Review

Microbial Poly-(glutamic acid): Production, Biosynthesis, Properties, and Their Applications in the Food and Biomedicals

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Microbial Poly-(glutamic acid): Production, Biosynthesis, Properties, and Their Applications in the Food and Biomedicals

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Abstract: This review offers an in-depth analysis of microbial γ-poly-glutamic acid (γ-PGA), highlighting its production, biosynthetic pathways, unique properties, and extensive applications in the food and health industries. γ-PGA is a naturally occurring biopolymer synthesized by various microorganisms, particularly species of *Bacillus*. The report delves into the challenges and advancements in cost-effective production strategies, addressing the economic constraints associated with large-scale γ-PGA synthesis. Its biocompatibility, biodegradability, and non-toxic nature make it a promising candidate for diverse industrial applications. In the food industry, γ-PGA's exceptional water-holding capacity and humectant properties are key to its utility. These features enable it to enhance the stability, viscosity, and shelf life of food products, making it a valuable ingredient in processed foods. The review highlights its ability to improve the textural quality of baked goods, stabilize emulsions, and act as a protective agent against staling. Beyond food applications, γ-PGA's role in health and pharmaceuticals is equally significant. Its use as a drug delivery carrier, vaccine adjuvant, and biofilm inhibitor underscores its potential in advanced healthcare solutions.

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1. Introduction

Biopolymers, defined as naturally occurring substances or polymeric biomolecules synthesized by living organisms during their entire life cycle, have emerged as a promising alternative to their synthetic counterparts. These biopolymers, categorized into three distinct classes i.e., polypeptide, polysaccharide, and polynucleotide based on their monomeric units, possess remarkable versatility and can be easily manipulated and modified to suit a wide range of applications [1].

The natural process of food and beverage fermentation involves the presence of both beneficial and non-beneficial microorganisms. This results in the product changing its composition, which can lead to improved access to nutrients, the breakdown of toxins, and the reduction of anti-nutritional components. The fermentation of whole cereal products has been linked to various functional benefits, including the reduction of cholesterol levels, enhancement of antioxidant and anti-inflammatory properties, and improvement in vascular function [2]. Depending on the fermentation condition and food substrate, various bioactive metabolites including biopolymers such as polyhydroxyalkanoates, polylactic acid, and polyglutamic acid produced by non-infectious *Bacillus* and *Lactobacillus spp.* strains [3]. Polypeptides (poly amino acids) that are produced naturally are of various kinds such as poly-γ-glutamic acid (γ-PGA), poly-ε-lysine (ε-PL), and cyanophycin [4]. γ-PGA an anionic, water-soluble, biodegradable, non-toxic extra-cellular viscous material is produced predominantly by *Bacillus* strains.

γ-PGA was discovered by Ivanovics as a *Bacillus anthracis* capsule, while autoclaving the aged and autolyzed cells, γ-PGA was released into the medium [5]. This edible biopolymer consists of D-

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and L-glutamic acid residues and has diverse applications as humectants, thickeners, cryoprotectants, edible films, heavy metal absorbents, drug carriers, and biological adhesives. Owing to its outstanding water solubility, biodegradability, edibility, and non-toxic nature, γ-PGA and its derivatives have found extensive use in various industrial sectors, including food and pharmaceutical industries [6,7].

Unlike other proteins and peptides that are normally composed of α-amino and α-carboxylic acid units which are prone to protease digestion, γ-PGA differs from them by possessing γ-amide linkages (α-amino and γ-carboxylic units) which prevents its degradation by the action of proteases [8]. Cost-effective substrates and efficient strains are required for the production of the biopolymers commercially through microbial fermentation [9]. Although α-PGA can also be synthesized chemically and through biotransformation these ways are neither economic nor ecological [10].

The preference for L-amino acids in cellular protein synthesis results in the production of proteins that lack D-amino acids. This homochirality is also reflected in the stereochemistry of γ-PGA, where the arrangement of D- and L- forms of glutamic acid is crucial [11]. The molecular weight of γ-PGA, which can range from 100 to 2500 kDa, significantly affects its chain length; as the molecular weight increases, so does the viscosity of the biopolymer, indicating a direct correlation between these two properties [12]. Furthermore, the stability of the α-helical conformation of γ-PGA is maintained within a pH range of 2.5 to 5.5, facilitating the formation of additional COO^- groups [13] in its side chains. An increase in pH beyond 5.5 results in the formation of aggregates, which can lead to arrangements resembling amyloid fibrils [14,15].

Biopolymers have been greatly gaining interest in polymer industries due to their non-chemical production. It is a direct yes to the industries looking for eco-friendly and green synthesis alternatives. Polymers produced biologically are not only environment-friendly ways over chemical-based polymers but are also easy to maintain and produce, and above all they are cost-effective. The gamma-polyglutamic acid and its derivatives have become eminent biopolymers due to their widespread applications in the fields of food to drug discovery [12]. The good physico-chemical properties like water solubility, water-holding capacity (WHC), and flowability make it a useful ingredient in food products over chemical-based ones.

2. Polyglutamic Acid (PGA)

PGA is a naturally existing polymer with anionic properties. It is made up of a highly viscous homopolyamide comprising D- and L- glutamic acid units. PGA is produced by different microorganisms, but for commercial applications, *Bacillus* spp. (specifically *Bacillus subtilis* and *Bacillus licheniformis*) are generally employed [6]. Two distinct types of PGA exist α-PGA and γ-PGA. These forms differ in their structural composition, with glutamic acid components connected either by α-amino or γ-carboxylic group linkages. The linkages between the units in γ-PGA are predominantly γ-amide linkages, involving both γ-carboxylic acid and α-amino and units [16]. α-PGA is chemically synthesized; however, γ-PGA is primarily produced by a wide variety of microbial species, particularly *Bacillus* sp. unlike other proteins, it is not synthesized by ribosomes [17]. Instead, it is generated as an extracellular polymer by gram-positive bacteria [18] and a few gram-negative bacteria [19]. *Bacillus subtilis* and *Bacillus licheniformis* are the most common strains utilized for fermentative production of γ-PGA. However, *B. anthracis, B. thuringensis, B. cereus, B. pumilis, B. megaterium, B. mojavensis, B. coagulans, Lysinibacillus sphaericus, Staphylococcus epidermidis,* and *Fusabacterium nucleatum* were correspondingly reported for γ-PGA production [20,21,22,23,24,25]. In addition, a halophilic (salt-tolerant) archaebacterium called *Natrailba aegyptiaca* sp. is capable of producing γ-PGA. However, the challenges associated with its production make it unsuitable for fermentative cultivation of γ-PGA. γ-PGA exhibits the ability to stimulate and enhance immune activity, and it possesses functional properties for the targeted delivery of chemotherapeutic agents [26].

Proteins and peptides typically consist of α-amino and α-carboxylic acid units, which are prone to digestion by proteases. However, the presence of γ-amide linkages (involving α-amino and γ-

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carboxylic units) in γ-PGA prevents its degradation by proteases. γ-PGA is a chiral polymer with an optically active core in each glutamate residue. Three distinct types of stereochemically unique γ-PGA have been identified as homopolymer composed of D-glutamate units or L-glutamate units or copolymers containing both types. As γ-PGA in the culture medium, it forms a viscous solution containing approximately 5000 – 10,000 units of D- and L- glutamic acids [8]. The enzymes involved in the synthesis of γ-PGA have played a significant role in the development of production systems [27, 28]. The biosynthesis pathway of γ-PGA utilizes L-glutamic acid units, which can be obtained either externally or internally, with α-ketoglutaric acid serving as a direct precursor [2]. Due to its inherent differences from α-PGA (such as resistance to chemical modification and protease degradation), γ-PGA holds greater potential for medical applications, including vaccines, drug delivery, γ-PGA nanoparticles for localized drug release in cancer chemotherapy, and tissue engineering. The microbial synthesis of γ-PGA has fascinated significant consideration in the advancement of molecular biology techniques. Additionally, both isoforms of PGA have a broad range of applications, many of which are still being explored and discovered [28].

3. Biosynthesis of γ-PGA

A biosynthetic pathway has been proposed for the production of γ-PGA. The monomeric units of L-glutamic acid that combine to form γ-PGA can be acquired by two biosynthetic pathways i.e., exogenous or endogenous pathway (Figure 1). α-Ketoglutaric acid is a substrate for glutamic acid synthesis in the TCA cycle. For the endogenous production of γ-PGA, a shift in carbon source involving acetyl-CoA and TCA cycle intermediates is necessary. Glutamine synthase facilitates the conversion of exogenous L-glutamic acid into L-glutamine, which acts as a pioneer for glutamic acid synthesis. The synthesis of γ-PGA occurs via four distinct stages such as γ-PGA racemization, γ-PGA polymerization, γ-PGA regulation, and γ-PGA degradation. [29,30,31].

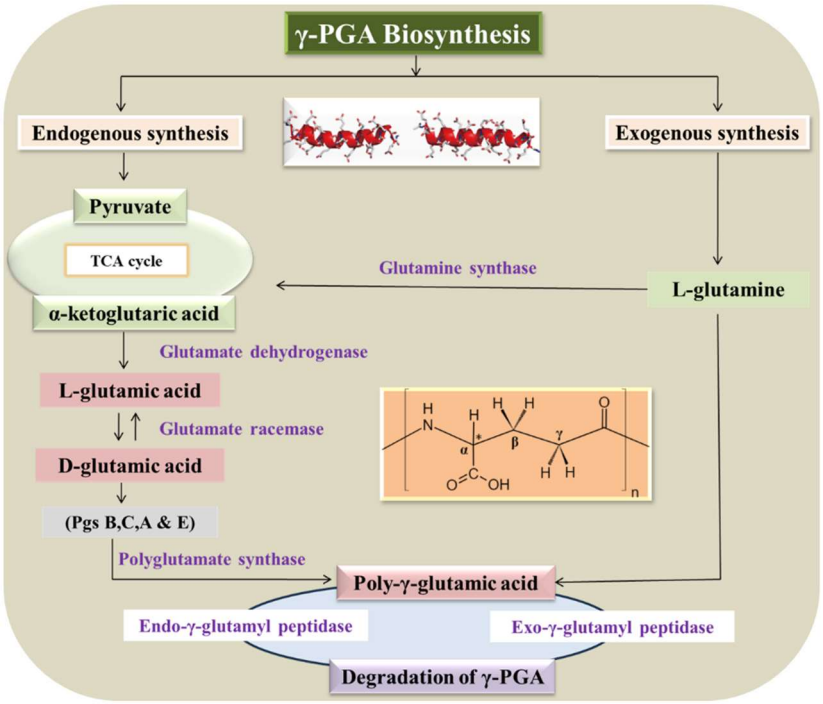

Figure 1. PGA Biosynthesis Pathway.

3.1. γ-PGA Racemization

γ-PGA is typically composed of different variants: D-glutamate, L-glutamate, or a grouping of both D- and L- enantiomers [32,33]. The process of incorporating D-glutamate into the growing L-glutamate chain is facilitated by a racemization reaction. This reaction converts L-glutamate, which

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can be obtained from external sources or produced internally, into D-glutamate. The conversion is carried out by the glutamate racemase gene (racE/glr), which plays a dynamic role in enabling the synthesis of γ-PGA. This enzymatic transformation allows the production of γ-PGA with a diverse composition of glutamate enantiomers [33].

3.2. γ-PGA Polymerization

Research studies have reported the existence of four genes (pgsB, C, A, and E) responsible for coding polyglutamate synthase (pgs), with ywsC, ywtAB, and capBCA identified as their *Bacillus* homologs [33,34]. Among these genes, PgsA plays a crucial role in removing the extended chain from the dynamic site, facilitating monomer expansion and the transportation of γ-PGA across the cell membrane. PgsB and PgsC are considered the key components of the catalytic site [28]. While PgsE is necessary for γ-PGA formation, high levels of pgsB, pgsC, and pgsA can contribute to the shaping of γ-PGA even in the non-availability of pgsE [34]. Additionally, the presence of Zn2+ is essential for γ-PGA production in B. subtilis, requiring the involvement of pgsE [35]. These studies demonstrated the intricate roles and interactions of multiple genes in the production of γ-PGA, highlighting the complex functions they perform collectively.

3.3. γ-PGA Regulation

The synthesis of γ-PGA involves the participation of two signal transduction systems: the ComP-ComA controller and the DegS-DegU, DegQ, and SwrA pathway [36]. The role of DegQ and its modification plays a crucial part in γ-PGA synthesis of γ-PGA and regulates the production of degradation enzymes [37]. It has been observed that the initiation of the pgs operon for γ-PGA synthesis occurs in the presence of SwrA and phosphorylated DegU (DegU-P), which triggers pgs expression when there are high levels of DegU-P, instead of swrA [38]. The transcriptional regulation of γ-PGA synthesis generally involves DegSU, DegQ, and ComPA, influenced by factors such as quorum sensing, phase variance signals, and osmolarity, while SwrA functions at a lesser extent [39].

3.4. γ-PGA Degradation

The degradation of γ-PGA is facilitated by two enzymes: Endo-γ-glutamyl peptidase and Exo-γ-glutamyl peptidase [4]. *B. licheniformis* and *B. subtilis* secrete Endo-γ-glutamyl peptidase, which breaks down high molar mass γ-PGA into smaller units ranging from 1000 Da to 20 kDa. This depolymerization process leads to a reduction in dispersity over time [40,41,18]. On the other hand, Exo-γ-glutamyl peptidase (Ggt), a vital enzyme in glutathione breakdown, is involved in the in vitro construction of γ-glutamic acid di- and tripeptides but is not directly involved in the *in vivo* synthesis of γ-PGA [33]. These enzymatic activities contribute to the degradation and metabolism of γ-PGA, influencing its molar mass and overall configuration.

4. Production of γ-PGA

Microbial production of γ-PGA is a promising approach for obtaining this biopolymer on a large scale. Various microorganisms, especially *Bacillus* species, have been studied and utilized for γ-PGA production. These microorganisms have the ability to synthesize γ-PGA through enzymatic pathways [10,34]. The production process involves the fermentation of selected microorganisms in a suitable growth medium. The growth medium composition is carefully designed to provide the necessary nutrients for the microbial cells to proliferate and produce γ-PGA. Typically, carbon and nitrogen sources, such as glucose, glycerol, or various amino acids, are included in the medium. In addition, other nutrients, vitamins, and minerals may be added to support cell growth and γ-PGA synthesis [10]. The fermentation process occurs in bioreactors under controlled conditions of temperature, pH, and oxygen supply. The microorganisms metabolize the nitrogen and carbon and sources in the growth medium, converting them into γ-PGA through enzymatic reactions. The synthesis of γ-PGA is regulated by specific genes and enzymes involved in its biosynthetic pathway

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[42]. During the fermentation process, the production of γ-PGA can be monitored by analyzing the culture samples at different time points. Techniques such as high-performance liquid chromatography (HPLC) or mass spectrometry (MS) can be used to quantify and characterize the γ-PGA produced. To optimize and improve γ-PGA production, various strategies can be employed. These include genetic engineering tactics to enhance the expression of genes involved in γ-PGA synthesis, optimization of fermentation conditions, and the use of advanced bioprocess technologies [10].

Four different approaches are employed for the production of γ-PGA: microbial fermentation, peptide synthesis, chemical synthesis, and biotransformation [43]. γ-PGA is a component commonly found in Japanese traditional food, specifically *natto*, which is prepared from fermented soybeans using *Bacillus* strains [44]. Research has been done to study the nutritional requirements for γ-PGA production in order to improve its fermentation productivity. *Bacillus* strains are proficient of making a huge amount of γ-PGA, up to 50 g, in the growth medium. Based on their nutritional requirements, bacterial species can be classified into two categories: one relies on L-glutamic acid as the nitrogen and carbon source for growth and γ-PGA production, while the other does not depend on L-glutamic acid. In either case, a sufficient amount of nitrogen and carbon and sources, typically in the range of 2-20 grams, is required by the bacteria for γ-PGA production. Different bacteria employ distinct γ-PGA production systems, indicating variations in the synthesis process [45]. *Bacillus subtilis* IFO3335, for instance, can synthesize a substantial amount of 0.96 g γ-PGA in a 100 mL medium containing 3 g of L-glutamic acid, 2 g of citric acid, and 0.5 g of ammonium sulfate, without any polysaccharide by-products [46]. When no or only 0.5 g of L-glutamic acid is added to the same medium with 2 g of citric acid and 0.5 g of ammonium sulfate, a small amount of PGA is produced. Interestingly, the addition of 0.01 g of L-glutamine to the medium leads to a significant increase in γ-PGA production, while the utilization of 0.1 g of yeast extract or glucose results in little to no γ-PGA production. Thus, the combination of citric acid and ammonium sulfate in the cultivation medium, supplemented with small amounts of specific components like L-glutamine, promotes efficient γ-PGA synthesis [47]. γ-PGA production has been also achieved through co-expression in *E. coli*. The γ-PGA synthase genes pgsBCA and racE from an L-glutamate-dependent γ-PGA producer *B. licheniformis* NK-03 and a non-L-glutamate-dependent γ-PGA producer *B. amyloliquefaciens*LL3 were cloned and co-expressed in *E.coli* JM 109 for evaluation of γ-PGA productivity. Results showed that pgsB and pgsC of both strains are highly similar with 93.13 and 93.96% resemblance, where the pgsA and racE presented 78.53 and 84.5% similarity, respectively [48].

5. γ-PGA from *Bacillus* spp.

Extensive research has been performed on the production and applications of γ-PGA (Table 1). PGA produced chemically results into the production of low molecular mass i.e., 10 kDa limiting its application. However, the γ-PGA (bacterial) varies from 10-100 kDa and may often reach as high as 10000 kDa [49]. Several micro-organisms are involved in the synthesis of γ-PGA. *Bacillus sp*. like *B. subtilis* and *B. licheniformis* are used to produce it commercially. γ-PGA production from *B. subtilis* (*natto*) proved that synthesis or lengthening of γ-PGA is coupled with its degradation and the resultant γ-PGA synthase complex is unstable. However, it has been found that *B. subtilis* (*chungkookjang*) produce an ultra-high molecular mass of γ-PGA in a medium containing a high concentration of ammonium sulphate. Without any by-products, the average high-molecular mass of γ-PGA obtained is 2×10^6. γ-PGA with a molecular mass exceeding 2×10^6 Da was challenging to measure accurately, and high-molecular-mass γ-PGA was estimated to be approximately 7×10^6 Da [50,51]. There are two types of microorganisms involved in the production of γ-PGA, namely L-glutamic acid-dependent and -independent bacteria [34]. L-glutamic acid-dependent bacteria include *B. subtilis* (*chungkookjang*) [52], *B. subtilis* (*natto*) ATCC 15245 [53], *B. subtilis* CGMCC 0833 [54], *B. licheniformis* NK-03 [55], and *B. licheniformis* 9945a [56]. On the other hand, non-glutamic-acid-dependent bacteria include *B. amyloliquefaciens* LL3 [22], *B. subtilis* C1 [57], and *B. subtilis* C10 [58].

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The bacterium *Bacillus subtilis* NRRL-B2612, when 200 g/L wheat gluten is used as a constituent and cultured at 33 ℃ for 2-3 days 10-14 g/L γ-PGA was produced [19]. *Bacillus licheniformis* ATCC 9945A was reported to produce 23 g/L of the product in the medium consisting 20g/L of glutamic acid,80g/L of glycerol, 12 g/L of citric acid, 7 g/L of ammonium chloride when cultivated at 37 ℃ for 4 days [59]. The strain *Bacillus subtilis* F-2-0, medium constitutes glutamic acid 70 g/L, glucose 1 g/L, veal infusion broth 20 g/L. 45.5 g of γ-PGA was produced when cultivated at 37 ℃ for 2-3 days [60]. Another bacterium employed was *Bacillus licheniformis* and the medium was LB agar slant composed of yeast extract 5 g/L, tryptone 10 g/L, NaCl 10 g/L, agar 15 g/L. Batch fermentation experiments were carried out in 250 mL Erlenmeyer flasks consisting of 50 mL of the sterile cultivation medium at 37±2 ℃ and 200 rpm for 72 h. The production medium was inoculated with 2% of 12 h inoculum and kept for fermentation. This was followed by centrifugation at 12000 rpm for 20 min at 4 ℃ to separate cells from the cultured broth. The cell-free supernatant containing γ-PGA was kept at 4 ℃ overnight after pouring it into 4 volumes of ice-cold ethanol with gentle stirring. The γ-PGA precipitate was collected by centrifugation at 12000 rpm for 20 min 4 ℃. The crude γ-PGA was resuspended in deionized water and any insoluble particles were pelleted by centrifugation at 12000 rpm at 4 ℃ for 20 min for its removal. The precipitation steps were followed thrice, and the resultant γ-PGA was centrifuged again and dried at 70 ℃ until it attained a constant weight [61]. Further purification was done by dialysis and lastly, γ-PGA slurry was freeze-dried to prepare powder and was estimated by ninhydrin with glutamic acid as a standard by TLC [62].

Table 1. Sources and properties of microbial PGA.

Sl. NO	NAME OF BACTERIA	LAB	SOURCES	PROPERTIES	REFERENCE
1	*Bacillus licheniformis* NCIM 2324	No	NCIM	Molecular weight determination, amino acid analysis, total sugar content	Bajaj et al. [64]
2	*B. licheniformis* & *B. subtilis*	No	Chunkookjang	Chemical and microbial synthesis, application of PGA in medicine as drug carrier & biological adhesives	Shih et al. (2004) [125]
3	*B. licheniformis* CCRC 12826	No	CCRC, Taiwan	Production of biodegradable & harmless PGA	Shih et al. (2001) [45]
4	*B. subtilis*	No	natto	Factors affecting production and agricultural applications	Yu et al. (2011) [126]
5	*B. subtilis* DYU1	No	Soil samples from a soy sauce manufacturing site	Flocculating activity and harmlessness to humans and environment	Wu & Ye (2007) [127]
6	*B. subtilis*	No	Soil sample of electroplating industry	Biodegradability, film-forming property, fibrogenicity, water-holding capacity	Zhang et al. (2020) [128]
7	*B. amyloliquefaciens* C06	No	Post-harvest fruit	Optimization of fermentation conditions to regulate stereochemical composition of γ-PGA & enhanced productivity of γ-PGA	Liu et al. (2011) [129]
8	*B. subtilis* ZC-5	No	CICC, China	Solid-state fermentation, low cost substrates, environmental friendly process, reduced energy requirement & waste-water production	Zhang et al. (2019) [130]
9	*B. licheniformis*	No	Applied Chemistry Research Center (Saltillo, Coahuila, Mexico)	Characterization of nanoparticles, encapsulation assays, bioactivity assays, *in vitro* release assays	Pereira et al. (2017) [131]
10	*B. subtilis* & *B. licheniformis*	No	reviewing different sources	Biopolymer Rheology & Viscosity-molecular weight correlation	Richard & Margaritis (2001) [132]
11	*B. subtilis*	No		Analysis of heavy metal distribution in soil	Yang et al. (2018) [133]
12	*B. licheniformis* ATCC 9945a	No	ATCC	Water absorption & solubility, graft content & efficiency, rheological behaviour	Xu et al. (2016) [134]
13	*B. subtilis*	No	Nattokinase	High safety, simple production process, drug delivery system, excellent water solubility, biocompatibility, biodegradability	Zhang et al. (2017) [135]

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14	*B. subtilis*	no	natto	cryoprotective effects of γ-PGA, Determination of dynamic rheological properties, Ca2+-ATPase activity, gel strength, salt-soluble protein content	Tao et al. (2020) [136]
15	*B. subtilis* ZJU-17	no	fermented bean curd	effects of carbon sources and influence of nitrogen source on gamma polyglutamic acid production	Shi et al. (2006) [137]
16	*B. licheniformis* 9945	no	ATCC	Production and purification and molecular size estimation	Kongklom et al. (2012) [138]
17	*B. methylotrophicus* , *B. subtilis* and *B. licheniformis*	no	Natto & rhizosphere of pepper, cabbage, sweet corn, fenugreek leaves, barley, tomato, and sugarcane plants	Use of methylene blue to differentiate the monomeric and the polymeric forms of glutamic acid	Chatterjee et al. (2018) [139]
18	*Natrialba aegyptiaca* & *N. asiatica*	no	beach sand (Egypt)	Analysis of the extracellular polymer	Hezayen et al. (2001) [140]
19	*Bacillus* natto 20646	no	Natto	PCR Analysis	Qi et al. (2013) [141]
20	*Bacillus sp.* SJ-10	no	Chungkookjang	physicochemical properties and biofunctionality of PGA, Molecular weight determination	Lee et al. (2018) [78]
21	*B. licheniformis* WBL-3 (mutant of 9945)	no	ATCC	Effect of glycerol on cell growth and g-PGA production	Du et al. (2005) [142]
22	*B. subtilis*	no	Natto	Culture conditions, PGA Analysis	Ogawa et al. (1997) [72]
23	*B. subtilis* C10	no	Sauce (from local supermarket, China)	Isolation and characterisation of exogenous glutamic acid-independent strain	Zhang et al. (2012) [143]
24	*Bacillus spp.* FBL-2.	no	Cheonggukjang	Optimization of medium components by central composite design (CCD)	Min et al. (2019) [144]
25	*B. amyloliquefaciens* C06	no	Mesophilic cheese starter	Molecular weight determination, UV scanning and amino acid analysis with paper chromatography	Liu et al. (2011) [129]
26	*B. licheniformis* A13	no	Isolated from a tannery effluent	optimization of PGA production	Mabrouk et al. (2012) [65]
27	*B. licheniformis* A35	no	Natto	Determination of amino acid	Cheng et al. (1989) [67]
28	*B. licheniformis* NRC20	no	Mine soil	Viscosity measurement, Molecular weight determination, Amino acid analysis	Tork et al. (2015) [26]
29	*B. subtilis*	no	Natto	Application of γ-polyglutamic acid (Na+ form) in skin care products	Ho et al. (2006) [76]
30	*B. licheniformis* and *B. subtilis*	no	Natto	biofilm formation, biosynthesis of PGA, genes involed, applications	Najar & Das (2015) [12]
31	*B. subtilis* NRRL B-2612	no	devitalized wheat gluten	Solubility in water, molecular weight determination,viscocity	Ward et al. (1963) [145]
32	*B. licheniformis* 9945a (NCIM 2324), *B. subtilis* ZJU-7	no	Reviewing many sources	Molecular mass determination, Amino acid analysis, biodegradability, edibility and mmunogenicity	Ogunleye, et al. (2015) [7]
33	*B. subtilis*	no	Natto	Rheology of biopolymers	Kreyenschulte et al. (2014) [146]
34	*B. licheniformis*	no	ATCC	production optimization	Giannos et al. (1990) [147]
35	*B. licheniformis* NBRC12107	no	Fermented locust bean products	Characterization, Tensile strength and porosity	Yu & Aubin (2020) [116]
36	*B. licheniformis* A14	no	Marine sands	Microbially derived biopolymers are renewable in nature	Ali et al. (2020) [148]
37	*B. subtilis* (CGMCC17326)	no	Natto	Film forming property, Reduced degree of browning in shiitake mushrooms	Tao et al. (2021) [117]
38	*B. subtilis* W-17 CICC 10260	no	CICC	Use of γ-polyglutamic acid waste biomass	Zhang et al. (2021) [75]

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5.1. B. licheniformis

B. licheniformis, particularly the strain *B. licheniformis* 9945a (NCIM 2324), is a well-known and extensively utilized bacterium for the production of γ-PGA. To achieve maximum yield, the production was optimized through solid-state fermentation. The impact of various factors such as substrates, carbon and nitrogen sources, moisture content, pH, amino acids, and TCA cycle intermediates on γ-PGA production was investigated using the "one factor at a time" approach. By employing optimized media, a yield of 98.64 mg (g dry solids)-1 γ-PGA was obtained through solid fermentation [63]. Bajaj et al. (2009) [64] also conducted research on optimizing the production of γ-PGA using *B. licheniformis* NCIM 2324, employing the "one factor at a time" method. They utilized response surface methodology to determine the optimal nutrient concentrations, which were then experimentally validated. The optimized medium, consisting of glycerol (62.4 g l^{-1}), citric acid (15.2 g l^{-1}), ammonium sulfate (8.0 g l^{-1}), and L-glutamic acid (20 g l^{-1}), resulted in a yield of 26.12 g l^{-1} of γ-PGA. In comparison, the yield obtained with the basal medium was 5.27 g l^{-1}. The γ-PGA produced had a molecular mass of approximately 2.1 x 10^5 Da.

B. licheniformis Al3, a producer independent of exogenous glutamate, achieved a γ-PGA yield of 28.2 g l^{-1} in an optimized medium. The optimized medium consisted of glucose (50 g l^{-1}), NH_4Cl (3 g l^{-1}), yeast extract (2 g l^{-1}), $MgSO_4.7H_2O$ (0.8 g l-1), NaCl (0.8 g l^{-1}), $CaCl_2.2H_2O$ (0.00084 g l^{-1}), K_2HPO_4 (6.4 g l^{-1}), $FeSO_4.4H_2O$ (0.006 g l^{-1}), 0.1 mL of trace element solution, and a culture volume of 23 mL. The Plackett-Burmann design was used up to 72 hours after inoculation to assess the effects of different factors on γ-PGA production [65]. The results indicated that yeast extract and medium volume were the two factors that significantly influenced γ-PGA production. For the bacteria *B. licheniformis* WBL-3, monthly subculture was performed on agar slants containing 2.0% agar. The slants consisted of 10 g citric acid, 10 g L-glutamic acid, 6 g NH_4Cl, 1 g K_2HPO_4, 0.05 g $MgSO_4.7H_2O$, 0.02 g $FeCl_3.6H_2O$, 0.2 g $CaCl_2$, and 0.05 g $MnSO_4.H_2O$ at pH 6.5 [66]. The same medium without agar was used for seed medium (50 mL) preparation and incubated at 37 °C for 24 hours. The flasks were placed in a rotary shaker at 200 rpm [67]. In the case of *B. licheniformis* A35, under denitrifying conditions, it produced 8 mg/mL of γ-PGA. The pre-cultured medium used in a liter of culture contained 10 g meat extract, 10 g peptone, 5 g sodium chloride, and 10 g glucose [68].

5.2. Bacillus subtilis

Bovarnick (1942) was the first to demonstrate that *B. subtilis* fermentation released the γ-PGA into the medium [69]. More emphasis has been placed on investigating *B. subtilis* strains for γ-PGA production compared to *B. licheniformis*. Scoffone et al. (2013) evaluated γ-PGA production by knocking out the pgdS and ggt genes, which are responsible for two important γ-PGA degradation enzymes, in the laboratory strain *B. subtilis* 168. The impact of single mutations (deletion of pgdS or ggt) and a double mutation (deletion of both pgdS and ggt) on γ-PGA production was assessed. While single mutations did not result in significant improvement in γ-PGA yield, the double mutant strain produced more than twice the amount (>40 g L-1) compared to the wild-type strain [70]. Shih et al. [71] presented findings on the high-yield, cost-effective, and large-scale production of γ-PGA from *B. subtilis* ZJU-7 (*B. subtilis* CGMCCl250). Their study demonstrated that using 40 g/L yeast extract, 30 g/L L-glutamate, and 20 g/L initial glucose, along with maintaining a glucose concentration in the range of 3-10 g/L through a fed-batch approach, significantly improved the yield of γ-PGA. Compared to batch fermentation, this approach resulted in a 1.4 to 3.2-fold increase in γ-PGA yield. The study recorded an overall γ-PGA concentration of 101.1 g/L and a productivity of 2.19 g/L. The strain *Bacillus subtilis* ZJU-7 is obtained from fermented bean curd. The culture medium used for slant preparation consists of glucose (10 g/L), tryptone (10 g/L), L-glutamic acid (10 g/L), and NaCl (5 g/L). The seed medium is composed of the same components as the slant medium, with the addition of 0.1 g/L $MgSO_4$ and 0.1 g/L $CaCl_2$. The basal medium is similar to the slant medium but contains a higher concentration of L-glutamic acid (20 g/L). To optimize the effects of these components on γ-PGA production, response surface methodology (RSM) is employed by changing the composition of the media. The pH of the media is adjusted to 7.0 using HCl or NaOH, and all media samples are

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sterilized by autoclaving at 121 °C for 20 minutes. For cultivation, the inoculated samples are transferred into 500 mL flasks and incubated at 37 °C with shaking at 200 rpm. After a fermentation period of 24 h, the culture is separated, and the γ-PGA is purified through methanol precipitation [46].

Batch cultures of *Bacillus subtilis* (natto) were conducted in a 5L laboratory fermenter system, while a 600-liter pilot plant fermenter system was employed for the development process. Agar plates for culturing were prepared using a 1.5% agar solution. The medium used consisted of 8% glucose, 10% sodium L-glutamate, 0.05% K_2HPO_4, 1.5% peptone, 0.02% $CaCl_2$, 50% biotin, 1.0% yeast extract, and 3.0% NaCl. Additionally, 0.05% silicone oil (Dow Corning Silicone) was included as an anti-foaming agent, and the temperature was maintained at 37 °C. The extracellular production of γ-PGA was observed with a molecular weight ranging from 100,000 to 2,500,000 Da. Alcohol was used to precipitate γ-PGA from the cell-free culture broth solution, followed by centrifugation and purification of the precipitates [67].

For *Bacillus subtilis* strain MR-141, derived from strain MR-1, spore formation was achieved by growing the strain on nutrient plates containing 1.5% agar at 40 °C for 7 days. Subsequently, the strain was transferred to MSG medium, which consisted of 6% maltose, 7% soy sauce, 3% sodium L-glutamate, 0.25% K_2HPO_4, 0.05% $MgSO_4.7H_2O$, and 3% NaCl. Alternatively, MSG medium with 6% glucose instead of maltose could be used, along with 0.1% silicone oil as an anti-foaming agent. The glutamic acid present in the broth was quantified using an amino acid analyzer [72].

5.3. Bacillus anthracis

B. anthracis, a known producer of enantiomer form of γ-PGA does not release γ-PGA into the medium as compared to other *Bacillus* species instead, it is peptidoglycan bound [73]. It is important to note that industrial production of γ-PGA by *B. anthracis* is not viable owing to its toxicity. γ-PGA aids in making the *B. anthracis* capsule non-immunogenic, which has been linked to the lethal toxin [74]. Hence, its cap gene responsible for the anchoring of γ-PGA onto its surface needs to be targeted to render *B. anthracis* immunogenic [27].

5.4. Bacillus thuringiensis

B. thuringiensis sv. monterrey strain BGSC 4AJ1 and *B. anthracis* (Ames) have four common alleles, gmk-1, pta-1, pur-1 and tpi-1, where other three alleles, glpF-57, ivld-52, and pycA-52, differ by 2, 2 and 3 nt respectively. Genes encoding the synthesis of γ-D-PGA showed similarity with those of *B. anthracis* and are present on a plasmid (pAJ1-1). The Discovery of a γ-PGA capsule in this *B. thuringiensis* strain is an indication of the ability of the bacteria to be pathogenic under certain conditions [7].

6. Structural and Physico-Chemical Properties of γ-PGA

γ-Polyglutamic acid (γ-PGA) exhibits diverse properties, including various conformational states, enantiomeric forms, and molecular weights. Its biodegradable, non-toxic, and non-immunogenic characteristics make it valuable in the food and pharmaceutical industries. For instance, Zhang et al. demonstrated that utilizing waste biomass hydrolysate and substituting tryptone in the γ-PGA production medium introduces a more sustainable production method. Applications of γ-PGA encompass protein crystallization, tissue adhesives for soft tissues, and non-viral vectors for gene delivery. Each unique property of γ-PGA aligns with specific applications, highlighting the need for further research to identify bacterial strains capable of producing high yields of γ-PGA with tailored properties [75]. Optimization of γ-PGA production concerning its production cost, molecular mass, and conformational or enantiomeric properties is crucial in bringing its application into practice. Knowledge of the enzymes and genes involved in γ-PGA production will not only aid in increasing productivity by reducing the production cost but also help in understanding the mechanism by which γ-PGA is beneficial in numerous applications [7].

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Physicochemical and functional characterization of γ-PGA molecules can be achieved using several modern techniques and instruments (Figure 2).

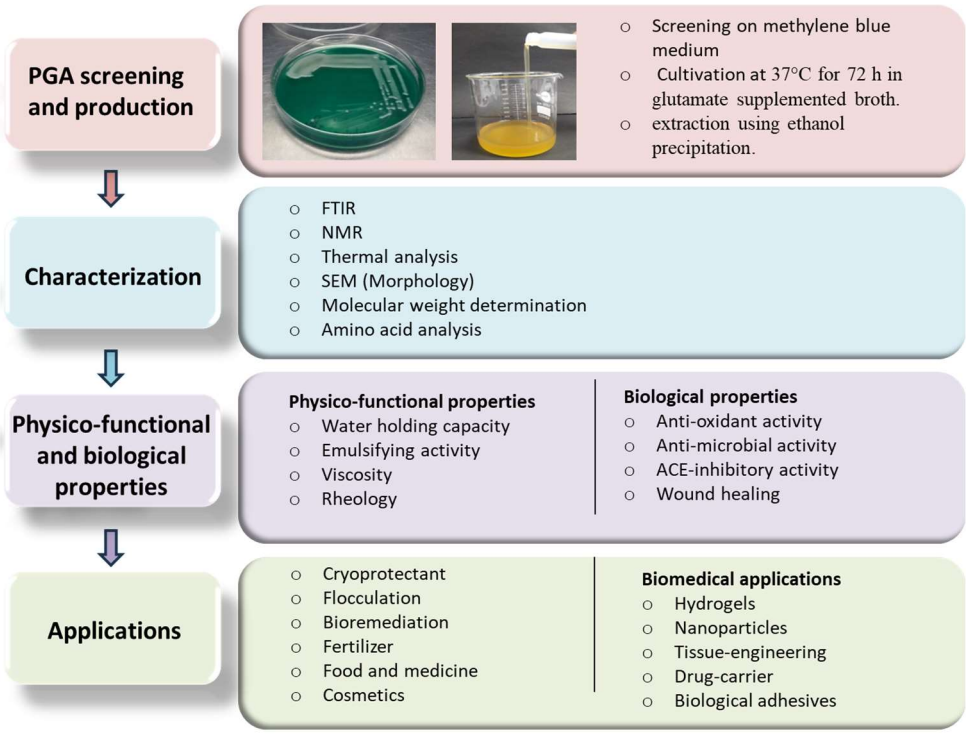

Figure 2. Overview of production, characterization, and properties of PGA molecule.

6.1. Fourier Transform Infrared (FT-IR) Spectroscopy

FT-IR spectroscopy is the measurement of the procedure applied to record IR spectra. FTIR interferograms expose the functional groups in the purified γ-PGA illustrates that can be resolved by recognizing the specific peak values in the graphical values of FTIR. FTIR spectroscopy was carried out for the detection of γ-PGA functional groups of 4000-400 cm^{-1} frequency. The sample pellet for the spectrum analysis was prepared using purified γ-PGA and dried potassium bromide (KBr) by compression and the functional group vibrations for C=O (carboxyl), -NH (amine), -OH (hydroxyl), and C-N (carbonyl) stretches were produced as various peaks and bendings [67,77].

6.2. Nuclear Magnetic Resonance (NMR) Analysis

Usually for spectroscopy ^{1}H- and ^{13}C-NMR are used to analyze the degree of esterification and homogeneity of γ-PGA. Following NMR spectra chemical shifts are standardized using the known standards. The samples were analyzed at 100 MHz, with a 30°pulse and a 4s cycle time. As well, solid state samples were analyzed at 50 MHz, and the spectra were kept under careful observation of cross-polarisation, magic angle sample spinning, and power decoupling circumstances with a 90° pulse and 4 s cycle time. To know the chemical composition comprehensively, analyses are done. ^{1}H NMR for γ-PGA in D_2O shows chemical shifts at 3.66 ppm for the α-CH proton, 2.08 ppm for the β-CH_2 proton, and 2.33 ppm for γ-CH_2 proton. The ^{13}C NMR spectra showed chemical shifts at 55.53 ppm for α-CH_2 group, 27.77 ppm for β-CH_2 group, 34.34 ppm for γ-CH_2 group, 175.21 ppm for the CO group, and 181.96 ppm for the COO^- group [67].

6.3. Thermal Analysis

In thermogravimetric analysis (TGA) of γ-PGA, thermal degradation temperature and thermal stability of biomolecules can be determined by using a thermal gravimetric analyzer. In dynamic experiments, usually, a powdered form of purified γ-PGA was used. The TGA was implemented at

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50-700 °C temperature and a heating rate of 10 °C min^{-1} at a nitrogen atmospheric rate of 25 mL min^{-1} [78]. TGA is carried to determine the thermal decomposition temperature (T_d). It expresses the thermal stability of γ-PGA and gives the thermal gravimetric curve and the percentage loss in weight was analyzed while raising the temperature from 50 °C to 700 °C. γ-PGA produced in SJ-10 was found to exhibit high resistance to high temperatures of thermal degradation. The decomposition temperature (T_d) and the temperature representing half of the initial weight ($T_{d50\%}$) were recorded at 320 °C and 455 °C, respectively. The T_d was the same as the sodium form of γ-PGA when compared to the normal one. The T_d of γ-PGA was different according to the combined cations. The T_d of γ-PGA in addition to Na^+, K^+ Ca^{2+}, and Mg^{2+} cations was more than those with H^+ and NH^+_4 [67,79].

6.4. Molecular Weight Determination

The γ-PGA synthesized by *Bacillus spp.* typically exhibits a high molecular weight ranging from 10^5 to 10^6 Da [34]. Molecular weight estimation of γ-PGA has predominantly been conducted using gel permeation chromatography (GPC), employing various mobile phases and calibration against different standards [56, 80]. Bajestani et al. reported utilizing DEAE cellulose-52 column resin to run ion-exchange chromatography. The column charged with γ-PGA was eluted with a gradient concentration of NaCl (0.1, 0.5, 0.75, and 1 M), and fractions were collected. Then, γ-PGA content was quantified using (GPC) as it detects UV-light roughly at 216 nm depicting the chromatogram, followed by lyophilization. The molecular weight of γ-PGA as a heavy weight fraction was estimated to be $7.7x10^6$ g/mol and $1.7x10^4$ g/mol as the average molecular weight number. Birrer et al. followed another approach of chromatography, high-performance liquid chromatography (HPLC), and GPC to determine the number (M_n) and weight average molecular weights (M_w) along with polydispersity of γ-PGA. A calibration curve was constructed using narrow polydispersity pullulan standards and molecular weight M (M_w and M_n) of γ-PGA was calculated to be 22000 g/mol and 266000 g/mol, respectively [56].

6.5. Amino Acids and Enantiomeric Composition Analysis

HPLC is usually used to serve this purpose. The γ-PGA hydrolysate chromatogram was detected at a position corresponding to D-glutamic acid having equal retention flow, no peak corresponding to L-glutamic acid was detected. The result indicates that separated biocompatible γ-PGA contains D-glutamic acid residues the most [7].

7. Physico-Functional Properties

7.1. Water Holding Capacity

A substance capacity to retain moisture is water holding capacity. γ-PGA is reported to have an excellent water-holding ability [82]. Apart from food applications, γ-PGA is used in cosmetic industries because of its significant water-holding capacity, and hydrogels are utilized for biomedical applications [83]. Additionally, the introduction of γ-PGA to sandy soils has been reported a significant lowering of the water insinuation competence, whereas the water holding capacity of the soil improved the saturated water content and effective water utilization. The results in soil suggest that γ-PGA can not only add to the water-holding capacity of soil but bring about an obvious change in the moisture distribution patterns thus, paving a way through agro-ecosystems as well [84]. The good water-binding capacity of γ-PGA results in an increase in moisture holding while reducing the oil uptake significantly [85]. However, the water holding capacity of γ-PGA was found to be dropped (56.9%) when the reaction time was increased up to 9 days [86].

7.2. Emulsifying Property

The study on the effect of γ-PGA addition on the emulsifying property of sponge cake revealed that the addition of γ-PGA significantly improved the emulsion activity and stability, and foam

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stability of sponge cake paste confirming the contribution of γ-PGA in delayed staling by [82]. A multiple layered oil-in-water emulsions of γ-PGA with soyabean oil showed that the emulsion ability was sturdily reliant on γ-PGA addition. A sheer increase in mean particle diameter was detected with a surge in γ-PGA concentration (0 to 0.01 w/v %) and an appreciable cream formation occurred at intermediate γ-PGA concentrations (0.023 w/v %) [89].

7.3. Rheology and Viscosity

The rheology studied by Zhang et al. focused on the rheological properties of γ-PGA produced by *Bacillus subtilis* 1006-3. The γ-PGA solution exhibited non-Newtonian fluid behavior, specifically pseudoplasticity, with shear-thinning properties. This behavior is described using the Ostwald-de Waele power law model. The apparent viscosity of the γ-PGA solution increased as its concentration was raised from 1-10%. Deviations from a neutral pH, as well as the addition of NaCl or $MgCl_2$, reduced the apparent viscosity of the γ-PGA solution. The solution was more sensitive to the addition of Mg^{2+} ions compared to Na^{+} ions. At concentrations of 4, 6, and 8%, the γ-PGA solution showed a predominantly viscous response (G”>G’) within the angular frequency range of 0.1-100 rad/s. The study indicated the potential application of γ-PGA as a thickening agent due to its rheological properties [88].

8. Biological Properties

8.1. Antioxidant Activity

The ABTS radical scavenging and phosphomolybdenum assay was accomplished to measure the total antioxidant activity of γ-PGA. The γ-PGA from *Bacillus sp.* SJ-10 with a molecular mass of 400 kDa unveiled a maximal scavenging activity at 1 mg/mL (20 μg ascorbic acid-equivalent). Additionally, it exhibits action on par with commercially available natural antioxidants [86]. The anti-oxidant activity of γ-PGA paves its way into various fields of food, cosmetic and biomedical industries.

8.2. Anti-Microbial Activity

The γ-PGA was reported to have an inhibition effect towards both Gram-positive and Gram-negative bacteria. *S. aureus* was inhibited strongly by the γ-PGA. γ-PGA exhibited an anti-bacterial activity against these bacteria at 1% concentration but showed no activity at 0.1%. Apart from that, the γ-PGA showed no activity against pathogenic yeasts, *C. albicans* [86]. Due to its excellent anti-microbial activity, γ-PGA is used as hydrogels of a new wound dressing type for wound healing also, superior effects in healing were observed when compared to sutures, component skin adhesives, and fibrin glue with reduced inflammatory response [90].

8.3. Angiotensin-Converting Enzyme (ACE) Inhibitory Activity

γ-PGA is reported to have significant ACE-Inhibitory activity, ACE inhibition regulates blood pressure by getting involved in the renin-angiotensin system therefore, is an important pathway in treating hypertension in humans. ACE-inhibitory activity in γ-PGA possibly due to oligosaccharides and protease inhibitors connected with hydroxyl groups to form hydrogen bonds with ACE, besides peptides that may be present in γ-PGA [91]. γ-PGA showed an increase in ACE inhibition in a concentration dependent manner. The highest ACE- inhibition activity was 100% observed from 1-1.8 mg/mL γ-PGA which endured persistent thenceforth [91]. The Inhibitory concentration (IC_{50}) value of γ-PGA was found to be 0.108 mg/mL which is lower than the standard ACE inhibitory drug named captopril (IC_{50}) 0.247 mg/mL [86].

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8.4. Wound Healing

γ-PGA with the other electrolytic materials like chitosan formed a polyelectrolyte composite and proved to be a potential wound dressing material by regulating the water uptake and thus minimizing the risk of dehydration on the wound. It also improved the structural stability of chitosan [92]. Also, γ-PGA-PEG (Polyethylene glycol) injectable complex was shown to be a promising hemostatic material for liver and spleen injuries (visceral hemorrhage) when compared to clinical fibrin glue [93].

9. Applications of γ-PGA

Poly-γ-glutamic acid (γ-PGA) is a biodegradable, water-soluble, and non-toxic biopolymer produced by various Bacillus species. Its unique properties make it suitable for diverse applications in food, pharmaceuticals, agriculture, and water treatment (Figure 3). Also, various commercial PGA have been studied for their functional properties and further applied in diverse applications (Table 2).

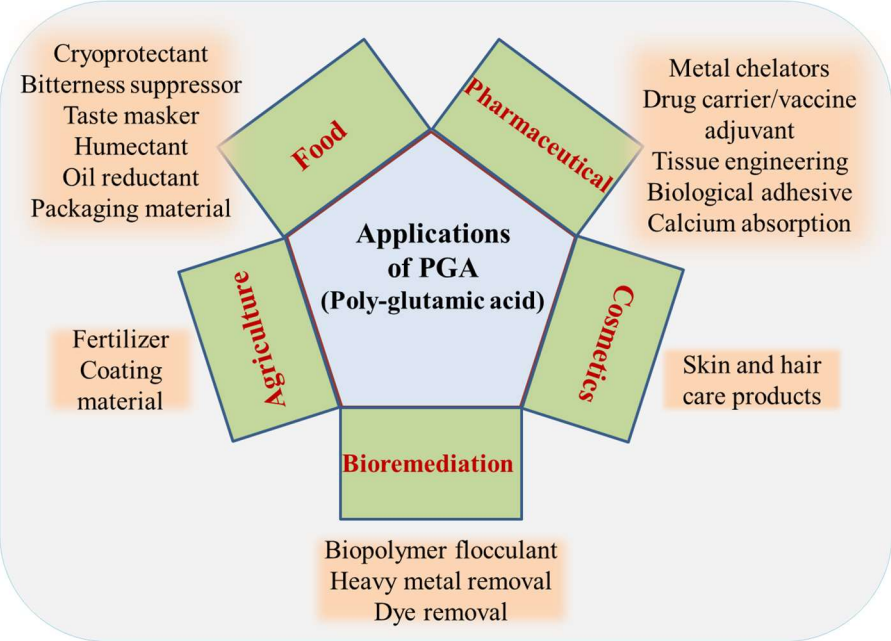

Figure 3. Applications of bacterial PGA in various fields.

Table 2. List of commercial PGAs and their applications.

SL NO	PGA	SOURCE	PROPERTIES	REFERENCE
1	Commercial PGA	Natto Biosciences (Montreal, Canada)	hydrophilicity, biodegradability, biocompatibility, immunogenicity and ionic nature	Manocha & Margaritis (2010) [149]
2	Commercial PGA	Sigma Aldrich	Detection of MCF-7 human breast cancer cells & MUC1 biomarker	Yazdanparast et al. (2018) [150]
3	Commercial PGA	VEDAN Co. (Taichung, Taiwan)	Polyelectrolyte complex formation	Tsao et al. (2011) [92]
4	Commercial PGA	IRIS Biotech gmbh (CAS No 26247-79-0)	Protective agent of protein aggregation, drug delivery, low physical stability	Oliveri et al. (2017) [151]
5	Commercial PGA	VEDAN Co. (Taichung, Taiwan)	Water-soluble properties, anti-cancer & antioxidant activties, increase biocompatible & biodegradable abilities, encapsulation efficiency	Ko et al. (2015) [152]

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6	Commercial PGA	Bioshinking Company (Nanjing, China	Biodegradability, physico-chemical characterization & evaluation of PGA bioflocculant in coagulation flocculation & sedimentation processes	Li et al. (2020) [153]
7	Commercial PGA	Sigma Aldrich	Antibacterial activity, low solubility in organic solvents, high positive potential, low sentivity	Santos et al. (2020) [154]
8	Commercial PGA	VEDAN Co. (Taichung, Taiwan)	Determination of Swelling Degree	Garcia et al. (2013) [155]
9	Commercial PGA	New England BioLabs, Hitchin, Hertfordshire, United Kingdom	Biodegradable polymer, increased rigidity, porosity & availailibity, rate of degradation	Rethore et al. (2009) [156]

9.1. Flocculation

γ-PGA can be used as a bio-flocculent in wastewater treatment, downstream processing in food industries, and pharmaceutical and medicine industries. γ-PGA is used for the flocculation of solid waste and metals in wastewater treatments [94]. The flocculation efficiency has a direct relation with the molecular mass. γ-PGA from *B. subtilis P-104* was shown to have good flocculating activity [95]. It can be improved by the addition of cations by stimulating the flocculation activity by nullifying and alleviating the negative charge on the functional group of bio-flocculent by establishing bridges amid elements. Cations, temperature, and pH are the major factors that affect the flocculation efficiency of γ-PGA. A new organic approach for solving consequential environmental issues generated by the use of massive quantities of liquid fertilizer in agriculture: limiting surplus ammonia in soil and thus nitrogen translation into γ-PGA has been reported. For cations like Fe^{2+}, Fe^{3+}, Mg^{2+}, $Ca^{2+,}$ and Mn^{2+}, that occur naturally, γ-PGA serves as a waste nitrogen transit base as well as an environmentally safe fertilizer/manure [96]. γ-PGA (9.9×10^5 Da) could be used for the elimination of basic dyes from aqueous solution. The progression is a result of the electrostatic interface of γ-PGA and dyes which initiates adsorption at pH >5 and the exclusion of dyes from γ-PGA takes place at extremely acidic (pH 1), facilitating the reuse of γ-PGA [97]. In addition to its benefit as a flocculating agent, PGA when used as an inorganic salt may result into the production of raw sludge that has to be managed later [98].

γ-PGA is a flocculant as it can play an important role in effluent treatment, and downstream processing in foods, pharmaceuticals, and drug industries therefore can replace synthetic flocculants. γ-PGA can be used as a bio-flocculant in food and fermentation industries to harvest microalgae. Reaping microalgae with PGA is lucrative, and during other harvesting techniques like centrifugation, loss of lipid is prevented due to algal cell breakage [99]. γ-PGA from *B. licheniformis* CCRC 12826 revealed efficient flocculation of numerous organic and inorganic compounds [45].

9.2. Bioremediation

Pollutants in the environment like heavy metals, radionuclides, and synthetic substances, endanger public health and upsurge universal lack of provisions because of contamination which leads to polluted water, diminished agricultural output, and adverse effects like acid rain. The remediation of polluted soils, residues, and streams includes the interaction of these contaminations with γ-PGA to implement new remediation strategies [100].

Removal of heavy metals: γ-PGA covalently combined into microfiltration layers through membrane pore surface attachment has a super-high heavy metal sorption capability. γ-PGA muddles and effectively eliminates >99.8% of lead ions from water using a low-pressure ultra-filtration system [101].

Dye removal: γ-PGA (9.9×10^5 Da) is an efficient method for removing simple dyes from hydrated solutions. At pH 1, it was found that 98% of the adsorbed dye on γ-PGA might be retrieved, allowing γ-PGA to be reused [97].

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9.3. Fertilizer

Plant growth and development are enhanced by adding fertilizers to the soil. To avoid environmental pollution, γ-PGA can be used as a bio-control agent and/or a synergist to chemical fertilizers in agriculture. It assists in enhancing growth by improving nutrient consumption even in exhausted nutrient situations. The enzymes from soil such as urease, sucrose, and catalase show an augmented activity after the supplementation of γ-PGA, nitrogen-immobilized microbes raise the total nitrogen accretion in soil [102]. γ-PGA was found to promote the growth of Chinese cabbage and increase the total nitrogen, soluble protein, and soluble amino acids content in leaves. The addition of γ-PGA brings upon a surge in the activity of enzymes involved in the breakdown and acclimatization of nitrogen. It facilitates the Ca influx in the cytoplasm which acts as a positive signal for nitrogen metabolism thus promoting the growth of plants [102]. Wang et al. (2024) reported the use of fermented grain broth BSG which is a good source of live *B. subtilis* and other metabolites beneficial to soil and plant, thus tends to be used as a modern functional bioorganic fertilizer [103]. *B. subtilis* B6-1 produces Lipopeptides and γ-PGA using soybean and sweet potato scums sufficiently repressed cucumber wilts, amplified the growth of cucumber seedlings and also increases nutrient consumption [104].

9.4. Cryoprotectant

γ-PGA possesses a high anionic amino acid composition and thus exhibits an antifreeze activity. Polymers having acidic amino acids possess high antifreeze activity in comparison to other polymers. γ-PGA with lower molecular masses <20kDa, demonstrated significant antifreeze activities than very effective antifreeze agents like glucose, without interfering with the taste of foods. The antifreeze activity is reduced in the sequence *Na salt = K salt > Ca salt > acidic form* [105]. During freeze drying, γ-PGA from *B. subtilis* has the potential to shield probiotic bacteria *Lactobacillus paracasei* remarkably better than sucrose. The probiotic strains of *Bifidobacteria* (*Bifidobacteria longum* and *Bifidobacteria breve*) have been in use for proper operation of the gastrointestinal tract. γ-PGA helps protect these cells in fruit juices and prevents their survival from harsh environments of the digestive tract [106].

9.5. In Food and Medicine

γ-PGA is utilized as a food constituent due to its functional and physico-chemical features. Consumption of γ-PGA improves intestinal calcium absorption in post-menopausal women by inhibiting the formation of an insoluble calcium complex with phosphate and can potentially be used for the treatment of bone disorders [107]. Supplementation of γ-PGA acts as a preventing agent for osteoporosis of bones by greatly improving in vitro and in vivo calcium solubility in rats and postmenopausal women respectively, also the calcium content of their bones [107, 7]. γ-PGA conjugates to produce increased absorption of vanadyl sulphate which is a mimetic insulin inorganic salt. γ-PGA has an anti-diabetic effect because it reduces the rate of intestinal absorption of glucose, as γ-PGA vanadyl complex having higher insulin – mimetic activity than free vanadyl sulfate. K-γ-PGA administration prevents a surge in blood pressure by tumbling sodium absorption and thus controls hypertension [108]. γ-PGA was found to improve the gut microbiota by increasing the abundance of *Lactobacillales* in the gut [109].

γ-PGA has a significant antifreeze activity that's why it acts as a cryoprotectant for frozen foods. As a cryoprotectant, during freeze-drying, the impact of γ-PGA probiotic microbes was found to be more effective than sucrose, sorbitol, and trehalose [110], and *Acetobacter xylinum* produces nata, bacterial cellulose [111]. Also, it is used as a thickening agent in foods/beverages improves the texture of foods, and also prevents aging. γ-PGA is demonstrated to have a positive effect in dropping oil uptake and moisture loss during deep fat frying of foods. γ-PGA is known to have water retention capacity therefore, it helps in controlling water loss and produces a dense matrix with improved integrity. Thus, γ-PGA can be utilized as a functional oil-reducing agent in deep-fat fried foods. During deep-fat frying, the impact of γ-PGA on the absorption of oil and loss of moisture content in

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doughnuts was found to be more effective and has preferred appearance and taste over ordinary doughnuts. Subsequently, in deep-fat fried foods, γ-PGA can be utilized as a functional oil-reductant [85].

9.6. Cosmetics

γ-PGA plays a significant role in cosmetics because γ-PGA improves the solubility of vitamin C when forms the PGA– vitamin C complex. Vitamin C is crucial for collagen creation which assists in skin repair. Due to its anti-oxidant activity, it acts as anti-aging. Therefore, it is a dynamic component in cosmetic compositions owning to its hygroscopic properties and skin compatibility. γ-PGA is a good hydrophilic humectant and has the potential to improve the production of urocanic acid, pyrrolidone carboxylic acid, and lactic acid compared to hyaluronic acid and soluble collagen as natural moisturizing agents. γ-PGA aids in enhancing the qualities of skincare and hair care products, such as exfoliating, nourishing, and taking away wrinkles [112]. The cosmetic constituent with γ-PGA-vitamin complex results in better firmness, enhanced absorption, and constant release of vitamins from the composite [11].

9.7. Biomedical Applications

γ-PGA gained its space in biomedical applications due to its glutamic acid composition which are natural excerpts of the human body [113].

9.7.1. Hydrogels

Hydrogel is a bioabsorbable product known to have the ability to swell in water and retain it inside its structure. It has paved the way for immense applications in the field of drug delivery and tissue engineering. Hydrogel preparation has various approaches, including γ-irradiation, chemical, or physical cross-linking. Microbial γ-PGA and L-lysine were cross-linked to prepare biodegradable hydrogels by amide bond in the presence of DMT-MM in water [114]. Using no chemical treatment γ-PGA reacted with polyvinyl alcohol (PVA) in aqueous solution to form hydrogel. The elongation and water retention ability of the hydrogels is increased with an increase in γ-PGA concentration. Protein adsorption and platelet adhesion on hydrogel have an inverse relation with γ-PGA concentration and thus help in improving the blood compatibility of the hydrogel. Due to its water resistance, mechanical properties, and blood compatibility, PGA–PVA hydrogel functions as a good biomaterial for medical devices that are used to carry blood [115]. The combination formed with bacterial cellulose and γ-PGA was found to have promising applications as bio-degradable structural high-performance materials, construction materials, and tissue engineering scaffolds (tendon, ligament, and skin) due to their biodegradability and good tensile toughness [116]. γ-PGA hydrogel showed a promising result as an edible coating material in shiitake mushrooms preserving its nutrient quality and extending the shelf-life [117].

9.7.2. Nanoparticles

Gene and Drug delivery could be made possible by nanoparticles. Due to the smaller size of the nanoparticle, it can easily escape from the reticuloendothelial system resulting in increased circulation time in blood. γ-PGA being hydrophilic and water soluble is used as a carrier for anti-cancer drugs. The γ-PGA and chitosan nanoparticles have been widely used for the oral conveyance of hydrophobic drugs and proteins. PGA-chitosan nanoparticles act as an efficient system for the delivery of insulin to diabetic patients for treatment [115,118,119].

9.7.3. Tissue Engineering

Tissue engineering is the process by which biological substitutes are developed to reinstate and sustain the functions of tissue. Due to its hydrophilic and cytocompatible nature γ-PGA/chitosan composite biomaterial exhibits latent application in tissue engineering than traditional chitosan

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matrices [120]. A PEC (poly electrolyte complex) of chitosan and γ-PGA shows a potential application in wound dressing. The complex holds the required moisture and has good mechanical properties, which allows easy removal of the dressing from the surface of the wound without destroying the renewed tissues [92].

9.7.4. Drug Carrier/Deliverer

As a drug delivery agent, the factor determining the drug delivery properties involves the molecular mass of γ-PGA, which helps to regulate the rate of drug discharge. γ-PGA with covalently attached cisplatin reduces cisplatin toxicity, improves tumor size retention in naked mice with xenografted human breast tumors, and extends the survival of bare mice with Bcap-37 tumor cells [121.

9.7.5. Metal Chelators

Heavy metals and radionuclides may be removed using metal chelators. PGA-coated super paramagnetic iron oxide NPs had a high efficiency in removing heavy metal from activated gastrointestinal fluid and a metal solution. [77]. For instance, γ-PGA molecular mass or sub-atomic mass of ~3-6 x 10^4 Da was utilized to deliver Paclitaxel poliglumex (a macromolecular form of paclitaxel and γ-L-PGA) exhibits advantages over ordinary paclitaxel. The active agent paclitaxel was eventually released from paclitaxel poliglumex as it accumulated in tumor tissue [123].

9.7.6. Biological Adhesive

A hydrogel formed by a mixture of γ-PGA aqueous solution and gelatin in the presence of water-soluble carbodiimide helps in lung adhesion and air-leak sealing than regular fibrin glue [124].

10. Conclusions

γ-PGA is a biopolymer that is edible, non-immunogenic, and therefore can be used without any worry in numerous different applications that are expanding rapidly. γ-PGA is an important polymer due to its various applications in the fields of medicine, agriculture, wastewater treatment, and food industries. In particular, γ-PGA is an enormously promising constituent in food. As a biodegradable substance, γ-PGA possesses a few preponderant features containing good water solubility, biocompatibility, degradability, and non-toxicity. Based on this, γ-PGA can be used in pharmaceuticals, such as drug carriers/deliverers, vaccine adjuvants, and coating material for microencapsulation, etc. PGA as humectants regulate water activity in foods, improving stability and viscosity, maintaining texture, and reducing microbial activity. Food additives help to reduce water activity while keeping foods moist and safe for a longer shelf life of processed foods. Also, many researchers have conducted extensive research on the cost-effective manufacture of γ-PGA.

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Reference

 doi:10.20944/preprints202502.2267.v1

1. Russo, T., Fucile, P., Giacometti, R., & Sannino, F. **2021**. Sustainable removal of contaminants by biopolymers: a novel approach for wastewater treatment. Current state and future perspectives. *Processes*, *9*(4), 719.
2. Jonnalagadda, S. S., Harnack, L., Hai, L. R., McKeown, N., Seal, C., Liu, S., et al. **2011**. Putting the whole grain puzzle together: health benefits associated with whole grains-summary of American Society for Nutrition 2010 satellite symposium. *Journal of Nutrition*.141, 1011S-1022S.
3. Ajayeoba, T. A., Dula, S., & Ijabadeniyi, O. A. **2019**. Properties of poly-γ-glutamic acid producing-Bacillus species isolated from ogi liquor and lemon-ogi liquor. *Frontiers in Microbiology*, *10*, 771.
4. Obst M, Steinbuchel A. **2004**. Microbial degradation of poly (amino acid)s. *Biomacromolecules*. 5:1166–76.
5. Ivanovics, G., & Bruckner, V. **1937**. The chemical nature of the immuno-specific capsule substance of anthrax *bacillus*. *Naturwissenschaften*, *25*, 250.
6. Bajaj, I., and Singhal, R. **2011**. Poly (glutamic acid) an emerging biopolymer of commercial interest. *Bioresource Technology* 102, 5551-5561.
7. Ogunleye, A., Bhat, A., Irorere, V. U., Hill, D., Williams, C., and Radecka, I. **2015**. Poly-γ-glutamic acid: production, properties and applications. *Microbiology* 161, 1-17.
8. Ashiuchi, M., Kamei, T., Baek, D. H., Shin, S. Y., Sung, M. H., Soda, K., et al. **2001**. Isolation of *Bacillus subtilis* (chungkookjang), a poly-γ-glutamate producer with high genetic competence. *Applied Microbiology and Biotechnology* 57, 764–769.
9. Yang, L. B., Zhan, X. B., Zhu, L., Gao, M. J., & Lin, C. C. **2016**. Optimization of a low-cost hyperosmotic medium and establishing the fermentation kinetics of erythritol production by *Yarrowia lipolytica* from crude glycerol. *Preparative Biochemistry and Biotechnology*, *46*(4), 376-383.
10. Hseu, Z., Guo, Y., Liu, J., Qiu, H., Zhao, M., Zou, W., & Li, S. **2016**. Microbial synthesis of poly-γ-glutamic acid: current progress, challenges, and future perspectives. *Biotechnology for Biofuels*, 9(1), 1-12.
11. Sung, M. H., Park, C., Kim, C. J., Poo, H., Soda, K., & Ashiuchi, M. **2005**. Natural and edible biopolymer poly-γ-glutamic acid: synthesis, production, and applications. *The Chemical Record*, *5*(6), 352-366.
12. Najar, I. N., & Das, S. **2015**. Poly-glutamic acid (PGA)-Structure, synthesis, genomic organization and its application: A Review. *International Journal of Pharmaceutical Sciences and Research*. *6*(6), 2258.
13. Wang, L. L., Chen, J. T., Wang, L. F., Wu, S., Zhang, G. Z., Yu, H. Q., & Shi, Q. S. **2017**. Conformations and molecular interactions of poly-γ-glutamic acid as a soluble microbial product in aqueous solutions. *Scientific reports*, *7*(1), 1-11.
14. Stanley, C. B., & Strey, H. H. **2008**. Osmotically induced helix-coil transition in poly (glutamic acid). *Biophysical Journal*, *94*(11), 4427-4434.
15. Gooding, E. A., Sharma, S., Petty, S. A., Fouts, E. A., Palmer, C. J., Nolan, B. E., & Volk, M. **2013**. pH-dependent helix folding dynamics of poly-glutamic acid. *Chemical Physics*. *422*, 115-123.
16. Jose, A. A., Anusree, G., Pandey, A., & Binod, P. **2018**. Production optimization of poly-γ-glutamic acid by *Bacillus amyloliquefaciens* under solid-state fermentation using soy hull as substrate. *Indian Journal of Biotechnology*. vol 17(1).
17. Oppermann-Sanio, F. B., and Steinbüchel, A. **2002**. Occurrence, functions and biosynthesis of polyamides in microorganisms and biotechnological production. Naturwissenschaften 89, 11-22.
18. Yao, J., Jing, J., Xu, H., Liang, J., Wu, Q., Feng, X., et al. **2009**. Investigation on enzymatic degradation of γ-polyglutamic acid from *Bacillus subtilis* NX-2. *Journa of Molecular Catalysis* B 56, 158-164.
19. Moraes, L. P., Brito, P.N., and Alegre, R. M. **2013**. The existing studies on biosynthesis of poly (γ-glutamic acid) by fermentation. *Food Public Health* 3, 28-36.
20. Cachat, E., Barker, M., Read, T. D., and Priest, F. G. **2008**. A *Bacillus thuringiensis* strain producing a polyglutamate capsule resembling that of *Bacillus anthracis*. *Federation of European Microbiological Societies Microbiology Letter*. 285, 220-226.
21. Candela, T., Moya, M., Haustant, M., and Fouet, A. **2009**. *Fusobacterium nucleatum*, the first gram-negative bacterium demonstrated to produce polyglutamate. *Canadian Journal of Microbiology* 55, 627-632.
22. Cao M., Geng W., Liu L., Song C., Xie H., Guo W., Jin Y., Wang S. **2011**. Glutamic acid independent production of poly-γ-glutamic acid by *Bacillus amyloliquefaciens* LL3 and cloning of pgsBCA genes. *Bioresource Technology* 102:4251–4257.

 doi:10.20944/preprints202502.2267.v1

23. Chettri, R., Bhutia, M. O., and Tamang, J. P. **2016**. Poly-γ-glutamic acid (PGA)-producing *Bacillus* species isolated from Kinema, Indian fermented soybean food. *Frontiers Microbioogy*. 7:971.
24. Kambourova, M., Tangney, M., and Priest, F. G. **2001**. Regulation of polyglutamic acid synthesis by glutamate in *Bacillus licheniformis* and *Bacillus subtilis*. *Applied and Environmental Microbiology*. 67,1004-1007.
25. Meerak, J., Yukphan, P., Miyashita, M., Sato, H., Nahagawa, Y., and Tahara, Y. **2008**. Phylogeny of (γ-polyglutamic acid-producing *Bacillus* strains isolated from a fermented locust bean product. *Journal of General and Applied Microbiology* 54, 159-166.
26. Tork, S. E., Aly, M. M., Alakilli, S. Y., and Al-Seeni, M. N. **2015**. Purification and characterization of gamma poly glutamic acid from newly *Bacillus licheniformis* NRC20. *International Journal of Biological Macromolecules* 74, 382-391.
27. Candela T., Fouet A. **2006**. Poly-gamma-glutamate in bacteria. *Molecular Microbiology* 60:1091–1098.
28. Buescher JM and Margaritis A. **2007**. Microbial biosynthesis of polyglutamic acid biopolymer and applications in the biopharmaceutical, biomedical and food industries. *Critical Reviews in Biotechnology*. 27:1–19.
29. Ko Y. H., Gross R. A. **1998**. Effects of glucose and glycerol on γ-poly(glutamic acid) formation by *Bacillus licheniformis* ATCC 9945a. *Biotechnology and Bioengineering*. 57:430–437.
30. Rehm B. **2009**. Microbial Production of Biopolymers and Polymer Precursors: Applications and Perspectives Caister: Horizon Scientific Press
31. Li, D., Hou, L., Gao, Y., Tian, Z., Fan, B., Wang, F., & Li, S. **2022**. Recent advances in microbial synthesis of poly-γ-glutamic acid: a review. *Foods*, *11*(5), 739.
32. Wu Q, Xu H, Xu L, Ouyang P. **2006**. Biosynthesis of poly (gamma-glutamic acid) in *Bacillus subtilis* NX-2: regulation of stereochemical composition of poly (gamma-glutamic acid). *Process Biochemistry*. 41:1650–5.
33. Ashiuchi M. **2004**. Enzymatic synthesis of high-molecular-mass poly-gamma-glutamate and regulation of its stereochemistry. *Applied and Environmental Microbiology*.70:4249–55.
34. Candela T, Fouet A. **2005**. *Bacillus anthracis* CapD, belonging to the gamma-glutamyl transpeptidase family, is required for the covalent anchoring of capsule to peptidoglycan. *Molecular Microbiology*. 57:717–26.
35. Yamashiro D, Yoshioka M, Ashiuchi M. **2011**. *Bacillus subtilis*pgsE (formerly ywtC) stimulates poly-γ-glutamate production in the presence of Zinc. *Biotechnology and Bioengineering*. 108:226–30.
36. Tran LSP, Nagai T, Itoh Y. **2000**. Divergent structure of the ComQXPA quorum-sensing components: molecular basis of strain-specific communication mechanism in *Bacillus subtilis*. *Molecular Microbiology*.37:1159–71.
37. Do TH. **2011**. Mutations suppressing the loss of DegQ function in *Bacillus subtilis* (natto) poly-γ-glutamate synthesis. *Applied and Environmental Microbiology*.77:8249–58.
38. Ohsawa T, Tsukahara K, Ogura M. **2009**. *Bacillus subtilis* response regulator DegU is a direct activator of pgsB transcription involved in gamma-poly-glutamic acid synthesis. *Bioscience, Biotechnology and Biochemistry*.73:2096–102.
39. Stanley NR, Lazazzera BA. **2005**. Defining the genetic differences between wild and domestic strains of *Bacillus subtilis* that affect poly-gamma-dl-glutamic acid production and biofilm formation. *Molecular Microbiology*. l.57:1143–58.
40. Kimura K, Tran LSP, Uchida I, Itoh Y. **2004**. Characterization of *Bacillus subtilis* gamma-glutamyl transferase and its involvement in the degradation of capsule poly-gamma-glutamate. *Microbiology*.150:4115–23.
41. King EC, Blacker AJ, Bugg TDH. **2000**. Enzymatic breakdown of poly-gamma-D-glutamic acid in *Bacillus licheniformis*: identification of a polyglutamyl gamma-hydrolase enzyme. *Biomacromolecules*.1:75–83.
42. Hsueh, Y. H., Huang, K. Y., Kunene, S. C., & Lee, T. Y. **2017**. Poly-γ-glutamic acid synthesis, gene regulation, phylogenetic relationships, and role in fermentation. *International Journal of Molecular Sciences*, *18*(12), 2644.
43. Sanda, F., Fujiyama, T., & Endo, T. **2001**. Chemical synthesis of poly-γ-glutamic acid by polycondensation of γ-glutamic acid dimer: synthesis and reaction of poly-γ-glutamic acid methyl ester. *Journal of Polymer Science Part A: Polymer Chemistry*, *39*(5), 732-741.
44. Inatsu, Y., Nakamura, N., Yuriko, Y., Fushimi, T., Watanasiritum, L., & Kawamoto, S. **2006**. Characterization of Bacillus subtilis strains in Thua nao, a traditional fermented soybean food in northern Thailand. *Letters in Applied Microbiology*, *43*(3), 237-242.

 doi:10.20944/preprints202502.2267.v1

45. Shih I. L., Van Y. T., Yeh L. C., Lin H. G., Chang Y. N. **2001**. Production of a biopolymer flocculant from *Bacillus licheniformis* and its flocculation properties. *Bioresource Technology*. 78:267–272.
46. Goto, A., and Kunioka, M. **1992**. Biosynthesis and hydrolysis of poly (γ-glutamic acid) from *Bacillus subtilis* IFO3335. *Bioscience, Biotechnology and Biochemistry*. 56, 1031-1035.
47. Kunioka, M. (1995). Biosynthesis of poly (γ-glutamic acid) from l-glutamine, citric acid and ammonium sulfate in *Bacillus subtilis* IFO3335. *Applied Microbiology and Biotechnology*, *44*, 501-506.
48. 48.Cao M., Geng W., Zhang W., Sun J., Wang S., Feng J., Zheng P., Jiang A., Song C. **2013**. Engineering of recombinant *Escherichia coli* cells co-expressing poly-γ-glutamic acid (γ-PGA) synthetase and glutamate racemase for differential yielding of γ-PGA. Journal of *Microbiology and Biotechnology* 6:675–684.
49. Ashiuchi, M. **2013**. Biochemical engineering of PGA. *Microbial Biotechnology*, *6*(6), 664-674.
50. Wei, X., Yang, L., Chen, Z., Xia, W., Chen, Y., Cao, M., & He, N. (2024). Molecular weight control of poly-γ-glutamic acid reveals novel insights into extracellular polymeric substance synthesis in *Bacillus licheniformis*. *Biotechnology for Biofuels and Bioproducts*, *17*(1), 60.
51. Zeng, W., Liu, Y., Shu, L., Guo, Y., Wang, L., & Liang, Z. **2024**. Production of ultra-high-molecular-weight poly-γ-glutamic acid by a newly isolated *Bacillus subtilis* strain and genomic and transcriptomic analyses. *Biotechnology Journal*, *19*(4), 2300614.
52. Ashiuchi M., Kamei T., Baek D.H., Shin S.Y., Sung M.H., Soda K., Yagi T., Misono H. **2001**. Isolation of *Bacillus subtilis* (*chungkookjang*), a poly-γ-glutamate producer with high genetic competence. *Applied Microbiology and Biotechnology*. 57:764–769.
53. 53.Bhat A.R., Irorere V.U., Bartlett T., Hill D., Kedia G., Morris M.R., Charalampopoulos D., Radecka I. **2013**. *Bacillus subtilis* natto: a non-toxic source of poly-γ-glutamic acid that could be used as a cryoprotectant for probiotic bacteria. AMB Express3:36.
54. 54**.** Wu Q., Xu H., Ying H., Ouyang P. **2010**. Kinetic analysis and pH-shift control strategy for poly (γ-glutamic acid) production with *Bacillus subtilis* CGMCC 0833. *Biochemical Engineering Journal*. 50:24–28.
55. Cao M., Song C., Jin Y., Liu L., Liu J., Xie H., Guo W., Wang S. **2010**. Synthesis of poly (γ-glutamic acid) and heterologous expression of pgsBCA genes. *Journal of Molecular Catalysis B: Enzymatic*. 67:111–116.
56. Birrer, G. A., Cromwick, A. M., & Gross, R. A. **1994**. γ-Poly (glutamic acid) formation by *Bacillus licheniformis* 9945a: physiological and biochemical studies. *International journal of biological macromolecules*, *16*(5), 265-275.
57. Shih I. L., Wu P. J., Shieh C. J. **2005**. Microbial production of a poly (γ-glutamic acid) derivative by *Bacillus subtilis*. *Process Biochemistry*. 40:2827–2832.
58. Zhang H., Zhu J., Zhu X., Cai J., Zhang A., Hong Y., Huang J., Huang L., Xu Z. (2012). High-level exogenous glutamic acid-independent production of poly-(γ-glutamic acid) with organic acid addition in a new isolated *Bacillus subtilis* C10. *Bioresource Technology*. 116:241–246.
59. Cromwick, A. M., Birrer, G. A., & Gross, R. A. **1996**. Effects of pH and aeration on γ-poly (glutamic acid) formation by *Bacillus licheniformis* in controlled batch fermentor cultures. *Biotechnology and Bioengineering*, *50*(2), 222-227.
60. Kubota, H., Matsunobu, T., Uotani, K., Takebe, H., Satoh, A., Tanaka, T., & Taniguchi, M. **1993**. Production of poly (γ-glutamic acid) by *Bacillus subtilis* F-2-01. *Bioscience, biotechnology, and biochemistry*, *57*(7), 1212-1213.
61. Shih, L., & Van, Y. T. **2001**. The production of poly-(γ-glutamic acid) from microorganisms and its various applications. *Bioresource Technology*, *79*(3), 207-225.
62. Yoon, W. K., Garcia, C. V., Kim, C. S., & Lee, S. P. **2018**. Fortification of mucilage and GABA in Hovenia dulcis extract by co-fermentation with *Bacillus subtilis* HA and *Lactobacillus plantarum* EJ2014. *Food Science and Technology Research*, *24*(2), 265-271.
63. Bajaj I. B., Lele S. S., Singhal R. S. **2008**. Enhanced production of poly (γ-glutamic acid) from *Bacillus licheniformis* NCIM 2324 in solid state fermentation. *J Ind Microbiol Biotechnol* 35:1581–1586.
64. Bajaj I. B., Lele S. S., Singhal R. S. **2009**. A statistical approach to optimization of fermentative production of poly (γ-glutamic acid) from *Bacillus licheniformis* NCIM 2324. *Bioresource Technology* 100:826–832.
65. Mabrouk M., Abou-Zeid D., Sabra W. **2012**. Application of Plackett–Burman experimental design to evaluate nutritional requirements for poly (γ-glutamic acid) production in batch fermentation by *Bacillus licheniformis* A13. *African Journal of Applied Microbiology Res.* 2:6–18.

 doi:10.20944/preprints202502.2267.v1

66. Wang, N., Yang, G., Che, C., & Liu, Y. **2011**. Heterogenous expression of poly-γ-glutamic acid synthetase complex gene of *Bacillus licheniformis* WBL-3. *Applied Biochemistry and Microbiology*, *47*, 381-385.
67. Ho, G. H., Ho, T. I., Hsieh, K. H., Su, Y. C., Lin, P. Y., Yang, J., & Yang, S. C. **2006**. γ-Polyglutamic acid produced by *Bacillus Subtilis* (Natto): Structural characteristics, chemical properties and biological functionalities. *Journal of the Chinese Chemical Society*, *53*(6), 1363-1384.
68. Cheng, C., Asada, Y., & Aida, T. **1989**. Production of γ-polyglutamic acid by *Bacillus licheniformis* A35 under denitrifying conditions. *Agricultural and biological chemistry*, *53*(9), 2369-2375.
69. Bovarnick, M. **1942**. The formation of extracellular d (-)-glutamic acid polypeptide by *Bacillus subtilis*. *Journal of Biological Chemistry*, *145*(2), 415-424.
70. Scoffone, V., Dondi, D., Biino, G., Borghese, G., Pasini, D., Galizzi, A., & Calvio, C. **2013**. Knockout of pgdS and ggt genes improves γ-PGA yield in *B. subtilis*. *Biotechnology and Bioengineering*, *110*(7), 2006-2012.
71. Shi, F., Xu, Z., & Cen, P. **2006**. Efficient production of poly-γ-glutamic acid by *Bacillus subtilis* ZJU-7. *Applied Biochemistry and Biotechnology*, *133*, 271-281.
72. Ogawa, Y., Yamaguchi, F., Yuasa, K., & Tahara, Y. **1997**. Efficient production of γ-polyglutamic acid by *Bacillus subtilis* (natto) in jar fermenters. *Bioscience, biotechnology, and biochemistry*, *61*(10), 1684-1687.
73. Zwartouw H.T., Smith H. **1956**. Polyglutamic acid from *Bacillus anthracis* grown *in vivo*: structure and aggression activity. *Biochemical Journal.* 63:437–442.
74. Ezzell J. W., Abshire T. G., Panchal R., Chabot D., Bavari S., Leffel E. K., Purcell B., Friedlander A. M., Ribot W. J. **2009**. Association of *Bacillus anthracis* capsule with lethal toxin during experimental infection. *Infection and Immunity.* 77:749–755.
75. Zhang, C., Zhong, C., & Wu, D. **2021**. Study on the reuse process of hydrolysate from γ-polyglutamic acid fermentation residues. *Arabian Journal of Chemistry*, *14*(5), 103145.
76. Mohanraj, R., Gnanamangai, B. M., Ramesh, K., Priya, P., Srisunmathi, R., Poornima, S., & Robinson, J. P. **2019**. Optimized production of gamma poly glutamic acid (γ-PGA) using sago. *Biocatalysis and Agricultural Biotechnology*, *22*, 101413.
77. Inbaraj BS, Chen B-H **2012**. *In vitro* removal of toxic heavy metals by poly (γ-glutamic acid)-coated superparamagnetic nanoparticles. *International Journal of Nanomedicine.* 7:4419.
78. Lee, J. M., Kim, J. H., Kim, K. W., Lee, B. J., Kim, D. G., Kim, Y. O., & Kong, I. S. **2018**. Physicochemical properties, production, and biological functionality of poly-γ-D-glutamic acid with constant molecular weight from halotolerant *Bacillus sp.* SJ-10. *International journal of biological macromolecules*, *108*, 598-607.
79. Gentilini, C., Dong, Y., May, J. R., Goldoni, S., Clarke, D. E., Lee, B. H., & Stevens, M. M. (2012). Functionalized Poly (γ-Glutamic Acid) Fibrous Scaffolds for Tissue Engineering. *Advanced healthcare materials*, *1*(3), 308-315.
80. Kunioka, M. **1995**. Biosynthesis of poly (γ-glutamic acid) from l-glutamine, citric acid and ammonium sulfate in *Bacillus subtilis* IFO3335. *Applied Microbiology and Biotechnology*, *44*, 501-506.
81. Bajestani, M. I., Mousavi, S. M., Mousavi, S. B., Jafari, A., & Shojaosadati, S. A. **2018**. Purification of extra cellular poly-γ-glutamic acid as an antibacterial agent using anion exchange chromatography. *International Journal of Biological Macromolecules*, *113*, 142-149.
82. Shyu, Y. S., & Sung, W. C. **2010**. Improving the emulsion stability of sponge cake by the addition of γ-polyglutamic acid. *Journal of Marine Science and Technology*, *18*(6), 14.
83. Johnson, L. C., Akinmola, A. T., & Scholz, C. **2022.** Poly (glutamic acid): From *natto* to drug delivery systems. *Biocatalysis and Agricultural Biotechnology*, *40*, 102292.
84. Shi, W., Liang, J., Tao, W., Tan, S., & Wang, Q. **2015**. γ-PGA additive decreasing soil water infiltration and improving water holding capacity. *Transactions of the Chinese Society of Agricultural Engineering*, *31*(23), 94-100.
85. Lim S., Kim J., Shim J., Imm B., Sung M., Imm J. **2012**. Effect of poly-γ-glutamic acids (PGA) on oil uptake and sensory quality in doughnuts. *Food Science and Biotechnology*. 21:247–252.
86. Lee, N., Go, T., Lee, S., Jeong, S., Park, G., Hong, C., et al. **2014**. In vitro evaluation of new functional properties of poly-γ-glutamic acid produced by *Bacillus subtilis* D7. *Saudi Journal of. Biological Sciences.* 21, 153–158.

 doi:10.20944/preprints202502.2267.v1

87. Mundo, J. L. M., Zhou, H., Tan, Y., Liu, J., & Mc Clements, D. J. **2020**. Stabilization of soybean oil-in-water emulsions using polypeptide multilayers: Cationic polylysine and anionic polyglutamic acid. *Food Research International*, *137*, 109304.
88. Zhang, R., Zhang, S., Jiang, G., Gan, L., Xu, Z., & Tian, Y. **2022**. Optimization of fermentation conditions, purification and rheological properties of poly (γ-glutamic acid) produced by *Bacillus subtilis* 1006-3. *Preparative Biochemistry & Biotechnology*, *52*(3), 302-310.
89. de Cesaro, A., da Silva, S. B., da Silva, V. Z., & Ayub, M. A. Z. **2014**. Physico-chemical and rheological characterization of poly-gamma-glutamic acid produced by a new strain of *Bacillus subtilis*. *European polymer journal*, *57*, 91-98.
90. Wang, R., Zhou, B., Xu, D. L., Xu, H., Liang, L., Feng, X. H., & Chi, B. **2016**. Antimicrobial and biocompatible ε-polylysine–γ-poly (glutamic acid)–based hydrogel system for wound healing. *Journal of Bioactive and Compatible Polymers*, *31*(3), 242-259.
91. Pihlanto, A., Akkanen, S., & Korhonen, H. J. **2008**. ACE-inhibitory and antioxidant properties of potato (*Solanum tuberosum*). *Food Chemistry*, *109*(1), 104-112.
92. Tsao C. T., Chang C. H., Lin Y. Y., Wu M. F., Wang J. L., Young T. H., Han J. L., Hsieh K. H. **2011**. Evaluation of chitosan/γ-poly (glutamic acid) polyelectrolyte complex for wound dressing materials. *Carbohydrate Polymers*. 84:812–819.
93. Ye, H., Xian, Y., Li, S., Zhang, C., & Wu, D. **2022**. In situ forming injectable γ-poly (glutamic acid)/PEG adhesive hydrogels for hemorrhage control. *Biomaterials Science*, *10*(15), 4218-4227.
94. Deng S, Bai R, Hu X, Luo Q **2003**. Characteristics of a bioflocculant produced by Bacillus mucilaginosus and its use in starch wastewater treatment. *Applied Microbiology and Biotechnology* 60:588–593.
95. Zhao C, Zhang Y, Wei X, Hu Z, Zhu F, Xu L, Luo M, Liu H **2013**. Production of ultra-high molecular weight poly-γ-glutamic acid with *Bacillus licheniformis* P-104 and characterization of its flocculation properties. *Applied Biochemistry and Biotechnology* 170:562–572.
96. Kinnersley, A., Strom, D., Meah, R.Y., and Koskan, C.P. **1994**. Composition and method for enhanced fertilizer uptake by plants (WO patent no. 94/09,628).
97. Inbaraj B. S., Chiu C. P., Ho G. H., Yang J., Chen B. H. **2006**. Removal of cationic dyes from aqueous solution using an anionic poly-γ-glutamic acid-based adsorbent. *Journal of Hazardous Materials.* 137:226–234.
98. Campos, V., Fernandes, A. R., Medeiros, T. A., & Andrade, E. L. **2016**. Physicochemical characterization and evaluation of PGA bioflocculant in coagulation-flocculation and sedimentation processes. *Journal of Environmental Chemical Engineering*, *4*(4), 3753-3760.
99. Zheng H, Gao Z, Yin J, Tang X, Ji X, Huang H **2012**. Harvesting of microalgae by flocculation with poly(γ-glutamic acid). *Bioresource Technology* 112:212–220.
100. Sheu, Y. T., Tsang, D. C., Dong, C. D., Chen, C. W., Luo, S. G., & Kao, C. M. **2018**. Enhanced bioremediation of TCE-contaminated groundwater using gamma poly-glutamic acid as the primary substrate. *Journal of Cleaner Production*, *178*, 108-118.
101. Hajdu I., Bodnár M., Csikós Z., Wei S., Daróczi L., Kovács B., Győri Z., Tamás J., Borbély J. **2012**. Combined nano-membrane technology for removal of lead ions. *Journal of Membrane Science.* 409–410:44–53.
102. Xu Z, Wan C, Xu X, Feng X, Xu H. **2013**. Effect of poly (γ-glutamic acid) on wheat productivity, nitrogen use efficiency and soil microbes. *Journal of Plant Nutrition and Soil Science* 13:744–755.
103. Wang, F., Chen, Y., Zheng, J., Yang, C., Li, L., Li, R., & Li, Z. **2024**. Preparation of potential organic fertilizer rich in γ-polyglutamic acid via microbial fermentation using brewer's spent grain as basic substrate. *Bioresource Technology*, *394*, 130216.
104. Wang Q., Chen S., Zhang J., Sun M., Liu Z., Yu Z. **2008**. Co-producing lipopeptides and poly-γ-glutamic acid by solid-state fermentation of *Bacillus subtilis* using soybean and sweet potato residues and its biocontrol and fertilizer synergistic effects. *Bioresource Technology*. 99:3318–3323.
105. Shih I. L., Van Y. T., Sau Y. Y. **2003**. Antifreeze activities of poly (γ-glutamic acid) produced by *Bacillus licheniformis*. *Biotechnology Letters*. 25:1709–1712.

 doi:10.20944/preprints202502.2267.v1

106. Bhat A, Irorere V, Bartlett T, Hill D, Kedia G, Charalampopoulos D, Nualkaekul S, Radecka **2015**. Improving survival of probiotic bacteria using bacterial poly-γ-glutamic acid. *International Journal of Food Microbiology* 196:24–31.
107. Tanimoto H., Fox T., Eagles J., Satoh H., Nozawa H., Okiyama A., Morinaga Y., Fairweather-Tait S. J. **2007**. Acute effect of poly-γ-glutamic acid on calcium absorption in post-menopausal women. *Journal of the American College of the Nutrition*. 26:645–649.
108. Kishimoto N, Morishima H, Uotani K. **2008**. Compositions for prevention of increase of blood pressure Japanese patent application. Publication (2008-255063).
109. Tamura, M., Watanabe, J., Hori, S., Inose, A., Kubo, Y., Noguchi, T., & Kobori, M. **2021**. Effects of a high-γ-polyglutamic acid-containing natto diet on liver lipids and cecal microbiota of adult female mice. *Bioscience of Microbiota, Food and Health*, 2020-061.
110. Siaterlis A., Deepika G., Charalampopoulos D. **2009**. Effect of culture medium and cryoprotectants on the growth and survival of probiotic *lactobacilli* during freeze drying. *Letters in Applied Microbiology*. 48:295–301.
111. Jagannath A., Raju P. S., Bawa A. S. **2010**. Comparative evaluation of bacterial cellulose (nata) as a cryoprotectant and carrier support during the freeze drying process of probiotic lactic acid bacteria. LWT – *Food Science and Technology*. 43:1197–1203.
112. Ben-Zur N., Goldman D. M. **2007**. γ-Polyglutamic acid: a novel peptide for skin care. *Cosmetics Toiletries.* 122:65–74.
113. Abd Alsaheb, R. A., Othman, N. Z., Abd Malek, R., Leng, O. M., Aziz, R., & El Enshasy, H. A. **2016**. Polyglutamic acid applications in pharmaceutical and biomedical industries. *Der Pharmacia Letter*, *8*(9), 217-225.
114. Murakami S, Aoki N, Matsumura S **2011**. Bio-based biodegradable hydrogels prepared by cross-linking of microbial poly (γ-glutamic acid) with l-lysine in aqueous solution. Polymer Journal. 43:414–420.
115. Lin W-C, Yu D-G, Yang M-C **2006**. Blood compatibility of novel poly (γ-glutamic acid)/polyvinyl alcohol hydrogels. *Colloids and Surfaces B: Biointerfaces*. 47:43–49.
116. Yu, K., & Aubin-Tam, M. E. (2020). Bacterially grown cellulose/graphene oxide composites infused with γ-Poly (glutamic acid) as biodegradable structural materials with enhanced toughness. *ACS Applied Nano Materials*, *3*(12), 12055-12063.
117. Tao, L., Long, H., Zhang, J., Qi, L., Zhang, S., Li, T., & Li, S. **2021**. Preparation and coating application of γ-polyglutamic acid hydrogel to improve storage life and quality of shiitake mushrooms. *Food Control*, *130*, 108404.
118. Lin Y-H, Chung C-K, Chen C-T, Liang H-F, Chen S-C, Sung H-W **2005**. Preparation of nanoparticles composed of chitosan/poly-γ-glutamic acid and evaluation of their permeability through Caco-2 cells. *Biomacromolecules* 6:1104–1112.
119. Mukhopadhyay P, Mishra R, Rana D, Kundu PP **2012**. Strategies for effective oral insulin delivery with modified chitosan nanoparticles: a review. *Progress in Polymer Science.* 37:1457–1475.
120. Hsieh C.-Y., Tsai S.-P., Wang D.M., Chang Y.N., Hsieh H.J. **2005**. Preparation of γ-PGA/chitosan composite tissue engineering matrices. *Biomaterials*. 26:5617–5623.
121. Ye H., Jin L., Hu R., Yi Z., Li J., Wu Y., Xi X., Wu Z. **2006**. Poly (γ,l-glutamic acid)–cisplatin conjugate effectively inhibits human breast tumor xenografted in nude mice. *Biomaterials*. 27:5958–5965.
122. Singer J. W. **2005**. Paclitaxel poliglumex (XYOTAX, CT-2103): a macromolecular taxane. J Control Release109:120–126.
123. Otani Y., Tabata Y., Ikada Y. **1999**. Sealing effect of rapidly curable gelatin-poly (l-glutamic acid) hydrogel glue on lung air leak. *Annals of Thoracic Surgery*. 67:922–926.
124. Shih, I. L., Van, Y. T., & Shen, M. H. **2004**. Biomedical applications of chemically and microbiologically synthesized poly (glutamic acid) and poly (lysine). *Mini reviews in medicinal chemistry*, *4*(2), 179-188.
125. Yu, X., Wang, M., Wang, Q. H., & Wang, X. M. **2011**. Biosynthesis of polyglutamic acid and its application on agriculture. *Advanced materials research*, *183*, 1219-1223.
126. Wu, J. Y., & Ye, H. F. **2007**. Characterization and flocculating properties of an extracellular biopolymer produced from a Bacillus subtilis DYU1 isolate. *Process Biochemistry*, *42*(7), 1114-1123.

 doi:10.20944/preprints202502.2267.v1

127. Zhang, C., Ren, H. X., Zhong, C. Q., & Wu, D. **2020**. Biosorption of Cr (VI) by immobilized waste biomass from polyglutamic acid production. *Scientific Reports*, *10*(1), 3705.
128. Liu, J., Ma, X., Wang, Y., Liu, F., Qiao, J., Li, X. Z., & Zhou, T. **2011**. Depressed biofilm production in *Bacillus amyloliquefaciens* C06 causes γ-polyglutamic acid (γ-PGA) overproduction. *Current microbiology*, *62*, 235-241.
129. Zhang, C., Wu, D., & Ren, H. **2019**. Economical production of agricultural γ-polyglutamic acid using industrial wastes by *Bacillus subtilis*. *Biochemical Engineering Journal*, *146*, 117-123.
130. Pereira, A. E. S., Sandoval-Herrera, I. E., Zavala-Betancourt, S. A., Oliveira, H. C., Ledezma-Pérez, A. S., Romero, J., & Fraceto, L. F. **2017**. γ-Polyglutamic acid/chitosan nanoparticles for the plant growth regulator gibberellic acid: Characterization and evaluation of biological activity. *Carbohydrate polymers*, *157*, 1862-1873.
131. Richard, A., & Margaritis, A. **2001**. Poly (glutamic acid) for biomedical applications. *Critical reviews in biotechnology*, *21*(4), 219-232.
132. Yang, Z. H., Dong, C. D., Chen, C. W., Sheu, Y. T., & Kao, C. M. **2018**. Using poly-glutamic acid as soil-washing agent to remediate heavy metal-contaminated soils. *Environmental Science and Pollution Research*, *25*, 5231-5242.
133. Xu, J., Krietemeyer, E. F., Finkenstadt, V. L., Solaiman, D., Ashby, R. D., & Garcia, R. A. **2016**. Preparation of starch–poly–glutamic acid graft copolymers by microwave irradiation and the characterization of their properties. *Carbohydrate Polymers*, *140*, 233-237.
134. Zhang, S. F., Gao, C., Lü, S., He, J., Liu, M., Wu, C., & Liu, Z. **2017**. Synthesis of PEGylated polyglutamic acid peptide dendrimer and its application in dissolving thrombus. *Colloids and Surfaces B: Biointerfaces*, *159*, 284-292.
135. Tao, L., Tian, L., Zhang, X., Huang, X., Long, H., Chang, F., & Li, S. **2020**. Effects of γ-polyglutamic acid on the physicochemical properties and microstructure of grass carp (*Ctenopharyngodon idellus*) surimi during frozen storage. *Lwt*, *134*, 109960.
136. Shi, F., Xu, Z., & Cen, P. **2006**. Optimization of γ-polyglutamic acid production by *Bacillus subtilis* ZJU-7 using a surface-response methodology. *Biotechnology and Bioprocess Engineering*, *11*, 251-257.
137. Konglom, N., Chuensangjun, C., Pechyen, C., & Sirisansaneeyakul, S. **2012**. Production of poly- γ-glutamic acid by *Bacillus licheniformis*: Synthesis and characterization. *Journal of Metals, Materials and Minerals*, *22*(2), 7-11.
138. Chatterjee, P. M., Datta, S., Tiwari, D. P., Raval, R., & Dubey, A. K. **2018**. Selection of an effective indicator for rapid detection of microorganisms producing γ-polyglutamic acid and its biosynthesis under submerged fermentation conditions using *Bacillus methylotrophicus*. *Applied biochemistry and biotechnology*, *185*, 270-288.
139. Hezayen, F. F., Rehm, B. H., Tindall, B. J., & Steinbüchel, A. **2001**. Transfer of *Natrialba asiatica* B1T to *Natrialba taiwanensis* sp. nov. and description of *Natrialba aegyptiaca* sp. nov., a novel extremely halophilic, aerobic, non-pigmented member of the Archaea from Egypt that produces extracellular poly (glutamic acid). *International Journal of Systematic and Evolutionary Microbiology*, *51*(3), 1133-1142.
140. Qi, H., Na, R., Xin, J., Xie, Y. J., & Guo, J. F. **2013**. Effect of corona electric field on the production of gamma-poly glutamic acid based on *Bacillus* natto. *Journal of Physics: Conference Series* (Vol. 418, No. 1, p. 012139).
141. Du, G., Yang, G., Qu, Y., Chen, J., & Lun, S. **2005**. Effects of glycerol on the production of poly (γ-glutamic acid) by *Bacillus licheniformis*. *Process Biochemistry*, *40*(6), 2143-2147.
142. Zhang, H., Zhu, J., Zhu, X., Cai, J., Zhang, A., Hong, Y., & Xu, Z. **2012**. High-level exogenous glutamic acid-independent production of poly-(γ-glutamic acid) with organic acid addition in a new isolated *Bacillus subtilis* C10. *Bioresource Technology*, *116*, 241-246.
143. Min, J. H., Reddy, L. V., Dimitris, C., Kim, Y. M., & Wee, Y. J. **2019**. Optimized production of poly (γ-glutamic acid) by *Bacillus sp.* FBL-2 through response surface methodology using central composite design.
144. Ward, R. M., Anderson, R. F., & Dean, F. K. **1963**. Polyglutamic acid production by *Bacillus subtilis* NRRL B-2612 grown on wheat gluten. *Biotechnology and Bioengineering*, *5*(1), 41-48.
145. Kreyenschulte, D., Krull, R., & Margaritis, A. **2014**. Recent advances in microbial biopolymer production and purification. *Critical reviews in biotechnology*, *34*(1), 1-15.

 doi:10.20944/preprints202502.2267.v1

146. Giannos, S. A., Shah, D., Gross, R. A., Kaplan, D. L., & Mayer, J. M. **1990**. Poly (glutamic acid) produced by bacterial fermentation. In *Novel biodegradable microbial polymers* (pp. 457-460). Dordrecht: Springer Netherlands.
147. Mahaboob Ali, A. A., Momin, B., & Ghogare, P. **2020**. Isolation of a novel poly-γ-glutamic acid-producing *Bacillus licheniformis* A14 strain and optimization of fermentation conditions for high-level production. *Preparative Biochemistry & Biotechnology*, *50*(5), 445-452.
148. Manocha, B., & Margaritis, A. **2010**. Controlled Release of Doxorubicin from Doxorubicin/γ-Polyglutamic Acid Ionic Complex. *Journal of Nanomaterials*, *2010*(1), 780171.
149. Yazdanparast, S., Benvidi, A., Banaei, M., Nikukar, H., Tezerjani, M. D., & Azimzadeh, M. **2018**. Dual-aptamer based electrochemical sandwich biosensor for MCF-7 human breast cancer cells using silver nanoparticle labels and a poly (glutamic acid)/MWNT nanocomposite. *Microchimica Acta*, *185*, 1-10.
150. Oliveri, V., Bellia, F., Viale, M., Maric, I., & Vecchio, G. **2017**. Linear polymers of β and γ cyclodextrins with a polyglutamic acid backbone as carriers for doxorubicin. *Carbohydrate polymers*, *177*, 355-360.
151. Ko, W. C., Chang, C. K., Wang, H. J., Wang, S. J., & Hsieh, C. W. **2015**. Process optimization of microencapsulation of curcumin in γ-polyglutamic acid using response surface methodology. *Food chemistry*, *172*, 497-503.
152. Li, M., Zhu, X., Yang, H., Xie, X., Zhu, Y., Xu, G., & Li, A. **2020**. Treatment of potato starch wastewater by dual natural flocculants of chitosan and poly-glutamic acid. *Journal of Cleaner Production*, *264*, 121641.
153. Santos, D. P., Bergamini, M. F., & Zanoni, M. V. B. **2008**. Voltammetric sensor for amoxicillin determination in human urine using polyglutamic acid/glutaraldehyde film. *Sensors and actuators B: Chemical*, *133*(2), 398-403.
154. Garcia, J. P. D., Hsieh, M. F., Doma Jr, B. T., Peruelo, D. C., Chen, I. H., & Lee, H. M. **2013**. Synthesis of gelatin-γ-polyglutamic acid-based hydrogel for the in vitro controlled release of epigallocatechin gallate (EGCG) from Camellia sinensis. *Polymers*, *6*(1), 39-58.
155. Rethore, G., Mathew, A., Naik, H., & Pandit, A. **2009**. Preparation of chitosan/polyglutamic acid spheres based on the use of polystyrene template as a nonviral gene carrier. *Tissue Engineering Part C: Methods*, *15*(4), 605-613.