International Food Research Journal 22(1): 23-30 (2015)
Journal homepage: *http://www.ifrj.upm.edu.my*

Mini Review

A natural preservative ε-poly-L-lysine: fermentative production and applications in food industry

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Article history

Received: 31 August 2013
Received in revised form: 12 July 2014
Accepted: 19 July 2014

Keywords

ε-Poly-L-lysine
Streptomyces albulus
Food preservative
Fermentation
Antimicrobial
Antiobesity

Abstract

ε-Poly-L-lysine (ε-PL) is a homopolymer linked by the peptide bond between the carboxylic and the epsilon amino group of adjacent lysine molecules. It is naturally occurring, water soluble, biodegradable, edible and nontoxic towards humans and environment. ε-PL shows a wide range of antimicrobial activity and is stable at high temperatures. This review focuses on various ε-PL producing strains, screening procedure, production, synthesis, antimicrobial activity, and its various applications in food industry.

Introduction

ε-Poly-L-lysine (ε-PL) is a homopolyamide with a single amino acid linked by peptide bonds. ε-Poly-L-lysine (ε-PL) consists of 25-35 L-lysine residues and is characterized by the peptide bond between the α-carboxyl and ε-amino groups of L-lysine (Figure 1). The isoelectric point for 25-35 L-lysine residues is approximately 9. It is basic in nature, highly soluble in water. On boiling a solution of ε–PL at 100°C for 30 minutes or autoclaving it for 20 minutes at 72°C, no degradation is observed and the polymer length is maintained. No structural change is reported upon heat treatment at pH 3.0 (Hiraki, 2000).

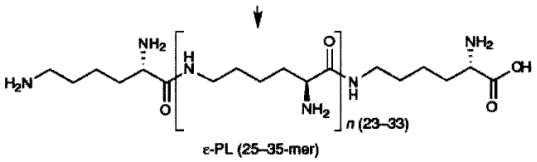

Figure 1. Epsilon poly–L-lysine

It is biodegradable, edible and non-toxic, and hence of great interest to the food and pharmaceutical industry (Shih *et al*., 2006).Currently ε–PL is mainly used as a natural preservative as it is harmless to human (Hiraki, 2000). The notable biological activity of ε-PL attracted a great deal of attention as a possible food preservative. The safety of ε-PL as a food additive was demonstrated by experiments using rats (Neda *et al*., 1999). In Japan, ε-PL has already entered the commercial market and is produced industrially by fermentation using a mutant derived from *S. albulus* (Hiraki, 2000). Apart from being used as a preservative in food industry, derivatives of ε–PL offers a wide range of applications such as emulsifying agent, dietary agent, biodegradable fibres, hydrogels, drug carriers, anticancer agent enhancer, biochip coatings, etc. Because of these wide array of applications in different sectors ε-PL has become a molecule of great interest. The present review focuses primarily on properties of ε–PL, its estimation, production parameters and its array of applications in food industry.

Organisms producing ε–PL

The most common organism producing ε-PL industrially is a filamentous bacterium known as *Streptomyces albulus*. But the main concern with respect to *S. albulus* is the enzymatic and pH induced degradation of secreted ε–PL in the culture medium (Hirohara *et al.*, 2006; Ouyang *et al*., 2006; Wang *et al.*, 2011). Hence different organisms like *Streptomyces diastatochromogenes* CGMCC 3145 (Wang *et al.*, 2011), *Streptomyces aureofaciens* (Takehara *et al*., 2010), *Streptomyces noursei* NRRL 5126 (Banker and Singhal, 2010), *Streptomyces griseofuscus* (Li *et al*., 2010), *Kitasatospora* sp. MY 5-36 (Zhang *et al*., 2010), *Kitasatospora kifunense* (Kobayashi and Nishikawa, 2007), *Kitasatospora* sp. PL6 (Ouyang *et al*., 2006), etc have been explored

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to substitute *S. albulus* for industrial production of ε-PL. An ergot fungus *Epichloe* sp. MN-9 producing ε-PL with 24-29 residues is the first ε-PL producer reported for eukaryotes (Nishikawa and Ogawa, 2002). Recently ε-PL was detected and produced using a novel marine bacteria *Bacillus subtilis* sp.(El-Sersy *et al.*,2012; Shukla and Mishra, 2013).

Qualitative and quantitative estimation of ε-PL

An attractive biopolymer, ε-PL, was discovered at first as a high molecular-weight compound secreted from a strain of *S. albulus* in the course of screening for Dragendorff positive substances (i.e., alkaloids or quaternary nitrogen compounds) from approximately 2,000 actinomycetes. The substance purified from the culture filtrates was identified as ε-PL by infrared spectra, paper chromatography, optical rotation, and chemical methods, and its degree of polymerization and the molecular weight were determined (Shima and Sakai 1977,1981).

Qualitative estimation of ε-PL was carried out using a simple and sensitive method using an acidic dye such as Poly R-478 (Nishikawa and Ogawa, 2002), methylene blue (Kobayashi and Nishikawa, 2007; Li *et al*., 2010). The method was based on interaction between basic charged groups of ε-PL with acidic dyes and was applicable to a solid culture medium hence it was possible to examine numerous microbes at one instance. Later Zhang *et al*.(2012) reported a novel agar diffusion method using methylene blue for both qualitative and quantitative determination of ε-PL in fermentation broth and foods. Studies revealed that easy and rapid quantification of ε-PL concentration can be done using spectrophotometric method which was based on precipitation of ε-PL with excess anionic dye methyl orange, followed by spectrophotometric determination of unbound dye (Itzhaki,1972). With this method 10 µg or less of ε-PL can be estimated. More sensitive method was developed by Shen *et al.*(1984) which could detect 10 fold lower concentrations (1-10 µg) of ε-PL. This method involved quantitative precipitation of ε-PL with trypan blue. The absorbance of unbound dye in the supernatant was inversely proportional to the concentration of ε-PL. Many other methods for qualitative measurement of ε-PL using HPLC have been reported. Kahar *et al.*(2001) used HPLC method for estimation of ε-PL using Tsk gel ODS-120T column (4.6 X 250). Samples were eluted with 0.1% H_3PO_4 at a flow rate of 0.4 ml/min; the detection wavelength was UV 215 nm. Another HPLC method for ε-PL estimation involved use of Hydrosphere C18 column maintained at 30^{o}C; mobile phase, a linear gradient of 0-40% v/v acetonitrile in 0.1% pentaflouropropionic acid. The peptides were monitored by measuring absorbance at 220nm (Kobayashi and Nishikawa, 2007).

Biosynthesis of ε-PL

ε-PL is a strong basic poly(aminoacid) secreted by various *Streptomycetaceae* and a few filamentous fungi. This linear polymer is constructed from L-lysine monomers by the formation of amide bonds between ε-amino and α- carboxyl groups. There are two possible mechanisms underlying the activation of aminoacids for peptide biosynthesis: adenylation (AMP forming) by nonribosomalpeptidesynthetases (NRPSs), where ATP is converted to AMP and pyrophosphate and phosphorylation (ADP forming) by amide ligases where the final products are ADP and phosphate (Nishikawa and Ogawa, 2006). The former catalyses a series of peptide bond formation between an aminoacid and a nascent peptide, both of which are anchored via thioester bonds; the latter catalyses condensation of an aminoacid into the carboxyl group of a free peptide (Kleinkauf and Dohren, 1996; Nishikawa and Ogawa, 2006). Recently Kawai *et al*. (2003) reported that ε-PLsynthesis was found to be non-ribosomally synthesized by catalysis of membrane bound enzymes of ε-PL producing bacteria and suggested that adenylated L-lysine is an activated reaction intermediate in ε-PL synthesis on the basis of L-lysine dependent AMP formation. Thus it could be concluded that lysine polymerization proceeds by iterative reactions between at least two active aminoacylthioester intermediates. Initially, two free lysine residues are loaded onto two free SH groups via lysyl-AMP formation and then the ε-amino group of one lysylthioesternucleophilically attacks the carbonyl of another lysylthioester to form one lysyl-lysylthioester and one free SH group (Kleinkauf and Dohren 1996; Kawai *et al*., 2003; Nishikawa and Ogawa,2006). In the next step, one free lysine is loaded onto the free SH group, and then the ε-amino group of the newly formed lysylthioester attacks the carbonyl group of lysyl-lysyl-thioester to form one lysyl-lysyl-lysyl-thioester and one free SH group. Thus lysine monomers are added to the carboxy terminus of nascent ε-PL, which remains bound to putative ε-PL synthetase. When the number of lysine residues is sufficient ε-PL is released by hydrolysis from ε-PL synthetase. Thus ε-PL synthetase act as ligase for peptide bond formation and the chain length diversity of ε-PL is directly generated by the synthetase rather than via the differential degradation of a uniform polymer by ε-PL degrading enzymes (Yamanaka *et al*., 2008).

In bacteria lysine is synthesized through the

diaminopimelate pathway (DAP) (Figure 2). DAP is formed via aspartate (Asp) produced by combining oxaloacetate (OXA) in tricarboxylic acid cycle with the ammonium ion of a nitrogen source. The first two enzymes in this pathway are aspartokinase (Ask) which catalyze the phosphorylation of L-aspartic acid to produce L-4-phosphoaspartic acid and Asp semialdehyde dehydrogenase (Asd) which reduces L-4-phosphoaspartic acid into L-Asp 4 semialdehyde. These two enzymes are responsible for complex regulation of the end product aminoacids (Hamano *et al*., 2007).

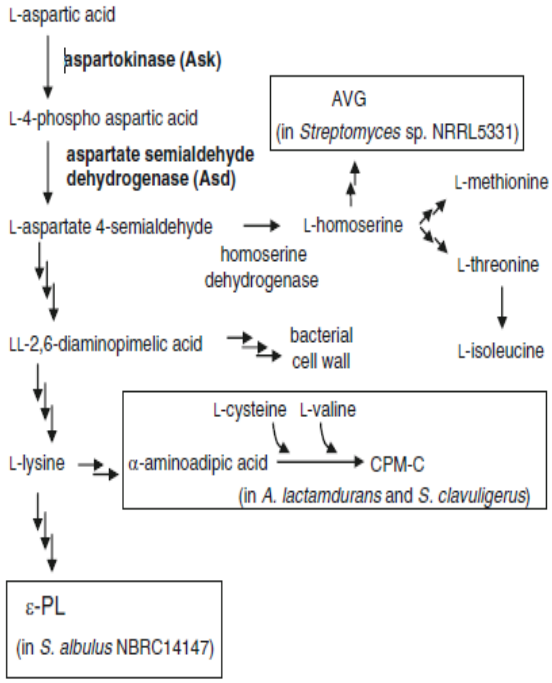

Figure 2. The amino acid biosynthetic pathway from L-aspartic acid (aspartate pathway).ε-PL-poly-L-lysine; AVG aminoethoxyvinylglycine ; CPM-C Cephamycin C

Reports reveal that metals such as iron, cobalt, manganese are involved in the expression of ε-PL synthesis genes. It was suggested that ferric uptake regulator (Fur) protein induce the expression of ε-PL synthesis genes or suppress the expression of genes that inhibit ε-PL synthesis (Kobayashi and Nishikawa, 2007). In correlation with this, studies also suggest that ferrous ion stimulates the activities of proteinase and promotes the activities of ammonia assimilating enzymes to convert ammonium ions into amino acids. Therefore the production of L-lysine is also promoted and the synthesis rate of ε-PL is accelerated (Wang *et al.*, 2011).

Microbial production of ε-PL

ε-PL was accidentally discovered as an extracellular material produced by filamentous bacterium *Streptomyces albulus* sp. *Lysinopolymerus* strain 346 more than 35 years ago. Since then extensive studies have been done to optimize its production. ε-PL is now industrially produced by aerobic fermentation, using a mutant derived from *S. albulus* 346, isolated from soil by Shima and Sakai. In the first fermentation study with the wild type of *S. albulus* 346, the accumulated concentration of ε-PL in the culture medium was 0.5 g/l under optimized culture conditions (Shima and Sakai 1977, 1981a,1981b). The maximum accumulation of ε-PL was observed during fermentation initiated at pH 6.0. After cell growth reached the stationary phase, the accumulation of ε-PL in culture broth was observed and lowering the pH value of the medium (pH 3.0–5.0) was essential for ε-PL production (Shima and Sakai 1977, 1981a). Kahar *et al*. (2001) suggested a pH control strategy for cell growth and ε–PL production. Optimized fermentation was divided into two phases. In phase I, cell growth was accelerated by maintaining the pH at higher than 5.0. In phase II, ε–PL production was increased by maintaining the pH at about 4.0. Glucose depletion caused an increase in the pH of the culture broth, resulting in the degradation of the produced ε-PL. To avoid increase in pH due to glucose depletion, glucose concentration was kept around 10 g/l. In this control system, ε-PL productivity in a fed batch culture was enhanced from 5.7 g/l to 48.3 g/l. In another study ε-PL production was carried out in air lift bioreactor and compared with jar fermentor to minimize the production cost including downstream processing of ε-PL. The production level of ε-PL was similar in both the fermentors but the power consumption was found to be more in jar fermentor. Moreover, the leakage of intracellular nucleic acid related substances into the culture broth in air lift bioreactor was less than jar fermentor thereby reducing the downstream processing cost (Kahar *et al.*, 2002). Banker and Singhal (2011), reported that aeration and agitation of the fermentation broth improved ε-PL production, cell mass formation and glycerol utilization. Fermentation studies showed that ε-PL is growth associated and agitation speed of 300 rpm and 2.0 vvm supports higher yields of ε-PL. Many other ε-PL producing strain such as *Streptomyces griseofuscus* (Li *et al*., 2010), *Streptomyces aureofaciens* (Takehara *et al.,* 2010), *Kitasatospora* sp.PL6 (Ouyang *et al.*, 2006), isolated from soil producing were explored. Further to enhance the productivity many statistical methods have been used to optimize the medium for ε-PL production. Shih and Shen (2006), used response surface methodology to optimize ε-PL production. In another study Plackett-

Burman design was used to screen and select the most significant culture variables further evolutionary operation was used for further optimization (Banker and Singhal, 2010). Metabolic precursors like amino acids, TCA acid cycle intermediates and cofactors have been investigated for improved production of ε-PL. Addition of citric acid after 24 hours and L-aspartate after 36 hours of fermentation medium had a significant effect on ε-PL production (Banker and Singhal, 2011). Other micronutrients such as iron, manganese, and cobalt promote ε-PL productivity and the most effective metal among these was iron (Kobayashi and Nishikawa, 2007). ε-PL production with immobilized Kitasatospora sp. MY 5-36 cells using inert supports such as baggase, synthetic sponge, macroporous silica gel, and loofah sponge was studied. The immobilized cells could be reused five times and thus this approach was found to be promising tool for industrial applications (Zhang *et al.*, 2010).

Antimicrobial and antiphage activity of ε–PL

Many peptide antibiotics have been isolated from microorganisms. However these antibiotics generally consist of more than two different amino acids and sometimes contain unusual amino acids. ε–PL a homopolymer shows strong antimicrobial activity against a wide spectrum of microorganisms. ε–PL inhibits the growth of gram positive and gram negative bacteria at concentration of 1-8 µg/ml. Some yeast were inhibited at low concentration of ε–PL, however most of fungi and yeast were not affected at much higher concentration of ε–PL.

The inhibition of spore germination by ε–PL on Bacillus strain showed that for spore germination the inhibitory concentration was 12.5 µg/ml for B. coagulans, 2.5 µg/ml for B. stearothermophilus and 12.5 µg/ml for B. subtilis (Hiraki, 1995). Bacteriophages that belong to long tail and non-contractile morphological types were inactivated by ε–PL (Shima *et al.,* 1984). ε–PL showed antiphage action, depending more on the phage morphology than the phage nucleic acid . Studies suggested that antiphage action was significantly enhanced by the presence of ferrous ions.

Mechanism of action of ε–PL

ε–PL molecules are cationic, surface active agent due to their positively charged amino groups in water. They show a wide antimicrobial spectrum against yeast, fungi, gram positive and gram negative bacterial species. The probable mechanism to explain the inhibitory effect of ε–PL on microbial growth is its electrostatic adsorption onto the cell surface of microorganism, leading to stripping of the outer membrane and abnormal distribution of the cytoplasm, which produce physiological damage to the cell (Shima *et al*., 1984).These effects are distinct from the mechanisms by which clinical antibiotics operate and are therefore suitable for food uses, where the development and spread of antibiotic resistance is a concern. It has been reported that at least 10 L-lysine residues appear to be necessary for ε-PL to show antimicrobial activity. It was suggested that the basic groups in the ε-PL molecule play an important role in determining antimicrobial activity. Therefore chemical modification of ε-PL would lower its antimicrobial activity (Shima *et al.*, 1984).

Applications of ε-PL in Food Industry

Food preservative

There is growing interest in preventing deterioration of food as well as in improving the shelf life along with enforcement of product liability law and the introduction of a consume by date. A wide variety of approaches to sanitize meat or poultry after harvesting include cold and hot water rinses, steam pasteurization or steam vacuum treatment, trimming, chemical rinses and organic acid rinses with or without surfactants. Freezing and low temperature preservation of food has markedly improved the quality of food but deterioration cannot be completely eliminated by these methods. There is a growing awareness that a widespread use of chemical preservatives may pose serious health problems. Hence there is a increasing demand for use of natural preservatives. Because of its excellent antimicrobial activity and heat stability (Hiraki, 2000), ε-PL has attracted a great deal of attention as a natural food preservative.

Acute oral toxicity study in rats and bacterial reversion assays with strains revealed ε-PL to be practically nontoxic and nonmutagenic respectively (Neda *et al*., 1999). In a two-generation reproduction study using rats, the nontoxic dosage level of ε-PL was concluded to be 10,000 ppm. No toxicity for reproduction, neurological function, embryonic and fetal development and growth was reported with ε-PL at 30,000 ppm (Neda *et al.*, 1999). The pharmacokinetics of ε-PL in vivo was also investigated (Hiraki *et al*., 2003). Absorption, distribution, metabolism and excretion (ADME) studies using14C-radiorabeled ε-PL revealed that ε-PL was poorly absorbed in the gastrointestinal tract and most of the dosed radioactivity was eliminated by excretion within 168 h. Furthermore, no accumulation of ε-PL in any tissue or organ was observed by whole body

autoradiography. Based on these results showing its safety, ε-PL was approved by the Japanese Ministry of Health, Labour and Welfare as a preservative in food in the late 1980s. Thereafter, ε-PL was listed in the Korea Food Additives Code and has been used in Korea. Since long ε-PL is safely used in Japan as a preservative for multiple foods including traditional dishes such as rice and noodles (Hiraki, 2000). In food systems generally usage of ε-PL include spraying or dipping sliced fish and fish sushi at levels of 1000-5000 ppm, preservation of boiled rice, noodles, soup stock and cooked vegetable at level of 10-500ppm (Otsuka *et al*., 1992;Hiraki, 2000). ε-PL has also been used in potato salad, steamed cakes, and custard cream (Hiraki *et al.*, 2003). Recently, the US Food and Drug Administration has given ε-PL GRAS status (generally recognized as safe). FDA approved the use of ε-PL as an antimicrobial agent in cooked or sushi rice at levels up to 50 mg/kg of rice (USFDA, 2004).For food preservation, ε-PL can be used alone or in combination with other food additives such as glycine, vinegar, ethanol and thiamine lauryl sulfonate (Hiraki, 2000; Shih *et al.,* 2006). The combination with other additives greatly enhanced the preservation efficacy of ε-PL.

According to the Economic Research Service of the United States Department of Agriculture, the estimated annual cost of foodborne illness caused by *E. coli* O157:H7, *L. monocytogenes*, and *S. typhimurium* totals $0.9, 2.3, and 2.9 billion, respectively (USFDA, 2004). Although there are guidelines to help reduce food borne illnesses, outbreaks continue to occur. The availability of ε-PL as an antimicrobial agent against food-borne pathogens of *E. coli* O157:H7, *Salmonella typhimurium*, and *Listeria monocytogenes* has been reported (Geornaras *et al.*, 2005; Geornaras *et al*., 2007; Chang *et al*., 2010). When these three pathogens were incubated with ε-PL in tryptic soy broth supplemented with 0.6% yeast extract, 200–400 mg/ml of ε-PL inhibited their growth. The antimicrobial activity of ε-PL was enhanced by combination with 0.25% sodium diacetate or 0.1% acetic acid. It has been reported that a combination ofε-PL with antimicrobial peptide nisin A, showed synergistic effect against food borne pathogens *Listeria monocytogenes* (Takahashi *et al*., 2012). The combination of ε-PL with the above mentioned additives can be considered an effective all-natural formulation for controlling a wide spectrum of food-borne pathogens, spores, and spoilage organisms, although more research is needed to determine the most effective combination or conditions for each food application.

Emulsifying agent

ε-PL can be used as a natural antimicrobial agent but it has a tendency to interact with proteins and acidic polysaccharides, leading to the possible loss of antimicrobial activity, which limits its use. Moreover ε-PL has poor emulsifying properties; therefore its usage has been restricted to starch based foods (Otsuka *et al*., 1992; Hiraki, 1995). Recently the emulsifying activity of ε-PL was improved by conjugating with dextran through Maillard reaction (Ho *et al*., 2000). The emulsifying ability of conjugated ε-poly-lysine was better than those of commercial emulsifiers Sunsoft SE11 and Q-18S, a sucrose-fatty acid ester and polyglycerine ester. The emulsifying ability of conjugate was high in neutral pH and was not affected even in presence of high concentration of salt (1.0M NaCl). The original antimicrobial activity was also retained in the conjugate with dextran. Thus the PL-dextran conjugate could be used as bi-functional additive performing the dual role of additive and emulsifier in food processing.

Dietary agent

Obesity is a serious disease that can lead to numerous health problems including diabetes, hypertension and atherosclerosis (Hill *et al*., 2000). Obesity can be prevented either by intake of low fat diet or ingestion of a natural product that selectively limits intestinal absorption of dietary fat. Studies reveal that pancreatic lipase plays an important role in lipid absorption from intestine (Duan, 2000); thus, it is suggested that natural products that inhibit the activity of pancreatic lipase may suppress dietary fat absorption from the small intestine. Recently, Kido *et al.* (2003) described the first report of lipase inhibitory activity and suppressive effect on postprandial hypertriacylglyceridemia of ε-PL. They showed that ε-PL inhibited human and porcine pancreatic lipase activity in substrate emulsions containing bile salts and phosphatidylcholine, in the concentration range of 10–1000 mg/l. At the same concentrations, it also destroyed the emulsifying activity, suggesting that lipase inhibitory activity and emulsion breakdown activity were associated. The same work also suggested that the breakdown of the substrate emulsion by interaction of ε-PL with bile salts was mainly responsible for lipase inhibition. These results suggested that ε-PL would inhibit lipase in the digestive tract (Kido *et al*., 2003). The effect of ε-PL on postprandial hypertriacylglyceridemia was investigated in rats, and the results showed that the plasma triacylglycerol concentration in rats intragastrically administered 15 mg/kg of both fat emulsion and ε-PL was significantly lower at 2 and

3h after administration than that in rats administered fat emulsion alone. These results strongly suggest that ε-PL is able to suppress dietary fat absorption from the small intestine by inhibiting pancreatic lipase activity (Kido *et al*., 2003).

In-vitro studies showed that, ε-PL strongly inhibits the hydrolysis of trioleoylglycerol emulsified with phosphatidylcholine and taurocholate by either pancreatic lipase or carboxylester lipase (Tsujita *et al*., 2003). Thus it was concluded that ε-PL act as an antiobesity agent. ε-PL resembles orlistat, a strong inhibitor of gastrointestinal lipase reaction, but differs in the mechanism of inhibition. The ε-PL concentration required was eight times lower than the concentration of orlistat required for the same effect (Tsujita *et a*l., 2006). Hence was found to be a promising tool for reducing intestinal lipolysis.

Conclusion

The antimicrobial property of ε-PL and its application as food preservative has been accepted world-wide since its discovery. ε-PL is industrially produced and used as food preservative in many countries. ε-PL is water soluble, polycationic, non-toxic and biodegradable and hence can be applicable in various other fields of food, medicine, environment, agriculture and electronics. Although ε-PL is industrially produced by microorganism but the yield is still low. Thus a new production process should be designed. Moreover there have been reports related to degradation of ε-PL from *S. albulus*, due to enzymes and induced by pH in culture medium. Therefore, it is necessary to explore alternative organisms for the productions of ε–PL. Also the biosynthetic mechanism has not been elucidated, though there are many hypothesis put forward by various scientists. On revelation of the enzymes present in biosynthesis of ε-PL and their genes, a more efficient system ε-PL production system can be developed. This review has highlighted the properties, fermentative production and wide applications of polylysine in food industry. Because of its wide array of applications it can be concluded that the development of this natural polymer is both economical and valuable and can be potential replacement for all existing chemical food preservatives.

References

Banker, S. B. and Singhal, R. S. 2010.Optimization of poly- ε-lysine production by *Streptomyces noursei* NRRL 5126. Bioresource Technology 101:8370-8375.

Banker, S. B. and Singhal, R. S. 2011.Improved poly-ε-lysine biosynthesis using *Streptomyces noursei* NRRL 5126 by controlling dissolved oxygen during fermentation. Journal of Microbiology and Biotechnology 21(6): 652–658.

Banker, S. B. and Singhal, R. S. 2011.Metabolic precursors enhance the production of poly-ε-lysine by *Streptomyces noursei* NRRL 5126. Engineering in Life Sciences 2: 1-6.

Chang, S. S., Lu, W. Y. W., Park, S. H. and Kang, D. H. 2010. Control of food borne pathogens on ready to eat roast beef slurry by ε-polylysine. International Journal of Food Microbiology 141: 236-241.

Duan, R. D. 2000. Enzymatic aspects of fat digestion in the gastrointestinal tract. In Christopher, A.B., Vriese, S. (Eds). Fat Digestion and Absorption. AOCS Press, p. 25–46. Champaign, IL.

El-sersy, N., Abdelwahab, A., Abouelkhiir, S., AbouZeid, D. and Sabry, S. 2012. Antibacterial and anticancer activity of ε-poly-L-lysine (ε-PL) produced by a marine *Bacillus subtilis* sp. Journal of Basic Microbiology 52: 513-522.

Georgnaras, I. and Sofos, J.N. 2005. Activity of ε-polylysine against *Escherichia coli* O157:H7, *Salmonella typhimurium*, and *Listeria monocytogenes*. Journal of Food Science 70(9) : 404-408.

Georgnaras, I., Yoon, Y., Belk, K. E., Smith, G. C. and Sofos, J. N. 2007. Antimicrobial activity of ε polylysine against *Escherichia coli* O157:H7, *Salmonella typhimurium*, and *Listeria monocytogenes* in various food extracts. Journal of Food Science 72(8) : 330-334.

Hamano, Y., Nicchu, I., Shimizu, T., Onji, Y., Hiraka, J. and Takagi, H. 2007. ε- Poly-L-lysine producer, *Streptomyces albulus*, has feedback inhibition resistant aspartokinase. Applied Microbiology and Biotechnology 76: 873-882.

Hill, J.O., Melansonand, E.L. and Wyatt, H. 2000. Dietary fat intake and regulation of energy balance: implication for obesity. Journal of Nutrition 130: 284–288.

Hiraki, J. 1995. Basic and applied studies on ε-polylysine. Journal of Antibacterial and Antifungal agents. 23: 349-354.

Hiraki, J. 2000. ε-Polylysine, its development and utilization. Fine Chemicals 29: 18–25.

Hiraki, J., Ichikawa, T., Ninomiya, S.I., Seki, H., Uoham, K., Seki, H., Kimura, S., Yanagimoto, Y., and Barnett, J.W. 2003. Use of ADME studies to confirm the safety of ε-polylysine as a preservative in food. Regulatory Toxicology and Pharmacology 37: 328–340.

Hirohara, H., Takehara, M., Saimura, M., Masayuki, A. and Miyamoto, M. 2006. Biosynthesis of poly(ε-L-lysine)s in two newly isolated strains of *Streptomyces* sp. Applied Microbiology and Biotechnology 73: 321-331.

Ho, Y.T., Ishizaki, S. and Tanaka, M. 2000. Improving emulsifying ability of ε-poly-lysine by conjugation with dextran through the Maillard reaction. Food Chemistry 68(4): 449-455.

Internet: US Food and Drug Administration (USFDA) (2004) GRAS Notice 000135: ε-Polylysine. Office of Food Additive Safety. Downloaded from *http://www.*

fda.gov. Cited 17 Nov 2009.

Itzhaki, F. R. 1972. Colorimetric method for estimating polylysine and polyarginine. Analytical Biochemistry 50: 569–574.

Kahar, P., Iwata, T., Hiraki, J., Park, Y.E. and Okabe, M. 2001. Enhancement of ε-polylysine production by *Streptomyces albulus* strain 410 using pH control. Journal of Bioscience and Bioengineering 91:190–194.

Kahar, P., Kobayashi, K., Iwata, T., Hiraki, J., Kojima, M. and Okabe, M. 2002. Production of ε-polylysine in an airlift bioreactor (ABR).Journal of Bioscience and Bioengineering 93:274–280.

Kawai, T., Kubota, T., Hiraka, J. and Izumi, Y. 2003 Biosynthesis of ε-poly-L-lysine in a cell-free system of *Streptomyces albulus*. Biochemical and Biophysical Communications 311: 635-640.

Kido, Y., Hiramoto, S., Murao, M., Horio, Y., Miyazaki, T., Kodama, T. and Nakabou, Y. 2003. ε-polylysine inhibits pancreatic lipase and suppresses postprandial hypertriacylglyceridemia in rats. Journal of Nutrition 133: 1887-1891.

Kleinkauf, H. andVon Döhren, H., 1996. A nonribosomal system of peptide synthesis. European Journal of Biochemistry 236(2): 335-351.

Kobayashi, K. and Nishikawa, M. 2007. Promotion of ε-poly-L-lysine production by iron in *Kitasatospora kifunense*. World Journal of Microbiology and Biotechnology 23: 1033-1036.

Li, S., Tang, L., Chen, X., Liao, L., Li, F. and Mao, Z. 2010. Isolation and characterization of a novel ε-poly-L-lysine producing strain: *Streptomyces griseofuscus*. Journal of Industrial Microbiology and Biotechnology 38: 557-563.

Neda, K., Sakurai, T., Stakahashi, M., Shiychi, M. and Ohgushi, M. 1999.Two generation reproduction study with teratology test of ε-poly ε-lysine by dietary administration in rats. Japanese Pharmacology and Therapeutics 27: 1139-1159.

Nishikawa, M. and Ogawa, K. 2002.Distribution of microbes producing antimicrobial ε-poly-L-lysine polymers in soil microflora determined by a novel method. Applied and Environmental Microbiology 68 : 3575–3581.

Nishikawa, M. and Ogawa, K. 2006.Inhibition of Epsilon-Poly-L-Lysine biosynthesis in *Streptomycetaceae* bacteria by short chain polyols. Applied and Environmental Microbiology 72(4) : 2306-2312.

Otsuka, N., Kuwahara, Y. and Manabe, K.1992. Effect of ε-poly-lysine on preservation of boiled noodles. Journal of Japanese Society of Food Science and Technology 39: 344–347.

Ouyang, J., Xu, H., Li, S., Zhu, H., Chen, W., Zhou, J., Wu, Q., Xu, L. and Ouyang, P. 2006. Production of ε-poly-L-lysine by newly isolated *Kitasatospora* sp.PL6-3. Biotechnology Journal 1(12): 1459-1463.

Saimura, M., Takehara, M., Mizukami, S., Kataoka, K.andHirohara, H. 2008 Biosynthesis of nearly monodispersed poly (ε-L-lysine) in *Streptomyces* species. Biotechnology Letters 30 : 377-385.

Shen, W.C., Yang, D. and Ryser, H.J. 1984. Calorimetric determination of microgram quantities of polylysine by trypan blue precipitation. Analytical Biochemistry 142: 521-524.

Shih, I., Shen, M.H. and Van, Y.T. 2006. Microbial synthesis of poly (ε-lysine) and its various applications. Bioresource Technology 97: 1148-1159.

Shih, I.L. and Shen, M.H. 2006.Application of response surface methodology to optimize production of poly ε-lysine by *Streptomyces albulus* IFO 14147. Enzyme and Microbial Technology 39 :15-21.

Shima, S. and Sakai, H. 1977.Polylysine produced by *Streptomyces*. Agricultural and Biological Chemistry 41: 1807–1809.

Shima, S. and Sakai, H. 1981a. Poly-L-lysine produced by *Streptomyces*. Part II. Taxonomy and fermentation studies. Agricultural and Biological Chemistry 45: 2497–2502.

Shima, S. and Sakai, H. 1981b. Poly-L-lysine produced by *Streptomyces*. Part III. Chemical studies. Agricultural and Biological Chemistry 45: 2503-2508.

Shima, S., Matsuoka, H., Iwamoto, T. and Sakai, H. 1984. Antimicrobial action of ε-poly-L-lysine. Journal of Antibiotics 37: 1449–1455.

Shukla, S.C. and Mishra, A. 2013.ε-PolyLysine production from sugarcane molasses by a new isolates of *Bacillus* sp. and optimization of fermentation condition. 2013. nnals of Microbiology 63(4) : 1513-1523.

Takahashi, H., Kashimura, M., Miya, S., Kuramoto, S., Koiso, H., Kuda, T., and Kimura, B. 2012.Effect of paired antimicrobial combination on *Listeria monocytogenes* growth inhibition in ready to eat sea food products. Food Control 26:397-400.

Takehara, M., Hibino, A., Saimura, M., and Hirohara, H. 2010. High yield production of short chain length poly (ε-L-lysine) consisting of 5-20 residues by *Streptomyces aureofaciens* and its antimicrobial activity. Biotechnology Letters 32: 1299-1303.

Tsujita, T., Sumiyoshi, M., Takaku, T., Momsen, W.E., Lowe, M.E. and Brockman, H.L. 2003 Inhibition of lipases by ε-poly-lysine. Journal of Lipid Research 44: 2278-2286.

Tsujita, T., Takaichi, H., Takaku, T., Aoyama, S., and Hiraki, J. 2006.Antiobesity action of ε-poly-lysine, a potent inhibitor of pancreatic lipase. Journal of Lipid Research 47: 1852-1858.

Wang, G., Jia, S., Wang, T., Chen, L., Song, Q.andLi, W. 2011. Effect of ferrous ion on ε-poly-L-lysine biosynthesis by *Streptomyces diastatochromogenes* CGMCC3145. Current Microbiology 62(3): 1062-1067.

Yakanama, K., Maruyama, C., Takagi, H. and Hamano, Y. 2008. ε-Poly-L-lysinedispersity is controlled by a highly unusual nonribosomal peptide synthetase. Nature Chemical Biology. 4(12) : 766-772.

Zhang, Y., Feng, X., Xu, H., Yao, Z. and Ouyang, P. 2010 ε-poly-L-lysine production by immobilized cells of *Kitasatospora* sp. MY 5-36 in repeated fed-batch cultures. Bioresource Technology 101: 5523-5527.

Zhang, Y., Zhang, Q., Feng, X., Li, S., Xia, J. and Xu, H. 2012.A novel agar diffusion assay for qualitative and quantitative estimation of ε-polylysine in fermentation broths and foods. Food Research International 48: 49-56.