albulus* 11011A, in a 3 l jar fermenter, 20 mg ε-PL/ml with an 8.9% yield was obtained from glucose and ammonium sulfate consumption in 120 h under continuous pH control [10]. In another study, optimal pH and glucose concentrations in ε-PL production using *S. albulus* 410 were evaluated by decreasing the pH of the broth culture from 6.8 to 4.8 over 36 h and gradually decreasing it to 3.2 in 96 h [11]. The results obtained in that study showed that, at pH below 4.2, ε-PL started to accumulate in the broth, so ε-PL productivity under strict pH control was evaluated. The optimized culture conditions were divided into two control phases. In the first phase, cell growth was accelerated by maintaining the pH value above 5.0. In the second phase, the ε-PL producing activity was highest at pH 4.0, and the glucose concentration was kept approximately 10 g/l. Glucose depletion caused an increase in the pH of the culture broth, resulting in the degradation of the produced ε-PL. In this control system, ε-PL concentration in a fed-batch culture was enhanced from 5.7 g/l to 48.3 g/l.

After the discovery of the *S. albulus* strain, a novel screening method isolated several strains of Streptomycetaceae and filamentous fungi [12]. This method detected basic polymers such as ε-PL that interact with a charged dye embedded in agar plate. Many ε-PL-producing microorganisms have been isolated and identified using this technique. The *Kitasatospora kifunense* strain MN-1 and an ergot fungus, *Epichloe* sp. MN-9, produced ε-PL with short chain lengths of approximately 29 residues. *Epichloe* sp. MN-9 was the first reported ε-PL producer among eukaryotes. Hirohara and co-workers reported that the two-stage culture (the first being cell growth culture and the second being the ε-PL production culture)