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POLY-GLUTAMIC ACID (PGA) - STRUCTURE, SYNTHESIS, GENOMIC ORGANIZATION AND ITS APPLICATION: A REVIEW

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ABSTRACT: Mankind has been exploring many new dimensions and techniques to obtain the most feasible and cost effective biopolymers. Biopolymers are the 21st century polymers and are in great demand as they are very easy to produce through the microbial partners and their conglomeration. Future is all about the emancipation of higher finesse quality products with an iota of eco-friendliness. Biodegradation and low pollutant output has made these polymers from the biological sources, a great eco-friendly substituent for the chemical based polymers. Poly-γ-Glutamic Acid (PGA) is a naturally occurring anionic polymer composed of extensively viscous homo-polyamide of D and L- Glutamic acid units. Its multi-functionality, biodegradability, non-toxicity, compatibility and edibility have made it a promising biopolymer for food, cosmetics and pharmaceutical industries. PGA has several applications in environmental, agricultural and biomedical products as well as it can be used as biodegradable packing material and in other applications including conductive display material, drug delivery, gene vector, dispersant and enzyme-immobilizing material. In this review, we have discussed about the history of PGA, its structure, synthesis, genes and its organization and the applications of PGA.

INTRODUCTION: Last few decades witnessed the development of several biopolymers which are earth friendly biodegradable polymers for several applications in comparison to those polymers which are derived from petroleum products. Some of these biopolymers belong to Poly-amide family. "Poly – amides" are enlarged family of amide linked monomeric chemical compounds, having either homogeneous or heterogeneous substituted molecule, constitutes up an intriguing and naturally degradable biopolymer.

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Categorically, the polyamides are divided into two types: A group of compounds constituting of monomeric and homogeneous poly amino acids, and another group of compounds constituting of heterogeneous protein molecules and substituted derivatives. [1, 2, 13, 25, 51, 54]

The bulk of polyamides are the co-polymeric proteins. A small group of polyamides are referred to as poly amino acids in order to distinguish them from the proteins due to different features of its biosynthesis. [1, 2] There are some major differences between them: (a) Poly-amino acids are composed of monomeric units of amino acids, at least in the backbone as compared to proteins which are composed by hetero – oligomeric units of amino acids, (b) Proteins are biosynthesized from DNA, through transcription and ribosomal mediated

translation mechanism whereas the biosynthesis of poly-amino acids are independent of transcription and translation machinery. Henceforth, the translational inhibitors, such as Chloramphenicol does not affect the biosynthesis of poly amino acids and some specific enzymes regulates and catalyses the biosynthesis of poly amino acid. (c) DNA being the genomic blueprint, determines the length of specific gene sequences which yields specific protein molecules of spatially spaced configured and conformed stable molecules having definite molecular weight whereas poly amino acids shows notable variation in the molecular weight. (d) Amide linkages in proteins are only formed between α-amino and α-carboxylic groups (α-amide linkages), whereas amide bonds in poly amino acids involve other side chain functions (i.e., β -and γ carboxylic and α-amino groups). (e) PGA can be stained with dyes like Methylene blue whereas proteins can be stained with Coomassie blue. [1, 2, 32]

Naturally, three different types of poly-amino acids exist, i.e., Poly-γ-Glutamic acid (γ-PGA), Poly-ε-lysine (ε-PL) and Cyanophycin. ε-PL is composed of lysine monomer and is formed by the linkage between α-carboxyl group and ε- amino group of Lysine. Generally, the length of the ε-PL polymer consists of only 25-30 *L*-lysine monomer units. [2, 3, 4] Cyanophycin, the third poly-amino acid, comprises of α-Aspartic residues containing suspended Arginine residues linked to the β-carboxyl group. [2, 5]

Poly-γ-Glutamic acid (γ-PGA):

PGA is a naturally occurring, infrequent anionic polymeric compound composed of extensively viscous homo-polyamide of D and L- Glutamic acid units [2, 13] and it is an extracellular polymer [2, 6, 7, 11] which is completely biodegradable and non-toxic to humans. [8] PGA is synthesized by several microorganisms; however for commercial proposes *Bacillus* species, (*B. licheniformis* and *B. subtilis*) are generally utilized to produce PGA. [6, 7, 11]

History:

PGA was first discovered in 1973 by Ivanovic and his co-workers in the form of capsule in *Bacillus anthracis,* (a sporulative Gram-positive bacterium and the causative agent of Anthrax) which was released in the medium during autoclaving or ageing or autolysis of cell.[13]

PGA has been reported to be the major component in the viscous sticky mucilage of *Natto* -a health food in Japan. *Natto*, has been in domestic and commercial use and consumed for more than a thousand years, which is produced by steaming small soybeans and fermenting them with starter culture of *Bacillus subtilis* (*natto*). [2,6,9,10,11,12,13,54]

Structure:

Poly-γ-Glutamic acid (PGA) is a homo-polyamide consisting of D- and L- Glutamic acid monomers, which are interlinked by the amide linkages formed between the α-amino and the γ-carboxyl groups and therefore it is resistant to proteases. PGA is an optically active biopolymer having a chiral centre in every glutamate unit (**Fig. 1 & 2**). [14, 8] This anionic polypeptide has an anomalous feature as the glutamate is polymerized via γ-amide linkage and can be readily synthesized by several microorganisms. It was found that three types of PGA are active stereo chemicals: the homopolymer composed of D- Glutamate (D- PGA), the homopolymer of L- Glutamate (L-PGA), and the copolymer composed of D- and L-Glutamate (D-L-PGA) (**Fig. 1 & 2**). [14] γ-(D)-Poly-Glutamic acid, γ-(L)-Poly-Glutamic acid, and γ-(D,L)-Poly-Glutamic acid are collectively called γ-Poly-Glutamic acid (γ-PGA). [11]

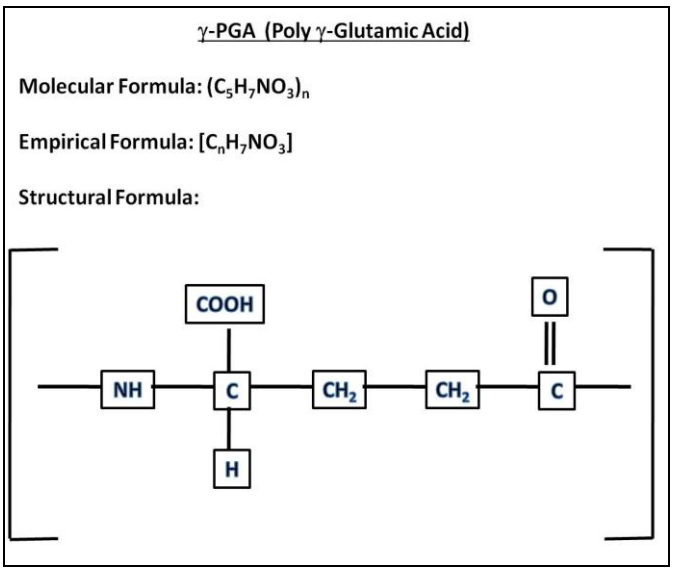

FIG.1: STRUCTURE OF PGA, ITS MOLECULAR FORMULA, EMPIRICAL FORMULA AND STRUCTURAL FORMULA

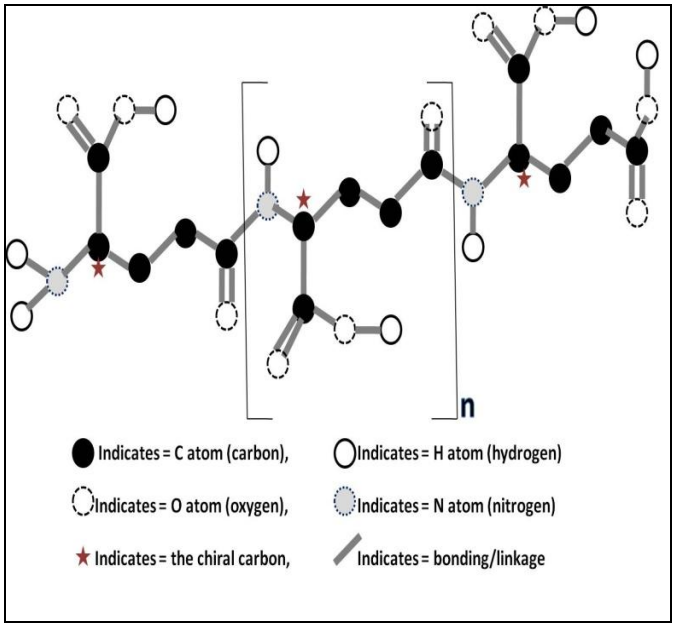

FIG.2: D, L STRUCTURE OF POLY-GLUTAMIC ACID (BALL AND STICK MODEL PRESENTATION).

Physical and Chemical Properties of PGA

γ-PGA is water-soluble and anionic polymer, however its free acid form (H^+) is insoluble in water, whereas the salt forms like K^+, Na^+, NH_4^+, Ca^{2+} and Mg^{2+} of γ-polyglutamates are completely soluble in water. [11, 12] It is completely biodegradable and nontoxic to humans [8] and thus can be used as biological glues. [16, 17] It acts as a natural moisturizer [14] and its hygroscopic and moisturizing effects were comparable with Hyaluronic acid (HA). [18] It has extensive adsorbing properties and therefore used as an absorbent molecules. [14] Cross-linked γ-PGA is one of the most useful Super Absorbent Polymers (SAP). [14]

The molecular weight of PGA ranges from 100 KDa to 2,500 KDa (Kilo Daltons). The ultimate molecular weight of PGA is dependent on many factors, such as an increase in fermentation time will decrease the molecular weight of PGA because of the production of an enzyme that catalyzes the hydrolytic breakdown of PGA. [12, 19]

The other factors like pH, aeration, agitation, ionic strength etc., also affect the molecular weight of PGA which is produced during fermentation. [6, 82] Molecular weight variation of PGA allows various kinds of modifications and thus can have broad range of applications. [18] The reported size of PGA filaments from *B. Subtilis* varies from 160 KDa to 1500 KDa (about 1240–11630 Glutamate residues). [23, 24]

Poly-γ-glutamate may assume quite a few different structures. The structure of PGA has been predicted and proposed by eliciting the peptides of 10 or 20 glutamate molecules. In aqueous solution, the conformational model consists of a left-handed helix which is stabilized by intra-molecular hydrogen bonds has been reported. [20]

Another study of PGA, purified from *Bacillus licheniformis* showed that its conformation is flexible, depending on the PGA concentration and pH of the solution. [21] At low concentration (0.1% w/v) when the pH is below 7.0, PGA adopts a conformation based largely on α-helices, whereas a β-sheet-based conformation predominates at pH>7.0. The β-sheet conformation exposes the negative charges of PGA most efficiently because at higher pH, i.e., at low H^+ concentration, the Glutamic acid tends to give or release charges and it has been proved that at higher pH, PGA possess sheet conformations. [21] It has been recently reported that an unordered conformation occurred in circular dichroism experiments, but the proposed mechanism was not completely elucidated and the test pH was also not validated. [20, 22]

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Organisms producing PGA:

Several microorganisms produce PGA which includes many varied species of bacteria, one archaea and one eukaryotic organism. Some of the organisms which produce PGA are *B. anthracis*, *B. subtilis*, *B. licheniformis*, *B. megaterium*, *Bacillus pumilus*, *Planococcus halophilus*, *Sporosarcina halophile*, *Staphylococcus epidermidis*, *Natrialba aegyptiaca* and Hydra. [2,13,25] It has been observed that only Gram positive bacteria produces PGA; more precisely they are the members of the Order Bacillales, Class: *Bacilli* and hence they are phylogenetically linked. [25]

The most noticeable bacteria producing PGA are *Bacillus subtilis* and *Bacillus licheniformis*. In *Bacillus anthracis* and *Bacillus megaterium*, PGA serves as a structural component and is covalently attached to the peptidoglycan and thus acts as a capsular polymer. [26,183] As mentioned earlier PGA is found in three different forms which are

produced by different types of microorganisms. D-PGA is produced by *Bacillus anthracis* and possibly only bacteria which produces D-PGA [54], whereas L-PGA is produced by extreme halophilic bacteria, i.e., *Natrialba aegyptiaca* and halobacterium *Bacillus megaterium, Bacillus halodurans* and *Natronococcus occultus*. [126,127,128,129] D-L-PGA is produced by several strains of *Bacillus subtilis* and *Bacillus licheniformis* and some strains of *Staphylococcus epidermidis*. [6, 9, 11, 13, 28, 31, 36, 54, 57, 108, 114, 130, 131]

Most of the soil bacteria belonging to the genus *Bacillus*, (excluding *B. anthracis*) use released PGA for the appropriation of toxic metal ions for increasing their resistance to adverse environments. PGA can form chelating compounds with the toxic metal ions and protects the bacteria from the adverse environments. PGA is the glutamate source for bacteria during the late stationary phase of life cycle where usually it starves for nutrition and energy.[27] *Planococcus halophilus, Sporosarcina halophile* and *Natrialba asiatica,* (till date, it is the only reported PGA producing archaea) which uses it to decrease high local salt concentrations, facilitating them to survive in a hostile environment. [27] PGA-producing bacteria are biochemically divided into two groups: Glutamate-dependent and Glutamate-independent producers. In the first case the biosynthesis and the production of PGA increases upon addition of glutamate to the medium, but the bacteria can produce considerable amount of PGA even in the absence of glutamate because of the operation of the de novo pathway of L-Glutamate synthesis. [6]

But for the second case, they do not require L-Glutamic acid for γ-PGA production. The L-Glutamic acid dependent bacteria are *B. anthracis* [28], *B. licheniformis* ATCC 9945A [29] and *B. subtilis* IFO3335 [12] whereas most notably L-Glutamic acid independent bacteria are *B. subtilis* 5E [30], *B. subtilis* TAM-4 [11], *B. licheniformis* A35.[31] *B. subtilis* 5E can produce γ-PGA from L-Proline as a sole carbon and nitrogen source in a minimum mineral medium whereas *B. licheniformis* A35 can produce γ-PGA from Glucose and Ammonium Chloride under denitrifying conditions.

Functions of PGA

There are many organisms which produce PGA for different functions. Some of the functions are:

Poly-γ-Glutamic acid helps in immune evasion and contributes to the virulence of PGA producing Bacteria:

Staphylococcus epidermidis is considered as one of the most common pathogen involved in nosocomial infection over the past few decades [33] which are quite often antibiotic resistant also. [34] It has been discovered recently that this organism evades host immune system by the formation of a special kind of exopoly-saccharide termed as Polysaccharide Intra-cellular Adhesions or PIA. [35] But, PIA is restricted to only a subpopulation of the microorganism. Therefore, there should be a wider mechanism which contributes to the resistance property of these microorganisms.

It has been shown that the cap locus of *S. epidermidis* is responsible for the PGA production which provides resistance to these bacteria against the host immune defence mechanism. Using cap mutant strains, it has been shown that these strains are more vulnerable to anti-bacterial peptides like LL–37 and defensin molecules. They also showed increased rate of phagocytosis by neutrophils in humans and thus PGA lends to the virulence factor of this specific microorganism. [36] Further, it has been found that PGA facilitates the survival of *S. epidermidis* in the host. To test this hypothesis, mice were infected with wild type strains and cap mutant strains of *S. epidermidis* and it was found that the mice which were infected by the wild type strains had large number of bacterial population on implanted catheters than the mice infected with the cap mutant strains. This shows the critical role of PGA in the survival of the bacterial cells on the indwelling medical devices. [36]

In *Bacillus anthracis,* the PGA capsule is considered as the major virulence factor. PGA capsule is very weak immunogen and it has antiphagocytic properties which helps the bacilli to disguise it from the host immune surveillance strategies. [37, 38] Like, other T-cell independent Polysaccharide Antigens (PA), the immunogenicity of PGA are enhanced when it is conjugated with other proteins such as PA. [39] Further, it has been

shown that PGA, as well as exotoxins are involves the regulation of the innate immune response. [40, 41, 42]

PGA helps in biofilm formation in *Bacillus subtilis:*

Biofilms are defined as structured communities of microbial species embedded in a biopolymer matrix on either biotic or abiotic substrata. [42, 43] Biofilms have been found to contain several compounds like proteins, polysaccharides and nucleic acids. [43] Exopolysaccharide (EPS) is an important component of the extracellular matrix in wild type strain of *B. subtilis* 3610. [44] However, PGA is an important factor for the formation of biofilms in some strains of *B. subtilis*. It has been demonstrated that without PGA formation the strain was either unable to produce biofilm or the amount of biofilm formation was very low. [45]

PGA contributes to resistance to high salt concentration in *S. epidermidis:*

It has been shown that the *S. epidermidis* can easily withstand high salt concentration (on natural environment like human skin) of up to 2M NaCl. In high salt concentration *cap* mutant showed minimum growth whereas they grew well in optimum physiological salt concentration compared to wild type strains which showed good growth in both the conditions. Furthermore, quantitative real-time PCR analysis and immuno dot blots demonstrated that *S. epidermidis*, up regulates the cap expression and PGA production in response to the high NaCl concentration which possibly suggest that PGA imparts the ability to *S. epidermidis* to survive on human skin. [36] PGA also helps some halophilic archae bacteria to survive in the extreme halophilic conditions. [46]

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PGA helps the Hydra to catch prey:

Cnidarian group of animals furnished with stinging cells called nematocysts, are the only eukaryotic organisms known to produce PGA. Cnidarians have some special organelles for their predation, which are explosive in nature. These specific organelles helps them in locomotion, capture prey and even uses it for defence during which they produce large amounts of PGA during this explosion. [32] Hydra basically possesses four different types of capsules [47]; the large stenoteles that exhibit prominent spines at the base of their tubules, the atrichous and holotrichous isorhizas that are used for attachment to surfaces and prey organisms, and the small desmonemes, which coil tightly around appendices of the prey after discharge. During the explosion process, an osmotic pressure is required which is present in the nematocysts matrix constituting of higher concentration of Poly-γ-Glutamate (PGA). [48, 32] As the osmotic pressure increases, the capsule gets swollen because after the invagination of the tubule, the biosynthesis of PGA occurs during the late phase of capsule formation. [49]

A hypothesis developed by Holstein and Tardent has successfully demonstrated that the PGA is driving force required for the explosion process in stenotele nematocysts which is a two step mechanism involving different kinetics. [50] During the initial steps, the stylet apparatus of the nematocysts comes out from the capsule within 10 µs (which occurs at a fast rate) and during the second step, the stylets protrudes and the tubule gets reverted by itself within 3 ms (which occurs at a slow rate and it is the rate determining step). The mechanism involved during the process of tubule reversion can be considered due to the fact that the PGA might induce the spread of stylets as it is present at the base within the tubules resulting the second phase of the explosion process. It was observed that PGA was the major component in the tubule lumen in stenoteles rather than at isorhizas and desmonemes. [32]

Biosynthesis of PGA:

The synthesis of γ-PGA is a ribosome independent process which is catalysed by enzymes and it occurs in two steps in bacteria: First step is the synthesis of D and L- Glutamic acid and in the second step, D and L- Glutamic acid units join to form PGA. [51,185] γ-Poly-Glutamic acid is initially biosynthesized inside the cell body and successively released into the fermentation broth. The salvage bioconversion pathway is shown as in the **Fig. 3**. For the production of γ-Poly-Glutamic acid, L- Glutamic acid serves as an inducer and feed stock. In this bioconversion pathway, most of the L- Glutamic acid is first racemized into D-Glutamic acid and then both L- and D- Glutamic acids are co-polymerized into the end product γ-

Poly-Glutamic acid. Both D- and L- Glutamic acid optical enantiomers exists in the γ-PGA, produced by the *B. subtilis* and were reported to be mostly in the ratio of 50-70% of D- Glutamic acid to 50-30% of L- Glutamic acid due to the specific Glutamate Racemase activity in the *B. subtilis* (natto) strains. It was described that the Mn^{2+} ion plays an important role in regulating the ratio of (D: L) in the γ-PGA biosynthesis. [6,52,53,54]

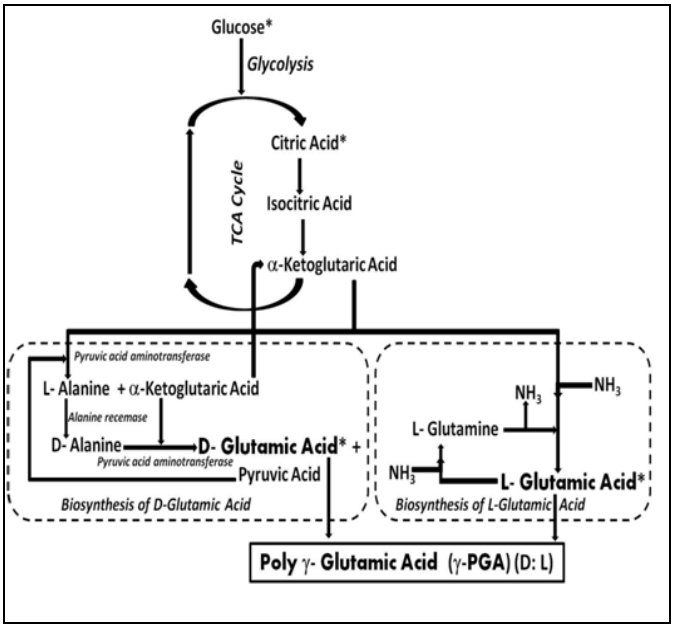

FIG. 3: SCHEMATIC FLOWCHART REPRESENTATION OF SALVAGE PATHWAY FOR PGA SYNTHESIS

Enzymes Involved In the Biosynthesis of PGA:

Many efforts have been done to clarify the enzymes and its roles related to the biosynthesis of γ-PGA in order to upsurge its productivity. The metabolic pathways for the γ-PGA synthesis are known. It has been indicated that the key intermediate of γ-PGA synthesis is 2-Oxoglutarate, which is a direct L-Glutamic acid precursor and is synthesized via glycolysis and TCA (Tricarboxylic acid) cycle.

It is found that in *B. licheniformis,* the two enzymes related to L- Glutamic acid, namely Glutamate synthase (EC1.4.1.13) and Glutamate dehydrogenase (EC 1.4.1.3) are impervious to the end-product concentration, which results in the high intracellular concentration of L- Glutamic acid that facilitates the production of γ- PGA.[7] A membrane bound γ-PGA synthetase system is recognized in *B. anthracis* which is involved in the biosynthesis pathway of γ-PGA. [55] This enzyme system contains at least three enzymes and a sequence-reaction.

Reaction steps are as follows:

L- Glutamic acid + ATP → γ-L- Glutamyl-AMP + Ppi (1)

γ-L- Glutamyl-AMP + SH-enzyme → γ-X-Glutamyl-S-enzyme + AMP (2)

γ-X-Glutamyl-S-enzyme → γ-D- Glutamyl-S-enzyme (3)

γ-D- Glutamyl-S-enzyme + $[\gamma\text{-D- Glutamyl}]_n$ → $[\gamma\text{-}D\text{- Glutamyl}]_{n+1}$ + SH-enzyme (4)

It is shown in the above reaction that the L-Glutamic acid is activated by the phosphorylation in the first step, for which energy must be supplied for the biosynthesis of the amide bond of γ-PGA. The isomerization reactions of L- Glutamic acid in *B. anthracis*, described as reaction (2) or (3), are still not clear, because in *B. subtilis* IFO3335, the results show that L- as well as D- Glutamic acid can be incorporated into the polymer. [1]

The first membrane-associated complex involved in PGA synthesis was described by Troy but the proteins involved were not purified in this study. [56] The membrane fractions of *B. Licheniformis* requires L- Glutamate, ATP and Mg^{+2}. [57] On the basis of the enzymatic steps a hypothetical model was developed to demonstrate the action of the complex. First step involves the activation of L-Glutamate by an ATP molecule. AMP is bounded to Glutamate by a γ-linkage.

The activated Glutamate is then transferred onto a protein that holds a thio-ester (S-protein) in second step. In the third step, isomerization takes place probably facilitating by S-protein. In the fourth step, Glutamate is then transferred from the S-protein to the newly synthesizing PGA filaments. [56] Within two step reaction, Glutamate can directly link with the newly synthesizing PGA filaments through Glutamate activation followed by the transfer and racemisation would then occur during the transfer and this complete process does not requires S- protein. It was reported that the incubation of membranes which carries the membrane anchored complex, forms Glutamyl-γ-hydroxamate with Hydroxylamine. [56,58] However, when the Hydroxylamine was used together with

the *in vitro* synthesized proteins, then it was unable to detect the Hydroxamate production and this concluded that another mechanism was involved in which PGA molecule was activated by a ATP molecule before the transfer of Glutamate. [58]

Genes involved in the biosynthesis of PG:

Bacillus group of bacteria, (like *B. subtilis* 168, *B. subtilis* (natto), *B. licheniformis*, *B. anthracis*) and *S. epidermidis*, possess PGA synthesizing genes. Among the Bacillus groups, a specific loci has been found extensively in *B. anthracis* and *B. subtilis*, a well defined and characterized sequence which encodes the Polyglutamate synthesis complex. [25] The PGA synthesis genes in the *B. anthracis* is carried out by the plasmid pXO2. [59] Four genes, *cap B*, *cap C*, *cap A* and *cap E* (encoding a 47-amino-acid peptide) are necessary for the Polyglutamate synthesis [50] (**Fig. 4**). The situation appears to be similar in the *B. subtilis*, in which three genes (*pgs B*, *pgs C* and *pgs AA*) were initially identified as involved in the Polyglutamate synthesis. [55, 61]

The naming for the genes encoding PGA synthesis is based on the mode of synthesis i.e. whether it is retained or released. [60] Genes responsible for the PGA capsulated bacterial surface are referred as *cap* (for "capsule") and extracellualry released PGA encoding genes are referred as *pgs* (for Polyglutamate synthase) (**Fig. 4**). The operon organization includes all the *cap* genes and the four *pgs* genes (*pgs B*, *pgs C*, *pgs AA*, *pgs E*). [61]

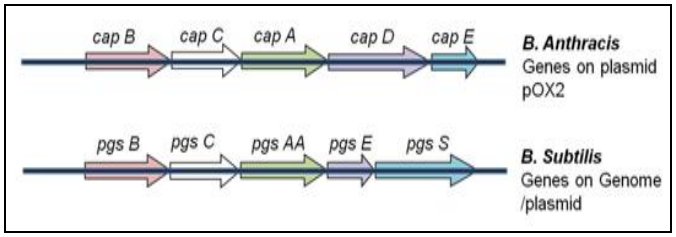

FIG. 4: THE GENETIC ORGANIZATION FOR PGA BIOSYNTHESIS IN *B. ANTHRACIS*, *B. SUBTILIS* AND *B. LICHENIFORMIS*

The genes related to the γ-PGA synthesis, in the *B. anthracis* lies on a large plasmid DNA. [62] In contrast, it was reported that the *B. subtilis* TAM-4 had no plasmid and the gene coding for the formation of γ-PGA lies on the genomic DNA. The genes related to the γ-PGA synthesis in *B. subtilis* (natto) has been designated and was proposed to have been carried out on the plasmids found in these organisms. [53] However, it was found that the plasmids did not encode any gene required for γ-PGA production and possibly present in the genomic DNA of *B. subtilis* (natto). [63] Although, several attempts have been made to isolate Poly-γ-glutamate synthetic system (PGS system) from the cells of the *Bacillus*, but unfortunately very little success has been made because of the extreme instability of this system.

A positive clone of *Escherichia coli* that produced γ-PGA extracellularly was recently screened from the *E. coli* clones consisting of the DNA genomic library of the *B. subtilis* IFO3336 (*B. subtilis* natto). [55] This clone was recognised as its three genes (newly designated as *pgs BCA*) for encoding a PGS system of *B. subtilis* IFO3336. The *pgs BCA* of *B. subtilis* IFO 3336 are highly homologous with the *cap BCA* of *B. anthracis*.

To further determine whether a γ-PGA synthetic system other than the *pgs B, C* and *A* system operate in *B. subtilis*, the *pgs B, C* and *A* gene was disrupted in *B. subtilis* (*chungkookjang*) and the new construct was made and the γ-PGA productivity of the mutant was examined. The results show that all the mutants lost their polymer synthesis ability.

They concluded that *pgs B, C* and *A* are exclusively responsible for γ-PGA biosynthesis. [59] The function of *pgs B, pgs C* and *pgs A* were also studied. The *pgs* A may function as a γ-PGA transporter. It seems likely that this function of *pgs* A is important for the extension of γ- PGA chain and secretion. A structural feature commonly seen in amide ligases is found in *pgs B* and the consensus sequence of the ATP-binding motif lies on the residues 37±42 (GIRGKS) of the protein. The *pgs C* may be essential in γ-PGA synthesis; however, the function of this gene is still unknown. [58]

Regulation of PGA biosynthesis:

Bacteria possess a large and elaborate family of two component signalling system in order to respond to environmental variations. In this two component signalling system, the kinase molecule phosphorylates its own histidine residue when it receives a signal. The phosphoryl group is then

transferred to an aspartate residue of a cognate response regulator, which in most of the cases act as a transcription factor. One of the two component system in *B. subtilis* is Deg S–Deg U system. [64]

When phosphorylated, Deg U (Deg U~P) activates the transcription of the various genes including genes responsible for the PGA synthesis (**Fig. 5).** Phosphorylated Deg U activates the expression of more than 120 genes. [65] The two component system Com A – Com P regulates the synthesis of γ-PGA (Trans). In this signalling process, extracellular and chemically modified peptide Com X triggers Com P autophosphorylation, after which Com P tranfers its phosphoryl group to Com A. [66] Com A acts as a transcription factor and activates transcription of the Deg Q which is a key regulator in the γ-PGA synthesis. [65,67] Deg Q enhances the efficiency of transfer of phosphate group from Deg S to Deg U. [68] Phosphorylated Deg U acts as a transcription factor and activate the transcription of *pgs B* which is an important catalytic component of the synthetase. [69]

Deg U binds to the promoter region of the *pgs* operon which is a 781bp 5' Untranslated region (UTR) and it lies between the *pgs B* and 5' upstream neighbour *rbs R.* [70] A putative Deg U binding consensus sequence of 5'-GWCATTTW-3' was proposed by Tsukuhara and Ogura (Fig. 5). [70] Deg U belongs to the Nar L response regulator family, which is characterised by a classical Helix-Turn-Helix domain that recognizes inverted repeats of the same motifs. [64, 71] Deg U~P has two recognition domains and binds to the differently arranged motif sequences. Out of the two domains, one has been speculated to bind to one cis sequence of the divergent repeat in the *pgs B* promoter. Deg U binds to a divergent repeats of the upstream half of Deg U inverted repeats in the *com K* promoter. [72]

Further, a swarming motility regulator *Swr* A, is also required for the γ-PGA synthesis (Fig. 5). [65,70,73] It was reported recently that the transcription of *Swr* A is partly dependent on Deg U~P. [74] *Swr* A is a small protein without any recognizable structural motif. It has been demonstrated that the effect of *Swr* A and Deg U~P is cooperative rather than additive and the contribution of *Swr* A is effective only in the presence of high levels of Deg U~P. [64, 74] The exact function of *Swr* A is not yet understood but there may be two possibilities of the function of *Swr* A in the γ-PGA production. First, *Swr* A may possess the ability to augment the transcription level of *pgs* operon by augmenting the Deg U~P function at the *pgs B* promoter.

This possibility is supported by the observation that γ-PGA production did not increase linearly with the transcription level of *pgs B*, suggesting that there must be a threshold level of *pgs B* transcription below which there is no PGA production.

Secondly, *Swr* A might have a role in post transcriptional regulation of *pgs B* expression as enhanced transcription of *pgs B* alone did not result in γ-PGA production. [69] The schematic depiction of the roles of Deg U and the two component signalling systems Deg S–Deg U and Com P–Com A in *pgs B* transcription is shown as in the **Fig.5**.

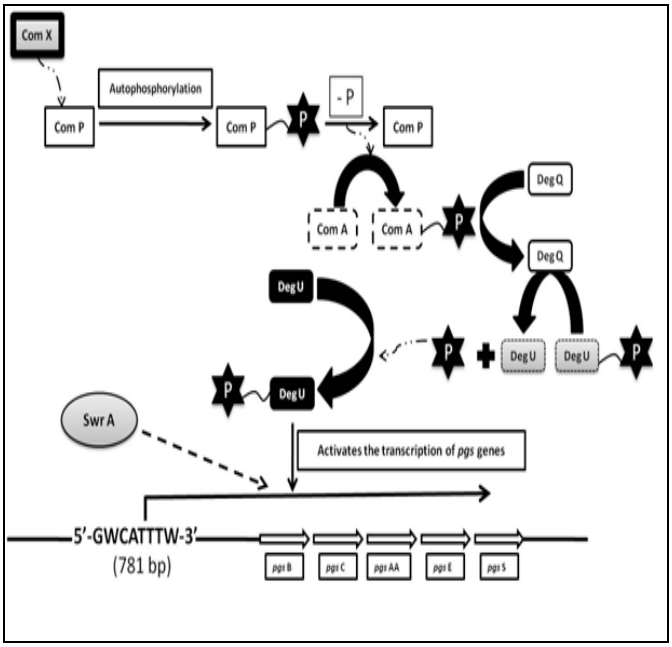

FIG. 5: SCHEMATIC REPRESENTATION AND THE ROLES OF TWO COMPONENT SIGNALING SYSTEM IN *PGS B* TRANSCRIPTION. "P" INDICATES PHOSPHORYL GROUP.

Production of γ-PGA:

Production of PGA can be carried out in two ways i.e., either by solid state fermentation or by submerged fermentation. There are only few strains which are utilized for PGA production in solid state fermentation (SSF) for example *B. subtilis* CCTCC 202048, *B. subtilis* B6-1, *B. subtilis* ME714 and *B.*

licheniformis NCIM 2324**.** Soybean cake powder, soybean meal, wheat bran, dairy manure, swine manure and sweet potato residues alone or in combination have been used for production of PGA by solid substrate fermentation. [51, 106]

Most of the reports for PGA production have been carried out by submerged fermentation. Even though several strategies have been utilized for PGA production by submerged fermentation (SmF) [8, 7, 107] however, the fermentation technology for PGA still remains difficult due to the significant increase in viscosity of the media results in uncontrollable foaming, limitation of the volumetric oxygen mass transfer which leads to insufficient cell growth and a decrease in PGA yield. [108, 184]

Furthermore, a relative high expenditure for SmF media hampers PGA becoming a prevalent commercial valuable product. Solid State Fermentation (SSF) is an important mode of fermentation where microorganisms grow on or within the substrates or supports, in the absence or near-absence of free water and excrete aimed products efficiently. [109, 110] Considering the lower energy requirement, simplicity of cultivation equipment and high product yield, this traditional method has been more prevalent and is more preferred technique by the researchers.

Through this fermentation technique many products like bio fuel, antibiotics, enzymes, alkaloids, organic acids, plant growth hormones, agents of biological detoxification of agriculture based wastes and biopharmaceutical products are being produced industrially. These are only few of the general products mentioned out of numerous ones. [111] SSF is a very promiscuous, stable, energy efficient technique which requires small fermenters and can be carried out in smaller volumes with less polluting effluent output. [111]

On comparing between SSF and Submerged Liquid Fermentation (SLF), SSF technique provides higher yields and has many other numerous advantages than SLF. SSF technique utilizes the fungal growth mostly, (but other microorganisms can also be used) on moisture containing solid materials or substrates without the presence of water. Cereal based substrates (like beans, corns, wheat, soybeans, rice, millets etc.) which were traditionally used are still favoured along with the non-traditional substrates like wastes from agricultural source, forest and food processing units for fermentation through SSF. [112]

Culture Conditions:

Depending on the strains of microorganisms used the media requirements and the culture conditions vary for PGA production. On the basis of the nutrient requirement, the PGA producing bacteria are classified into two groups as Glutamic acid dependant bacteria and Glutamic acid independent bacteria. [1] For the production of γ-Poly-Glutamic acid, L- Glutamic acid serves as an inducer and feed stock which also interact with other medium component. Since it is relatively costly component and affects production of PGA to a large extent it concentration must be optimized.

In most of the cases 20–30 g/L of L- Glutamic acid was utilized for the production of PGA. Also, In addition to Glutamic acid, several other factors such as carbon and nitrogen sources, ionic strength, aeration, agitation and medium pH affected the productivity and quality of PGA. [52,109] Along with glutamic acid in most of the fermentation for PGA production also use citric acid. It was reported that through tricarboxylic acid cycle (TCA), citric acid get converted to isocitrate and α-ketoglutarate which subsequently gets converted to glutamic acid and finally PGA. [6]

The selection of carbon source for PGA production is strain dependent. Glucose and glycerol are reported to support PGA production in most of the strains where glucose act as better carbon source for cell growth and utilized at a faster rate than Glycerol, Citrate, or Glutamate but higher concentration of glucose can lead to production of polysaccharides rather than PGA production. [113] However, by using mixtures of Glucose or other saccharides and Glycerol in medium formulations, the efficiency of γ-PGA production increased. [7] The saccharide includes monosaccharide or disaccharide such as Fructose, Lactose, Glucose, Sucrose, Maltose or Galactose, most preferably Glucose. When Glucose concentration was maintained at 10g/L or lower, 24g/L Poly-Glutamic

acid by *Bacillus sp.* was produced without formation of polysaccharide side-products, which is 1.6 fold higher than 15 g/L of Poly-Glutamic acid concentration obtained using E medium. [113] Quiet often a high concentration of glycerol (80g/l) is used for production of PGA production. It was found that glycerol stimulates PGA production by stimulating polyglutamyl synthetase and decreasing the molecular weight of PGA which leads to decrease in fermentation viscosity and enhancement of substrate uptake, thus, improving cell growth and PGA production. [56, 180]

Yield of PGA with organic nitrogen sources is generally lower as compared to that of inorganic nitrogen sources. It is suggested that free NH^{+4} ions are essential for PGA production. [12, 51] Inorganic salts like $CaCl_2$ and $MnSO_4$ have a significant effect on yield as well as stereo-chemical composition of PGA. [52, 56] It is found that an increase in oxygen supply doubled the dry cell weights of *B. licheniformis* ATCC 9945A. At high aeration, Glycerol, L-Glutamic acid and Citric acid were more rapidly depleted and PGA yields increased to 23 g/L as compared to 6.3 g/L at low oxygen content. [109] It has been proposed that the use of various types of precursors (citric acid, a-ketoglutarate, glutamine, etc.), and agents which can improve cell membrane permeability and by redistribution of metabolic flux for PGA biosynthesis, can lead to increase in production of PGA. [6, 51, 177, 179] Various strains producing PGA with their cultural conditions and yield is given in **Table 1**.

Large Scale Production of γ-PGA:

It becomes necessary to enhance the productivity of PGA in large amounts for industrial and commercial applications. Many researchers have demonstrated strategies for the production of PGA with high productivity. [8] Yoon, *et al.* cultured *B. licheniformis* ATCC 9945 A in 2.5 lt. bioreactor containing 950 mL of E-medium (pH 6.5) at 37°C where citric acid was added at the feeding rate of 0.2 mL/min for 3 hr and the maximum production of PGA was estimated as 35g/L. Further, using 300 lt. bioreactor with 200 lt. of E media, it has been reported that using monosaccharides at low concentration (2-3 g/L) in fed batch culture of *B. licheniformis* ATCC 9945A, the production of PGA reaches 57 g/L in a very short period. [113] Large scale production of PGA has also been reported by Ogawa and his co-workers by culturing *B. subtilis* (natto) strain MR-141 in 30 lt. jar fermenter. [9] They cultivated the mentioned strain in 20 lt. of Maltose-Soy sauce-Glutamate (MSG) medium and used NaCl as an antifoaming agent at pH 8 and the maximum yield was 35 g/L. Another strain *B. subtilis* F-2-01 was also reported by Kubota, *et al.* 1993, to large scale production of PGA. [114] The production of PGA by this strain was found to be 50 g/L when grown in 5×10^3 m^3 medium containing Glucose, Glutamic acid, Urea, Peptone, KH_2PO_4 for 6 days at pH 7.5 and at a temperature of 37°C as shown in **Table 1**.

Immobilization of Cell Strains for Efficient Production:

Immobilization of cells has received a lot of attention in the production of various bio-products. The use of immobilized cells is very common for industrial application in order to reduce the costs of biopolymer production as well as purification. The production of broad range of bio-products e.g. Amylase, CGTase, Prednisolone *etc*. by immobilized *Bacillus* has been reported. [115, 116] For immobilization, cells can be entrapped into various kinds of gels or adsorbed on different solid supports. There are different gel materials which were used for immobilization of *B. licheniformis* such as, Na-alginate, Agar alginate, K-Carrageenan and Agar. [117] It was found that highest PGA production (36.75 g/L) was obtained when aliginate agar gel was used. *B. licheniformis* were also adsorbed on different solid supports such as luffa pulp, sponge cubes, wood pieces, and pumice particles and it was shown that PGA production was highest (50.4 g/L) in luffa pulp as a solid support. [117]

Purification and Characterization of PGA:

Purification of γ-PGA by Goto and Kunioka is the best developed method till date. γ-PGA can be purified by the methanol precipitation method [12] or Copper Sulphate method. [56]

Purification of γ-PGA is straight forward involving three steps:

(a) Removal of cells by centrifugation or filtration

(b) Precipitation of the product from cell-free medium by ethanol, methanol, or 1-propanol

(c) Dialysis of low-molecular weight impurities below a nominal molecular weight limit [12,118]

The purified γ-PGA is usually characterized by amino acid analysis [12], thin layer chromatography (TLC). [76] For product homogeneity proton (H^1) - and Carbon (C^{13}) - NMR are use [118] and GPC is used for molecular weight and polydispersity.

TABLE 1: PGA PRODUCING STRAINS WITH THEIR NUTRIENT CULTURE CONDITION AND PGA YIELD.

Strain	Key nutrient (g)	PGA yield (g/L)	Remarks	Reference
B. licheniformis ATCC 9945A	Glycerol, Glutamic acid, Citric acid, NH_4Cl	15	Glutamic acid and Citrate are important for PGA production	56
	Glucose and Glycerol	29	Glucose is utilized more frequently than Glycerol	182
	*E medium without $MnSO_4$ and $MnSO_4$	14	pH and ionic strength effects PGA production	118
	E medium	23	Citrate plays productive role at pH 6.5	107
	E medium with various concentrations of $MnSO_4$	17	33μM of $MnSO_4$ produce max PGA	107
	*E medium (** LSP- Fed batch)*	53.4	Citrate used at different feeding rates produce max PGA	8
B. licheniformis SAB-26	Glycerol, Citrate, Casein hydrolysates, $(NH)_2SO_4$	53.4	In absence of Glutamate and Glycerol, pulsed citrate yields high production	26
B. licheniformis NCIM 2324	Glycerol, Citric acid, Glutamic acid, NH_4Cl	35.98	Better yield by combined effect of Citrate and Glutamate	177
B. subtilis IFO3335	Glutamic acid, Citric acid, $(NH_4)_2SO_4$	23	Presence of Citric acid and Ammonium sulphate is important for higher yield	181
B. subtilis chungkookjang	***Basal medium	13.5	Glutamate increases the yield	108
B. subtilis TAM-4	Fructose, NH_4Cl	22.1	Fructose is more favorable, D/L ratio of Glutamic acid constituting PGA remains constant	11
B. subtilis CGCMM 0833	Tween 80 , DMSO, glycerol as additive in Basal media	34.3	Tween 80 and DMSO increases productivity	179
B. subtilis NX2	Basal medium and Glycerol	31.7	Glycerol influence the yield as well as molecular weight of PGA	180
	Glucose and Glutamate	42	Glutamate was considered as the main substrate for PGA production	178
B. subtilis (natto) MR-141	Maltose, soy sauce, sodium glutamate	35	Glutamic acid and NaCl both affect the PGA yield	9
B. subtilis ZJU-7	Sucrose, Tryptone, Glutamic acid	54.4	Both sucrose and Tryptone has positive affect	1
B. subtilis F-2-01	Glutamic acid, Peptone, Glucose, KH_2PO_4 (LPS)	50	Production is high under optimum conditions	114
Bacillus sp. SW1-2	Na-citrate, (NH4)2SO4, Glutamic acid and $CaCl_2.2H2O$	36.5	Na-citrate, (NH4)2SO4, Glutamic acid and $CaCl_2.2H_2O$ were most significant variables that enhanced PGA production	121

* E- medium = *L*-Glutamic acid 20 g/L, Citric acid 12 g/L, Glycerol 80 g/L, NH_4Cl 7 g/L, $MgSO_4$ 0.5g/L, $FeCl_3$ 0.04 g/L,$K_2HPO_4$0.5 g/L

**LSP = Large scale production

***Basal medium = 5% Sucrose, 2% $(NH_4)_2SO_4$, 0.27% KH_2PO_4, 0.42% Na_2HPO_4, 0.05% NaCl, 0.5% $MgSO_4$. $7H_2O$, and the MS vitamin solution

Applications of PGA:

Poly-γ-glutamate (PGA) possesses multi-functionalities. It offers a wide range of unique applications and the potential applications of PGA and its derivatives have been of interest in the past few years in a broad range of fields such as food, cosmetics, medicine and water treatment. Some of the applications are:

Biodegradable substitutes:

PGA and its derivatives can be used as thermoplastics, fibres, films and membranes. [13] By calorimetric assay it was demonstrated that a complex namely PGAIC has a potential to form thermoplastics. PGAIC is comprised of L-PGA and HDP^+ (Hexadecylpyridinium cation) where HDP^+ serves as a potent candidate to suppress the extreme hydrophilicity of PGA. The PGAIC complex can be easily processed into a variety of the shapes and sizes via a simple pressurization. [119] Also by using ethanol solution of PGAIC, a stable PGA-based nano-fiber without a covalent cross linking has been successfully synthesized. [120]

Although, PGA is a novel nylon (polyamide plastic), but under ambient humidity it does not act as a thermoplastic. Thus, the usage of PGA as a water insoluble material, such as plastics, fibers and films has been mainly unsuccessful. [119]

Recently, Chung, *et al.* 2013, studied and designed a new protein based biopolymer responsive system, through temperature modification or solvent interaction which changed its structure, geometry and chemistry. They used an electro spun modified γ-PGA and the responsive material was fabricated to create a fibrous mat of sub-micron level. Thus through this sort of controlled modification and alteration, many interesting applications can be possible like it can be used in the dressing of a wound, produce compressed materials and self tightening knots. [175]

Hydrogels with very high water-absorption capability:

Hydrogel is a semi-rigid jelly-like colloid. Most hydrogels contain more than 90% of water by volume. Recently, hydrogels derived from natural polymers have received attention for their environmental and preservation applications. When water exceeds 95% of the total weight (or volume), the hydrogel is called super absorbent. Cross-linked γ-PGA is one of the most useful super absorbent polymers (SAP). Cross-linked γ-PGA can be utilized in various ways. Among them, the most advanced field of γ-PGA utilization is in the water treatment. [13]

Microscopic examination of the PGA hydrogel reveals a multi bag like structure that enables it to absorb moisture 5000 times than its own weight. The amount of water contained within the PGA hydrogel is influenced by pH and salt content. [168] Yang *et al*. 2002, introduced a novel, photo initiated cross linking methodology for the preparation of poly (L-Glutamic acid) (PLG) and Polyethylene glycol) (PEG) with different methacrylate concentrations and found that in this modified type of hydrogels, due to the reduction of cross linking density, it showed higher increase in the degree of swelling.

On further study, it was understood that the degree of swelling was directly pH dependent i.e. on increase in pH, the degree of swelling increases as the amount of ionized acidic side chains gets increased. The rate of release of drug from these modified hydrogels was proportional to the molecular weight of the protein and its cross linking density. Thus this type of modified hydrogel will be an easy choice for drug delivery applications. [169]

Flocculants:

Flocculation can be considered as alternative method for centrifugation and filtration which is used for garnering microbial cells from broths in food and fermentation industries. [52] It is common and operative method for removing suspended solids and metal ions in waste water treatment and wide range of industrial downstream processes. [75] It has been shown recently that Poly-Glutamic acid produced by *Bacillus sp.* PY90 [76] and *B. licheniformis* CCRCC 12826 [54] possess high flocculating activities which shows synergistic effect with cationic concentration and pH of reaction mixture. [54, 76]

Taniguchi *et al.* 2005, prepared cross linked Poly-γ-Glutamic acid (C-L γ-PGA) with γ-PGA irradiated

with γ-rays at various kGy values and found that its flocculation activity was influenced by pH, temperature and the concentration of PGA. Also, the flocculation activity increases following the addition of only trivalent cations (Fe^{3+} and Al^{3+}) whereas monovalent and divalent cations showed no effect. Further, the viscosity of C-Lγ-PGA increased with the increasing dose of γ-irradiation, although the water absorption capacity of C-Lγ-PGA remains unaffected. The flocculating activity of C-L-γ-PGA depends on the water absorption capacity and not on the viscosity because during the experimental study it was found that the flocculating activity and the water absorption capacity parallelly decreased when there was increase in the NaCl concentration. [162]

Later on, the flocculating activity of cross-linked Poly-γ-Glutamic Acid against Bentonite and *E. coli* Suspension pre-treated with $FeCl_3$ and its interaction with Fe^{3+} was studied. [163] The results showed that in the above two suspensions pre-treated with $FeCl_3$, small visible floats appeared in the early stage of incubation due to the direct interaction between $FeCl_3$ and C-L γ-PGA, indicating the formation of a water-insoluble complex. It was suggested that this complex was formed due to the interaction between Fe^{3+} in $FeCl_3$ and COO^- in the C-Lγ-PGA molecule, but also Fe_2O_3 and $Fe(OH)_3$ might be entrapped in this complex and hence it can be applied to scavenge metal ions including Fe^{3+} from polluted water. This effect of cross-linked PGA occasionally required pre-treatment with Poly-Aluminium Chloride (PAC). The mode of action of cross-linked PGA was thought to be based on electrostatic interaction between flocculent and PAC, and the surface of polluted water components. [163]

Recently, the oleaginous microalgae was studied which was harvested by flocculation with a commercially available microbial flocculant poly (γ-Glutamic acid) (γ-PGA). [164] They optimized conditions for flocculation of marine *Chlorella vulgaris* and freshwater *Chlorella protothecoides* by Response Surface Methodology (RSM). By applying these optimized condition they noticed more than 90% flocculation efficiency of γ-PGA and a concentration factor greater than 20 in *Nannochloropsis oculata* LICME 002, *Phaeodactylum tricornutum*, *C. vulgaris* LICME 001, and *Botryococcus braunii* LICME 003. Micrographs of the harvested micro-algal cells showed no damage to cell integrity as well as lipid loss during the process. Thus these results showed that flocculation with γ-PGA is feasible for harvesting microalgae for biodiesel production. [164]

Heavy metal and radionuclide binding agents:

The anionic γ-PGA of *B. licheniformis* has found to have affinity for binding to a variety of metals ions including Ni^{+2}, Cu^{+2}, Mn^{+2}, Al^{+3}, and Cr^{+3}. [77] It has also been reported that a wide variety of inexpensive functionalized micro-filtration membranes containing covalently attached PGA, have extremely high capacities for binding to heavy metals. [78] The high affinities of PGA for binding to heavy metals and metal ions can be utilized for bio-availability of cations like Ca^{2+}, Fe^{2+}, Fe^{3+}, Zn^{2+} and Mn^{2+} to plant rhizospheres and thus can be used as fertilizers. Also, PGA can be used as bio-flocculants for wastewater treatment and water purification. [13, 54, 76, 122, 123]

It was studied and evaluated that γ-PGA obtained from *Bacillus licheniformis* ATCC 9945 acts as a potential biosorbent material for use in the removal of heavy metals from aqueous solution. They selected copper (Cu^{2+}) as the model heavy metal since it is extensively used by electroplating and other industries. Their results showed that the PGA possessed a very good copper adsorption capacity approaching 77.9 mg/g and a binding constant of 32 mg/l at pH 4.0 and 25ºC. [165] Based on complexation of PGA with bivalent lead ion (Pb^{2+}) Bodnar *et al*, prepared novel biodegradable particles. They indicated the strong complexation capacity of PGA for lead ions indicated a promising sorbent for removal of heavy metals in polluted water. [166]

Inbaraj and Chen, (2012) demonstrated that the poly (γ-Glutamic acid)-coated super paramagnetic nanoparticles played an important role in the discharge of heavy metals from the simulated gastrointestinal fluid and metal solutions. In this study, through co-precipitation method, edible biopolymer PGA was used to modify Super Paramagnetic Iron Oxide Nanoparticles (SPIONs).

They showed that upon coating with PGA, the zeta potentials (potential difference between the dispersion medium and the stationary layer of fluid attached to the dispersed particle) got shifted from positive value to a negative value during the environmental pH of 3.0-8.0 and at biological pH of 1.0-8.0 which means that it shows good dispersion in the aqueous suspensions and this led to complimentary conditions for heavy metal removal (147.71 mg/g for lead and 23.15 mg/g for cadmium). Also the rate of discharge of lead and cadmium was faster when detected through batch studies as it followed a pseudo second order rate and its kinetic rates were 0.212 and 0.424 g/mg.min, respectively. For the clinical treatment of metal intoxication, cadmium in contrast to other metals did not had any impact on PGA-SPIONs bound lead, which is therapeutically considered as beneficial for the selective removal of lead from the biological matrix. [167]

Cryoprotectant:

To protect frozen food from deterioration or to stabilize cryo-preserved biomaterials, the addition of cryoprotectants has been widely used. It was shown that γ-PGA sodium salt produced by *B. licheniformis* CCRC 12826 has substantial antifreeze activity. Although γ-PGA sodium salt showed lower antifreeze activity than that of Glutamate (Na salt) and Glucose, it is still probably an effective cryoprotectant for frozen food because it carries a weaker taste than the commonly used lower molecular weight cryoprotectant and thus can be added to foods in larger quantities without a serious change in the taste. [79, 80, 124,125]

Drug delivery:

There are several reports which suggest that PGA can be also used as a drug carrier.[186, 187, 188, 189, 190] PGA paclitaxel (PG-TXL), prepared by covalent bonding of native Paclitaxel to a Glutamic acid polymer, is a water soluble form of Bristol-Myers Squibb's well known cancer drug Taxol. Preclinical data suggested that the uptake of PG-TXL by tumour cells was ~5 fold greater than that of Paclitaxel when equivalent doses were used. Once in the cells, the polymer is digested, delivering a higher and more potent dose of Paclitaxel directly to the tumour. [81, 82] Other PGA drug conjugates have been synthesised and tested for anti-cancerous activities such as Doxorubicin (DOX). DOX was conjugated with PG (Poly-Glutamic acid) either directly or through oligopeptide spacers via amide bonds. [152] It was shown that the conjugates were less cytotoxic than free drug *in vitro* in L1210 leukemia and B16 melanoma cells. [153, 154] PG-oligopeptides-DOX *in vivo* were active and showed increased anti-cancerous activity with increasing oligopeptide length (when the molecular weight increased from 14,000 to 60,000 Da at an equivalent DOX dose of 30 mg/kg) and its degradab ility. [154,155]

Also some DNA binding drugs such as L-phenylananine mustard (Melphalan) [156] and Mitomycin C (MMC) [157] have been conjugated by amide linkages to PGA. These conjugates also show less cytotoxicity as in case of Mitomycin C and were significantly more efficacious than the free drug as in case of Melphalan. [156,157] Anti-metabolite conjugates such as 1-β-D-Arabinofuranosylcytosine (Ara-C) linked with PGA via amide bonds showed markedly decreased cytotoxicity against Murine leukemia L1210 cells when compared with that of free Ara-C. However, they exhibited antitumor activity that was greater than, or equal to, that of free Ara-C in mice bearing L1210 tumor inoculated intra peritoneally. [158]

The characterization and the synthesis of branched poly-L-Glutamic acid were described by Tansey, *et al.*, 2004, in which it was found that it contains multiple PG chains centred on a poly (amido amine) (PAMAM) dendrimer or Polyethyleneimine (PEI) cores. Branched PG possessed some features that would make it a favourable carrier for targeted drug delivery. These polymers are water-soluble, biodegradable and have relatively narrow molecular distribution.

They hold multiple terminal amines that can be used for attachment of homing ligands. Moreover, they contain many functional groups on the side chains of PG arms different from those of the terminal groups, allowing simultaneous attachment of homing moieties and diagnostic or therapeutic agents. During this experiment it was found that through the initiator molecule like PEI or PAMAM along with the ring-opening polymerization of benzyl ester of L-Glutamic acid N-carboxy-

anhydride, branched PG polymers were obtained. These branched PG polymers reduced undesirable nonspecific interaction of diagnostic and therapeutic agents with non target cells and that the binding affinity of the homing moiety was preserved when the targeting moieties were attached to the termini of the polymers. [159]

Recently PGA has been used as oral drug delivery. Sonaje, *et al*. 2010, prepared Enteric-coated capsules filled with pH sensitive freeze-dried chitosan/poly γ-Glutamic acid nanoparticles for oral insulin delivery. It has been found that these nanoparticles without chitosan/ poly γ-Glutamic acid are retained in stomach for longer time which leads to degradation of these nanoparticles as well as insulin. However coating of these nanoparticles with Chitosan/poly γ-Glutamic acid decrease the pH sensitivity of NPs but also disrupt the internal structure of NPs.

These freeze dry nanoparticles were more efficient in reaching proximal segment of small intestine and the release and absorption of insulin in this part of intestine were higher. [160] Further, when Diethylene triamine pentaacetic acid (DTPA) were conjugated with γPGA to form γPGA-DTPA conjugate, these conjugates inhibited the intestinal proteases substantially compared to DTPA alone. Also, it was observed that the paracellular permeability of small intestinal cells were enhanced and it is transient and reversible. These results clearly indicate that conjugation of insulin nanoparticles with γ-PGA can promote the insulin absorption throughout the entire small intestine which can be even detected in kidney and bladder and it also protects these molecules from the acidic environment of stomach. [161]

Applications in Food industry:

In food industry γ-PGA has an inordinate diversity of applications. γ-PGA can be used as a bitterness relieving agent and for prevention of aging and improvement of textures of starch-based bakery products, noodles and stabilizer for ice cream. [83] Low concentrations of γ-PGA were testified to mend the taste and drinkability of juices and other drinks. [52] It also accelerates the absorption of minerals from the small intestine when added to the high mineral food preparations and can be used as animal feed additives. [84] It has been found that proper quantity of γ-PGA as food supplements increases the solubility of Ca^{2+} both *in vitro* and *in vivo* and thus better absorption. [132] Moreover, the significant uptake of physiologically active substances, such as vitamins, polyphenols, or carotenoids was found in the small intestine, when γ-PGA was added to the food products containing these substances. [79] Many salts of γ-PGA such as Ca^{+2}, Mg^{+2}, Na^{+} etc, were found to have antifreeze properties.

This antifreeze property can be utilized in the process of preserving foods, microorganisms and enzymes. Lower molecular weight PGA have better antifreeze activity and this activity was also dependent upon the cation used ($Mg^{+2} > Ca^{+2} \sim Na^{+} > K^{+}$) but was indifferent to the D/L ratio of Glutamic acid monomers. [140]

PGA in Agriculture Industry:

Wang, *et al.* 2008, proved that lipopeptides and PGA produced by *B. subtilis* B6-1 with soybean and sweet potato residues as substrate, effectively suppressed cucumber wilts and significantly increased the dry weight of both roots and shoots. [85]

The overuse of chemical fertilizers and wrong agricultural practices lead to numerous environmental problems. Thus in order to decline the environmental problems due to high concentration of ammonium in the manure bring in from animal breeding, many developing countries are using different alternatives. One of the desired processes is the conversion of manure to slow release fertilizer carried out in solid state fermentation. Recently, it was shown that *B. licheniformis* was able to grow in liquid swine manure in the presence of sodium gluconate or citrate and glycerol. [142, 141]

Their experiments indicated that the ammonia content was severely reduced and resulted in production of 0.16–0.85 g/l γ-PGA. [142, 141] The similar kind of work was done by Chen, *et al.* 2005 where they have shown the average PGA yield of 6% and which would lay a foundation for lessening the pollution of swine manure, increasing fertilizer efficiency and exploring a late-model organic

fertilizer that retains water and nutrients. [141] Further, it was demonstrated that the use of γ-PGA can lead to increase in winter wheat, number of tillers, seed number per spike, yield, Soil Microbial Biomass Nitrogen (SMBN), and soil enzymes after γ-PGA application. [144] The highest grain yield of 7435.69 ± 55.91 kg/Ha was obtained after γ-PGA application in the field experiment, which was 7.17% higher than the urea control. These results demonstrate that γ-PGA can also be used as a fertilizer synergist. [144] King, *et al.* 2007, developed a fertilizer composition comprising a water-insoluble slow-release reacted nitrogen fertilizer and an effective amount of PGA. [86]

PGA - a Bioclogging and Biocementation agent of soil:

Microbial Geotechnology is an advanced geotechnical engineering which uses the microbial polymers and exoenzymes to engineer the geological materials. [87] Bioclogging is the production of pore-filling materials through microbial means so that the porosity and hydraulic conductivity of soil can be reduced and also can be applied to reduce the erosion of the drain channels whereas in biocementation process the particle-binding material through microbial processes *in situ* promotes the shear strength of soil. [87] It can be used to prevent soil avalanching, mitigate the liquefaction potential of sand, and compact soil on reclaimed land sites. [88]

During chemical grouting the soil void spaces are filled with fluid grouts which are used to control water flow [89,90] and some of the commonly use chemical grouts are, solutions or suspensions of Sodium silicate, Acrylates, Acrylamides and Polyurethanes. The Xantan, Chitosan, Poly-Glutamic acid (PGA), Sodium alginate, and Poly-Hydroxy butyrate (PHB) are industrially produced water-insoluble gel-forming biopolymers of microbial origin which are used as grouts for enclosing of bioremediation zone, soil erosion control and mitigating soil liquefaction. [87, 89, 90, 91] The bacterial biomass accumulation forms huge amount of insoluble bacterial slime which acts as poorly soluble biogenic gas bubbles in soil that makes the soil more impermeable for water [91] and it enhances oil recovery or soil bioremediation.[87, 88, 93, 94, 95, 96, 97]

Gene Vectors:

It was shown by Dekie and his co-workers, that biodegradable derivatives of poly L-Glutamic acid are suitable vectors for gene therapy. They mixed DNA with new polymers (including γ-PGA derivatives) and showed that DNA and polymers self assemble and form poly electrolyte complexes which are fairly stable towards serum albumin. PGA derivatives reduce the surface charge and size of the DNA by forming such polyelectrolyte complexes with DNA. Such complexes are able to protect DNA from digestion by DNase I and also facilitate the uptake of DNA by cells. Preliminary transfection data showed that these complexes are less toxic and able to transfect spontaneously 293 cells, thus can be used for gene delivery. [103]

Some experiments were also conducted in which negatively charged poly (γ-Glutamic acid) was incorporated into CS/DNA complexes in order to modify their internal structures. [151] Chitosan (CS) is a biodegradable, non-toxic and tissue compatible cationic polysaccharide and it has a potential to condense DNA into compact structure and has been considered as a non-viral vector for gene delivery. [146, 147, 148] Although advantageous for DNA packing and protection, yet CS complexes leads to problems in DNA release once arriving at the site of action intracellularly, thus limiting their transfection efficiency.

However, after incorporating γ-PGA, analysis of the internal structure of CS/DNA/γ-PGA complexes by Small Angle X-ray Scattering (SAXS) unveiled that CS formed complexes with DNA and γ-PGA separately and produced two types of domains, leading to the formation of compounded nanoparticles. Thus improving the dissociation capacity of CS and DNA and enhancing the efficacy of gene expression by disintegrating into a number of even smaller sub-particles after cellular internalization and it also markedly increased their cellular uptake; whereas the endetailed mechanism is yet to be deciphered. [150]

Further, it was found that the γ-Glutamyl transpeptidase which is present in the cell membrane recognizes these complexes through its free γ-glutamic acid at the N-terminal region which

promotes its internalization in the cell via macro pinocytosis or caveolae mediated pathways but with lesser entry into lysosomal pathways. [151]

Vaccine Adjuvant:

Several studies suggest that PGA can be used as vaccine adjuvant. [104, 105, 143,144] In one of the study PGA used as adjuvant for recombinant Transmissible Gastro Enteritis virus nucleocapsid protein (TGEN) which was injected into rabbits. The group of rabbits that received PGA with TGEN produced significantly more antibodies than the control group and it was dependent on molecular size of PGA. Immunogenic compositions containing PGA was invented for eliciting an immune response against *S. epidermidis* and related staphylococci. PGA showed high antibody titre even when it was used with an antigen having poor immunogenicity. [104]

It was also shown that the antibody titre was enhanced by a single dose of GE vaccine with PGA-NPs (PGA-Nanoparticles) and all the immunized mice survived a lethal GEV infection, while only 50% of the mice that received a single dose of JE vaccine without PGA-NPs could survive. [105]

Uto, *et al.* 2007, studied the immune response in mice due to the effect of (antigen) Ag-carrying biodegradable γ-PGA nanoparticles (NPs) targeting dendritic cells (DCs). The DCs up took NPs efficiently, in the lysosomal compartments where they were localized. Also, γ-PGA NPs strongly induced cytokine production, up-regulated co-stimulatory molecules and enhanced T cell stimulatory capacity in DCs which indicated that the Ag-carrying γ-PGA NPs are strong inducers capable of inducing CMI and humoral immune response and has the potential of being tried as adjuvants in vaccine development for therapeutic treatment of infectious diseases. [144]

It was found that the nanoparticles prepared with EphA2-derived peptide vaccine with amphiphilic γ-PGA generates more EphA2- specific type-I CD8+ T cells compared to EphA2-derived peptide and complete Freund's Adjuvant (Eph+ CFA) and thus provides higher degree of anti MC38 liver tumor protection. Further, nanoparticles of EphA2-derived peptide vaccine with amphiphilic γ-PGA induces much lower liver damage compared to Eph+ CFA as demonstrated through the reduction in the level of serum alanine aminotransferase. Thus, it was proposed that NPs prepared with EphA2 and γ-PGA can be used as vaccines for the treatment of liver cancer. [143]

It was also found that the biodegradable nanoparticles (NPs) expounded with γ-PGA induces both adaptive and innate immune responses through signalling pathways involving MyD88 molecules and Toll-like receptor 4 (TLR4) which proves the NPs as potential antigen carriers which can not only act as potent adjuvant but also can elicit adaptive immune response against antigens through first-line host sensor mechanism. [145]

Contrast Agent:

In clinical medicine Non-invasive Imaging techniques are gaining importance. Some of these require the administration of contrast agents; for example, paramagnetic ions such as gadolinium. With the help of blood-pool contrast agents, imaging using Computed Tomography (CT), Ultrasonography, Gamma Scintigraphy and Magnetic Resonance Imaging (MRI) can provide minimally invasive angiography, assess angiogenesis, quantify the number and spacing of blood vessels, and measure blood volume and blood flow. The paramagnetic Gadolinium chelates of high molecular weight polymers are used as MRI blood pool agents as they gets easily retained within the intravascular space during MRI tests. [170, 171, 176]

It was found that when a conjugate PGA-Cystamine-Gd-DOTA was prepared by the conjugation of DOTA (Gadoterate meglumine) to PGA via cystamine, enhanced the contrast of MRI blood pool in nude mice bearing OVCAR-3 human ovarian carcinoma xenographs. [172]

In one of the study it was observed that PG chelates of Gadolinium (PG-Bz-DTPA-Gd) which was synthesized directly from PG and mono-functional *p*-aminobenzyl-DTPA (acetic acid-*tert*-butyl ester), can be easily degraded and cleared from the body and thus can be used as contrast agent. [174]

PGA complex also reported to be superior to many polymers previously used in MRI contrast agents. For example, unlike ε-PL, which has multiple positive charges, PGA has multiple negative charges which are less biologically disruptive and can increase safety, relaxivity, blood circulation time along with other desirable qualities. [173]

CONCLUSION: Biopolymers have been a huge boon for mankind and an immediate answer for the polymer industry which focuses on environmental friendly applications and green chemistry. Biodegradation and low pollutant output has made these polymers from the biological sources, a great eco-friendly substituent for the chemical based polymers. Microbial based polymers are not only easy for production and maintenance but the most important of all, they are cost effective. The Poly (γ-Glutamic acid) or Gamma Poly-Glutamic acid (γ-PGA) or Gamma Poly-glutamate, is an exciting biopolymer which has been recently in limelight as the compound and its derivatives have a broad spectrum applications ranging from drug delivery to heavy metal absorbers.

The high water retaining capacity of γ-PGA is enhanced by UV cross linking. Coupled with its non-toxicity, this makes γ-PGA very useful in environmental applications. The various applications are totally based on the molecular weight and also on the viscosity. The solubility and easy absorptivity enables it in various use in the field of agriculture. PGA is the world's current obsession as a wonder amino polymer with lots of potential. The polymer industry is the new age industry with more sophistication and easy handling.

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REFERENCES:

1. Shi F, Zhi-Nan X and Pei-Lin C: "Microbial production of natural polyamino acid," Sci China Ser B-Chem. 2007, 50(3): 291-303.
2. Oppermann-Sanio FB and Steinbuchel A: "Occurrence, functions and biosynthesis of polyamides in microorganisms and biotechnological production," Naturwissenschaften 2002, 89: 11–22.
3. Shima S and Sakai H: "Polylysine produced by Streptomyces," Agric Biol Chem. 1977, 41(9): 1907-1909.
4. Shima S and Sakai H: "Poly-L-lysine produced by Streptomyces: Part-III- Chemical studies," Agric Biol Chem. 1981, 45(11): 2503-2508.
5. Simon RD and Weathers PJ: "Determination of the structure of the novel polypeptide containing aspartic acid and arginine which is found in cyanobacteria," Biochim Biophys Acta.1976, 420(1): 165-176.
6. Kunioka M and Goto A: "Biosynthesis of poly (γ-glutamic acid) from L-glutamic acid, citric acid, and ammonium sulfate in Bacillus subtilis IFO3335," Appl. Microbiol. Biotechnol. 1994, 40: 867-872.
7. Young HKO and Gross RA: "Effects of glucose and glycerol on γ - Poly (glutamic acid) formation by Bacillus licheniformis ATCC 9945a," Biotechnol. Bioeng. 1997, 57: 430-437.
8. Yoon SH, Yoon JH, Do SY and Lee Chang HN: "Production of poly-γ-glutamic acid by fed-batch culture of *Bacillus licheniformis,"* Biotechnol. Lett. 2000, 22: 585-588.
9. Ogawa Y, Yamaguchi F, Yuasa K and Tahara Y: "Efficient production of γ-polyglutamic acid by Bacillus subtilis," Biosci. Biotech. Biochem. 1997, 61(10): 16-84.
10. Guan HH, Tong IH, Kuo HH, Yuan CS, Pi YL, Jeng Y, Kun HY and Shih CY: "Polyglutamic Acid Produced by Bacillus subtilis (natto): Structural characteristics, chemical properties and biological functionalities," Journal of the Chinese Chemical Society. 2006, 53(6): 1363-1384.
11. Ito Y, Tanaka T, Ohmachi T and Asada Y: "Glutamic acid independent production of poly (γ-glutamic acid) by Bacillus subtilis TAM-4," Biosci Biotech Biochem. 1996, 60: 1239-1242.
12. Goto A and Kunioka M: "Biosynthesis and hydrolysis of poly (γ-glutamic acid) from Bacillus subtilis IFO3335," Biosci Biotechnol Biochem. 1992, 56(77): 1031-1035.
13. Shih IL and Van YT: "The production of poly (gamma-glutamic acid) from microorganisms and its various applications," Bioresour Technol. 2001, 79: 207-225.
14. Moon HS, Chung P, Chul JK, Haryoung P, Kenji S and Makoto A: "Natural and Edible Biopolymer Poly-γ-glutamic Acid: Synthesis, production, and applications," The Chemical Record. 2005, 5: 352-366.
15. Makoto A, Hisaaki N, Takashi Y, Tohru K, Kenji S, Chung P, Moon-Hee S, Toshiharu Y and Haruo M: "Poly-γ-glutamate depolymerase of *Bacillus subtilis*: production, simple purification and substrate selectivity," Journal of Molecular Catalysis B: Enzymatic. 2003, 23: 249-255.
16. Iwata H, Matsuda S, Mitsuhashi K, Kenji I, Itoh E and Ikada Y: "A novel surgical glue composed of gelatin and N-hydroxysuccinimide activated poly (L-glutamic acid)," Biomaterials. 1998, 19: 1869-1876.
17. Otani Y, Tabata Y and Ikada Y: "Sealing effect of rapidly curable gelatin- poly (L-glutamic acid) hydrogel glue on lung air leak." Ann. Thorac. Surg. 1999, 67: 922-926.
18. Bae SR, Park C, Choi JC, Poo H, Kim JC and Sung MH: "Effects of ultra-high molecular weight PGA from *Bacillus subtilis* (chungkookjang) on corneal wound healing," J. Microbiol Biotechnol. 2010, 20: 803-808.
19. King EC, Blacker AJ and Bugg TDH: "Enzymatic breakdown of poly-γ-D glutamic acid in Bacillus licheniformis: Identification of a polyglutamyl γ-hydrolase enzyme," Biomacromolecules 2000, 1: 75-83.
20. Zanuy D and Aleman C: "Poly (gamma-glutamic acid) in aqueous solution: molecular dynamics simulations of 10- and 20- residue chains at different temperatures," Biomacromolecules 2001, 2: 651-657.

21. He LM, Neu MP and Vanderberg LA: "*Bacillus licheniformis* γ-glutamyl exopolymer: physicochemical characterization and UV interaction," Environ Sci Technol. 2000, 34: 1694-1701.
22. Joyce JG, Cook J, Chabot D, Hepler R, Shoop W, Xu Q, Stambaugh T, Aste-Amezaga M, Wang S, Indrawati L and Bruner M: "Immunogenicity and protective efficacy of *Bacillus anthracis* poly-gammaD-glutamic acid capsule covalently coupled to a protein carrier using a novel triazine-based conjugation strategy," J Biol Chem. 2006, 281: 4831-4843.
23. Shi F, Xu ZN and Cen PL: "Efficient production of poly-γ -glutamic acid by *Bacillus subtilis* ZJU-7," Appl Biochem Biotechnol 2006, 133(3): 271-282.
24. Suzuki T and Tahara Y: "Characterization of the *Bacillus subtilis* ywt D gene, whose product is involved in γ-polyglutamic acid degradation," J Bacteriol. 2003, 185: 2379-2382.
25. Thomas C and Agnes F: "Poly-gamma-glutamate in bacteria," Molecular Microbiology 2006, 60(5): 1091-1098.
26. Soliman NA, Berekaa MM and Abdel-Fattah YR: "Poly glutamic acid (PGA) production by *Bacillus* sp. SAB-26: application of Plackett–Burman experimental design to evaluate culture requirements," Appl. Microbiol. Biotechnol. 2005, 69: 259-267.
27. Hezayen FF, Rehm BHA, Eberhardt R, and Steinbuchel A: "Polymer production by two newly isolated extremely halophilic Archaea: application of a novel corrosion-resistant bioreactor," Appl Microbiol Biotechnol. 2000, 54: 319-325.
28. Thorne CB, Gomez CG, Noyes HE, and Housewright RD: "Production of glutamyl polypeptide by *Bacillus subtilis*," J. Bacteriol. 1954, 68: 307-315.
29. Thorne CB, Gómez CG, Blind GR, and Housewright RD: "Synthesis of glutamic acid and glutamyl polypeptide production by *Bacillus anthracis* III, factors affecting peptide production in synthetic liquid media," J. Bacteriol. 1953, 65: 472–478.
30. Murao S: "On the polyglutamic acid fermentation," Koubunshi. 1969, 16: 1204-1212.
31. Cheng C, Asada Y and Aaida T: "Production of γ- PGA by *Bacillus subtilis* A35 under denitrifying conditions," Agri. Biol.Chem. 1989, 53: 2369-2375.
32. Weber J: "Poly (gamma)-glutamic acids are the major constituents of nematocysts in Hydra (Hydrozoa, Cnidaria)," J Biol Chem 1990, 265: 9664-9669.
33. Vuong C and Otto M: "*Staphylococcus epidermidis* infections," Microbes Infect. 2002, 4: 481-489.
34. Gotz F: "*Staphylococcus* and biofilms," Mol. Microbiol. 2002, 43: 1367-1378.
35. Vuong C: "Polysaccharide intercellular adhesion (PIA) protects *Staphylococcus epidermidis* against major components of the human innate immune system," Cell. Microbiol. 2004, 6: 269-275.
36. Kocianova S, Vuong C, Yao Y, Voyich JM and Fischer ER: "Key role of poly-γ-glutamic acid in immune evasion and virulence of *Staphylococcus epidermidis*," J. Clin. Invest. 2005, 115: 688-694.
37. Ezzell JW and Welkos SL "The capsule of *Bacillus anthracis*," J. Appl. Microbiol. 1999, 87: 250.
38. Leppla SH, Robbins JB, Schneerson R and Shiloach J: "Development of an improved vaccine for anthrax," J. Clin. Investig. 2002, 110: 141-144.
39. Chabot DJ, Scorpio A, Tobery SA, Little SF, Norris SL and Friedlander AM: 2004. "Anthrax capsule vaccine protects against experimental infection," Vaccine 2004, 23: 43-47.
40. Kim TW, Lee TW, Bae HC, Hahm HJ, Kim HY, Park C, Kang HT, Sung HM and Poo H: "Oral administration of high molecular mass poly-γ-glutamate induces NK cell-mediated antitumor immunity," J. Immunol. 2007, 25: 231-250.
41. O'Toole G, Kaplan HB and Kolter R: "Biofilm formation as microbial development," Annu. Rev. Microbiol. 2000, 54: 49-79.
42. Parsek MR and Greenberg EP: "Sociomicrobiology: the connections between quorum sensing and biofilms," Trends. Microbiol. 2005, 13: 27-33.
43. Branda SS, Gonzalez-Pastor JE, Ben-Yehuda S, Losick R and Kolter R: "Fruiting body formation by *Bacillus subtilis*," Proc Natl Acad Sci. 2001, 98: 11621-11626.
44. Branda SS, Vik SL, Friedman L and Kolter R: "Biofilms: the matrix revisited," Trends Microbiol. 2005, 13: 20-26.
45. Morikawa M, Ito M and Imanaka T: "Isolation of a new surfactin producer *Bacillus pumilus* A-1 and cloning and nucleotide sequence of the regulator gene, psf-1," J. Ferment. Bioeng. 1992, 74: 255-261.
46. Anton J, Meseguer I and Rodriguez-Valera F: "Production of an extracellular polysaccharide by *Haloferax mediterranei*," Appl Environ Microbiol. 1988, 54: 2381-2386.
47. Holstein T: "The morphogenesis of nematocysts in Hydra and Forskalia: an ultrastructural study," J. Ultrastruct. Res. 1981, 75: 276-290.
48. Weber J. "Nematocysts (stinging capsules of Cnidaria) as Donnan potential-dominated osmotic systems," Eur. J. Biochem. 1989, 184: 465-476.
49. Susanne S, Cikala M and David CN: "PG synthesis during formation of nematocyst capsule in hydra," J. Cell Sci. 2001, 115: 745-751.
50. Holstein T and Tardent P: "An ultrahigh-speed analysis of exocytosis: nematocyst discharge," Science 1984, 223: 830-833.
51. Bajaj I and Singhal R: "Poly (glutamic acid) an emerging biopolymer of commercial interest," Bioresource Technology. 2011, 102: 5551-5561.
52. Shigeru Y and Reiko K: "New gamma-polyglutamic acid, production thereof and drink agent containing the same," Japanese Patent JP3047087, 1991.
53. Hara T, Fuzio Y and Ueda S: "Polyglutamic acid production by *Bacillus subtilis*. (natto)," J. Appl. Biochem. 1982, 4: 112.
54. Ashiuchi M and Misono H: "Biochemistry and molecular genetics of poly--glutamate synthesis," Appl. Microbiol. Biotechnol. 2002, 59: 9-14.
55. Ashiuchi M, Soda K and Misono H: "A poly-γ- glutamate synthetic system of *Bacillus subtilis* IFO 3336: gene cloning and biochemical analysis of poly-γ-glutamate produced by *Escherichia coli* clone cells," Biochem Biophys Res Commun. 1999, 263(1): 6-12.
56. Troy FA "Chemistry and biosynthesis of the poly (γ-D-glutamyl) capsule in *Bacillus licheniformis*. I. Properties of the membrane mediated biosynthetic reaction," J Biol Chem. 1973, 248: 305-315.
57. Leonard CG and Housewright RD "Polyglutamic acid synthesis by cell-free extracts of *Bacillus licheniformis*," Biochim Biophys Acta. 1963, 73: 530-532.
58. Ashiuchi M, Nawa C, Kamei T, Song JJ, Hong SP, Sung MH, Soda K, Yagi T and Misono H: "Physiological and biochemical characteristics of poly γ-glutamate synthetase complex of *Bacillus subtilis*," Eur J Biochem. 2001, 268: 5321-5328.

59. Green BD, Battisti L, Koehler TM, Thorne CB and Ivins BE: "Demonstration of a capsule plasmid in *Bacillus anthracis*," Infect Immun. 1985, 49, 291-297.
60. Candela T, Mock M and Fouet A: "Cap E, a 47amino-acid peptide, is necessary for *Bacillus anthracis* polyglutamate capsule synthesis," J Bacteriol. 2005, 187: 7765-7772.
61. Urushibata Y, Tokuyama S and Tahara Y: "Characterization of the *Bacillus subtilis* yws C gene, involved in γ-polyglutamic acid production," J Bacteriol 2002, 184: 337-343
62. Vietri NJ, Marrero R, Hoover TA and Welkos SL: "Identification and characterization of a trans-activator involved in the regulation of encapsulation by *Bacillus anthracis*," Gene 1995, 152: 1-9.
63. Nagai T, Koguchi K and Itoh Y: "Chemical analysis of poly-γ-glutamic acid produced by plasmid-free *Bacillus subtilis* (natto): evidence that plasmids are not involved in poly-g-glutamic acid production," J. General Appl. Microbiol. 1997, 43: 139-143.
64. Fabret C, Feher VA and Hoch JA: "Two component signal transduction in *Bacillus subtilis* How one organism sees its world," J. Bacteriol. 1999, 181: 1975-1983.
65. Stanley NR and Lazazzera BA: "Defining the genetic differences between wild and domestic strains of *Bacillus subtilis* that affect poly-γ-glutamic acid production and biofilm formation," Mol. Microbiol. 2005, 57: 1143-1158.
66. Okada M, Sato I, Cho SJ, Iwata H, Nishio T, Dubnau D and Sakagami Y: "Structure of the *Bacillus subtilis* quorum-sensing peptide pheromone Com X," Nat. Chem. Biol. 2005, 1: 23-24.
67. Msadek T, Kunst F, Klier A and Rapoport G: "Deg S-Deg U and Comp-Com A modulator-effector pairs control expression of the *Bacillus subtilis* pleiotropic regulatory gene deg Q," J. Bacteriol. 1991, 173: 2366-2377.
68. Kobayashi K: "Gradual activation of the response regulator Deg U controls serial expression of genes for flagellum formation and biofilm formation in *Bacillus subtilis*," Mol. Microbiol. 2007, 66: 395-409.
69. Ohsawa T, Tsukahara K and Ogura M: "*Bacillus subtilis* response regulator Deg U is a direct activator of pgs B transcription involved in poly-γ-glutamic acid synthesis," Biosci. Biotechnol. Biochem. 2009, 73: 2096-2102.
70. Kimura K, Tran LS, Do TH and Itoh Y: "Expression of the pgs B encoding the poly-γ-glutamate synthetase of *Bacillus subtilis* (natto)," Biosci. Biotechnol. Biochem. 2009, 73: 1149-1155.
71. Harrison SC and Aggarwal AK: "DNA recognition by proteins with the Helix-Turn-Helix motif," Annu. Rev. Biochem. 1990, 59: 933-969.
72. Tran LS, Nagai T and Itoh Y: "Divergent structure of the com QXPA quorum sensing components: molecular basis of strain-specific communication mechanism in *Bacillus subtilis*," Mol. Microbiol. 2000, 37: 1159-1171.
73. Osera C, Amati G, Calvio C and Galizzi A: "Swr AA activates poly-γ-glutamate synthesis in addition to swarming in *Bacillus subtilis*," Microbiol. 2009, 155: 2282-2287.
74. Calvio C, Osera C, Amati G and Galizzi A: "Autoregulation of Swr AA and motility in *Bacillus subtilis*," J.Bacteriol. 2008, 190, 5720-5728.
75. Deng SB, Bai RB, Hu XM and Luo Q: "Characteristics of a bioflocculants produced by *Bacillus mucilaginosus* and its use in starch wastewater treatment," Agricultural and Biological Chemistry. 2003, 60: 588-593.
76. Yokoi H, Natsuda O, Hirose J, Hayashi S and Takasaki Y: "Characteristics of a biopolymer flocculant produced by *Bacillus* sp.PY-90.J," Ferment. Bioeng. 1995, 79: 378-380.
77. McLean RJ, Beauchemin D, Clapham L and Beveridge TJ: "Metal-binding characteristics of the gammaglutamyl capsular polymer of *Bacillus licheniformis* ATCC 9945," Appl Environ Microbiol. 1990, 56: 3671-3677.
78. Bhattacharyya D, Hestekin JA, Brushaber P, Cullen L, Bachas LG and Sikdar SK: "Novel polyglutamic acid functionalized microfilteration membranes for sorption of heavy metals at high capacity," J. Membrane Sci. 1998, 141: 121-135.
79. Shih IL, Van YT and Sau YY: "Antifreeze activities of poly glutamic acid produced by *Bacillus licheniformis*," Biotechnology Letters 2003, 25: 1709-1712.
80. Seguro K, Tamiya T, Tsuchiya T and Matsumoto JJ: "Cryoprotective effect of sodium glutamate and lysine-HCl on freeze denaturation of lactate dehydrogenase," Cryobiology 1990, 27, 70-79.
81. Li C, Wallace S, Yu DF and Yang D: "Water soluble paclitaxel prodrugs," US Patent No. 5977163; 1999.
82. Li C, Yu DF, Newman A, Cabral F, Stephens C, Hunter N, Milas L and Wallace S: "Complete regression of well-established tumors using novel water-soluble polyglutamic acid-paclitaxel conjugate," Cancer Res. 1998, 58, 2404-2409.
83. Akai K, Sonoda C and Murase K: "Bitterness relieving agent," WO0021390: 2000.
84. Animoto H, Fox T, Eagles J, Satoh H, Nozawa H, Okiyama A, Morinaga Y, Susan J and Fairweather-Tait SJ: "A cute effect of poly-glutamic acid on calcium absorption in post-menopausal women," J. Am. College Nutr. 2007, 26(6): 645–649.
85. Wang Q, Chen S, Zhang J, Sun M, Liu Z and Yu Z: "Co-producing lipopeptides and poly-glutamic acid by solid-state fermentation of *Bacillus subtilis* using soybean and sweet potato residues and its biocontrol and fertilizer synergistic effects," Bioresour. Technol. 2008, 99(8): 3318–3323.
86. King WE, Fister RP and Norris SJ: "Slow-release fertilizer and method of making and using same," WO/024753: 2007.
87. Ivanov V and Chu J: "Applications of microorganisms to geotechnical engineering for bioclogging and biocementation of soil in situ," Rev Environ Sci Biotechnol. 2008: 1-15.
88. Ross N, Villemur R, Deschenes L and Samson R: "Clogging of limestone fracture by stimulating groundwater microbes," Water Res. 2001, 35: 2029-2037.
89. Momemi D, Kamil R, Martin R and Yen TF: "Potential use of biopolymer grouts for liquefaction mitigation," Columbus, Batelle Press, 1999.
90. Karol RH "Chemical grouting and soil stabilization," CRC Press, 270 Madison Avenue, New York, NY, 2003.
91. Baveye P, Vandevivere P, Hoyle BL, DeLeo PC and de Lozada DS: "Environmental impact and mechanisms of the biological clogging of saturated soils and aquifer materials," Crit Rev Environ Sci Technol. 1998, 28: 123-191.
92. Murphy EM and Ginn TR: "Modeling microbial processes in porous media," Hydrogeol J. 2000, 8, 142-158.
93. Stewart TL and Fogler HS: "Biomass plug development and propagation in porous media," Biotechnol Bioeng. 2001, 72, 353-363.
94. Ravenscroft N, Walker SG, Dutton GG and Smit J: "Identification, isolation, and structural studies of extracellular polysaccharides produced by *Caulobacter crescentus*," J Bacteriol. 1991, 173: 5677-5684.

95. Tsang PH, Li G, Brun YV, Freund LB and Tang JX: "Adhesion of single bacterial cells in the micronewton range," Proc Natl Acad Sci 2006, 103: 11435-11436.
96. Harada T: "Special bacterial polysaccharides and polysaccharases," Biochem Soc Symp. 1983, 48: 97-116.
97. Portilho M, Matioli G, Zanin GM, de Moraes FF and Scamparini AR: "Production of insoluble exopolysaccharide *Agrobacterium* sp. (ATCC 31749 and IFO 13140)." Appl Biochem Biotechnol 2006, 131(1-3): 864-869.
98. Kenyon WJ, Stephen WE and Buller CS: "The curdlan-type exopolysaccharide produced by *Cellulomonas flavigena* KU forms part of an extracellular glycocalyx involved in cellulose degradation," Antonie Van Leeuwenhoek. 2005, 87, 143-148.
99. Stehr G, Zoner S, Bottcher B and Koops HP: "Exopolymers: an ecological characteristic of a floc-attached, ammonia oxidizing bacterium," Microb Ecol. 1995, 30: 115-126.
100. Ivanov V, Wang JY, Stabnikova O, Krasinko V, Stabnikov V, Tay STL and Tay JH: "Iron-mediated removal of ammonia from strong nitrogenous wastewater of food processing," Water Sci Technol. 2004, 49: 421-431.
101. Ivanov V, Stabnikov V, Zhuang WQ, Tay STL and Tay JH: "Phosphate removal from return liquor of municipal wastewater treatment plant using iron-reducing bacteria," J Appl Microbiol. 2005, 98: 1152-1161.
102. Ivanov V, Wang XH, Tay STL and Tay JH: "Bioaugmentation and enhanced formation of microbial granules used in aerobic wastewater treatment," Appl Microbiol Biotechnol. 2006, 70: 374-381.
103. Dekie L, Toncheva V, Dubruel P, Schacht EH, Barrett L and Seymour LW: "Poly-L-glutamic acid derivatives as vectors for gene therapy," J. Controlled Release. 2000, 65: 187-202.
104. Sung MH, Kim CJ, Poo HR, Hong SP, Lee JS and Kim JY: "Composition for adjuvant containing poly-gamma-glutamic," WO090968: 2006.
105. Okamoto S, Yoshii H, Ishikawa T, Akagi T, Akashi M, Takahashi M, Yamanishi K and Mori Y: "Single dose of inactivated Japanese encephalitis vaccine with poly (c-glutamic acid) nanoparticles provides effective protection from Japanese encephalitis virus," Vaccine 2008, 26(5): 589-594.
106. Bajaj B, Lele SS and Singhal RS: "Enhanced production of poly (γ-glutamic acid) from *Bacillus licheniformis* NCIM 2324 in solid state fermentation," J. Ind Microbiol Biotechnol. 2008, 35: 1581-1586.
107. Cromwick AM, Birrer GA and Gross RA: "Effects of pH and aeration on γ-poly (glutamic acid) formation by *Bacillus licheniformis* in controlled batch fermentor cultures," Biotechnol Bioeng. 1996, 50: 222-227.
108. Ashiuchi M, Kamei T, Baek DH, Shin SY, Sung MH, Soda K and Yagi T, Misono H: "Isolation of *Bacillus subtilis* (chungkookjang), a poly-γ-glutamate producer with high genetic competence," Appl Microbiol Biotechnol. 2001, 57: 764-769.
109. Veronique BM, Olivier O and Pierre C: "Sensors and measurements in solid state fermentation: a review," Process Biochem 2003, 38: 881-896.
110. Ashok P, Carlos RS and David M: "New developments in solid state fermentation: I- bioprocesses and products," Process Biochem. 2000, 35: 1153-1169.
111. Guerra NP, Agrasar AT, Macias CL and Pastrana L: "Main characteristics and application of solid substrate fermentation," Electron. J. Environ. Agric. Food Chem. 2003, 2: 343-350.
112. Raimbault M: "General and microbiological aspects of solid substrate fermentation," J. Biotechnol. 1998, 3: 1-15.
113. Lee SY, Chang HN, Thakur N and Do JH: "A method for manufacturing highly concentrated polyglutamic acid with additional supply of saccharides," U.S. patent appl. 10/300,711 (2002.11.19), registered US 6989251 (2006.01.24), 2006.
114. Kubota H, Matsunobu T, Uotani K, Takebe H, Satoh A, Tanaka T and Taniguchi M: "Production of poly (d-glutamic acid) by *Bacillus subtilis* F-2-01," Biosci Biotechnol Biochem. 1993, 57(7): 1212-1213.
115. Dobreva E, Tonkova A, Ivanova V, Stefanova M, Kabaivanova L and Sapasova D: "Immobilization of *Bacillus licheniformis* cells, producers of thermostable α-amylase, on polymer membranes," J. Industr. Microbiol. Biotechnol. 2004, 20(3-4): 166-170.
116. El-Hadi AA: "Factors affecting the production of prednisolone by immobilization of *Bacillus pumilus* E601 cells in poly (vinyl alcohol) cryogels produced by radiation polymerization," Process. Biochem. 2003, 38(12): 1659-1664.
117. Berekaa MM, El-Assar SA, El-Sayed SM and El-Borai AM: "Production of poly-γ- Glutamate (PGA) biopolymer by batch and semi continuous cultures of immobilized *Bacillus licheniformis* strain-R," Brazilian Journal of Microbiology 2009, 40: 715-724.
118. Birrer G, Cromwick AM and Gross R: "γ-Polyglutamic acid formation by *Bacillus licheniformis* 9945A: physiological and biochemical studies," Int. J. Biol Macromol. 1994, 16: 265-275.
119. Ashiuchi M, Fukushima F, Oya H, Hiraoki T, Shibatani S, Oka N, Nishimura H, Hakuba H, Nakamori M and Kitagawa M: "Development of antimicrobial thermoplastic material from archaeal poly-g-L-glutamate and its nanofabrication," ACS Appl Mater Interfaces. 2013, 5: 1619–1624.
120. Wang S, Cao X, Shen M, Guo R, Bányal I and Shi X: "Fabrication and morphology control of electrospun poly (γ-glutamic acid) nanofibers for biomedical applications," Colloids Surf B. 2012, 89: 254–264.
121. Berekaa MM and Al-Otaibi SM: "Enhanced production of poly glutamic acid by *Bacillus* sp. SW1-2 using statistical experimental design," African Journal of Biotechnology. 2013, 12: 481-490.
122. Yokoi H, Arima T, Hirose J, Hayashi S and Tatasaki Y: "Flocculation properties of poly (γ-glutamic acid) produced by *Bacillus subtillis*," J. Ferment. Bioeng. 1996, 82, 84-87.
123. Kinnersley A, Storm D, Meah RY and Koskan CP: "Composition and method for enhanced fertilizer uptake by plants," WO patent no. 94/09, 628, 1994.
124. Mitsuiki M, Mizuo A, Tanimoto H, and Motoko M: "Relationship between the antifreeze activities and the chemical structures of oligo- and poly (glutamic acid)," J. Agric Food Chem. 1998, 46: 891-895.
125. Yamasaki D, Minouchi Y and Ashiuchi M: "Extremolyte like applicability of an archeal exopolymer, poly-γ-L-glutamate," Environ Technol 2010, 31: 1129-1134.
126. Hezayen FF, Rehm BHA, Tindall BJ, Steinbuchel, A. "Transfer of *Natrialba asiatica* B_1T to *Natrialba taiwanensis* sp. Nov, a novel extremely halophilic, aerobic, non pigmented member of Archea from Egypt that produces extracellular poly (glutamic acid)," Int. J. Syst Envol Microbiol 2001, 51, 1133-1142.
127. Shimizu K, Nakamura H and Ashiuchi M: "Salt inducible bio-nylon polymer from *Bacillus megaterium*," Appl. Environ. Microbiol. 2007, 73, 2378-2379.

128. Aono, R. "Characterization of structural component of cell walls of alkalophilic strain of *Bacillus* sp. C-125," Biochem. J. 1987, 245, 467-472.
129. Niemetz R, Karcher U, Kandlera O, Tindall BJ and Konig H: "The cell wall polymer of the extremely halophilic archeon, *Natronococcus occultus*," Eur. J. biochem. 1997, 249, 905-911.
130. Cromwick AM and Gross RA: "Effect of mangense (II) on Bacillus licheniformis ATCC9945A - Physiology and g-poly (glutamic) formation," Int. J. Biol. Macromol. 1995, 17: 259-267
131. Perez-Camero G, Congregado F, Bou JJ and Munoz-Guerra S: "Biosynthesis and ultrasonic degradation of bacterial poly (γ-glutamic acid)," Biotechnol. Bioengg. 1999, 63: 110-115.
132. Tanimoto H, Mori M, Motoki M, Torii K, Kadowaki M and Noguchi T: "Natto mucilage containing poly-γ-glutamic acid increases soluble calcium in the rat small intestine," Biosci. Biotechnol. Biochem. 2001, 65, 516-521.
133. Ashiuchi M: "Microbial production and chemical transformation of poly- γ -glutamate," Microbiol. Biotechnol. 2013, 1-11.
134. Lee EH, Son WC, Lee SE and Kim BH: "Anti-Obesity Effects of Poly-γ-glutamic Acid with or without Isoflavones on High-Fat diet induced obese mice," Biosci Biotechnol Biochem. 2013, 77(8), 1694-702.
135. Bae KC, Park JH, Na AY, Kim SJ, Ahn S, Kim SP, Oh BC, Cho HC, Kim YW and Song DK: "Effect of Green tea extract/Poly-γ-Glutamic acid complex in obese type 2 diabetic mice," Diabetes Metab J. 2013, 37(3): 196-206.
136. Park JH, Kim SJ, Hwang I, Bae KC, Bae JH and Song DK: "Green tea extract co-administered with a polymer effectively prevents alcoholic liver damage by prolonged inhibition of alcohol absorption in mice," Alcohol 2013, 48(1): 59-67.
137. Jeon YH, Kwak MS, Sung MH, Kim SH, Kim MH and Chang MJ: "High-molecular-weight poly-gamma-glutamate protects against hypertriglyceridemic effects of a high-fructose diet in rat," J. Microbiol. Biotechnol. 2013, 23(6): 785-793.
138. Kashima N, Furuta K and Tanabe I: "Permeability Enhancer," United States Patent 20060025346, 2006.
139. Chen X, Chen S, Sun M and Yu Z: "High yield of poly-γ-glutamic acid from *Bacillus subtilis* by solid-state fermentation using swine manure as the basis of a solid substrate," Bioresource Technology 2005, 96: 1872–1879.
140. Hoppensack A, Oppermann FB and Steinbuchel A: "Conversion of the nitrogen content in liquid manure into biomass and polyglutamic acid by a newly isolated strains of *Bacillus licheniformis*," FEMS Microbiol. Lett. 2003, 218, 39–45.
141. Potter M, Oppermann FB and Steinbuchel A: "Cultivation of bacteria producing polyamino acids with liquid manure as carbon and nitrogen source," Appl. Environ. Microbiol. 2001, 62: 617–622.
142. Zongqi X, Chuanbao W, Dan Z, Xiaohai F and Hong X: "Effect of Poly (γ-glutamic acid) on Wheat Productivity and Soil Microbes," http ://www.paper.edu.cn/en_ releasepaper/content/4482952
143. Yamaguchi S, Tatsumi T, Takehara T, Sasakawa A, Yamamoto M, Kohga K, Miyagi T, Kanto T, Hiramastu N, Akagi T, Akashi M and Hayashi N: "EphA2-derived peptide vaccine with amphiphilic poly (γ-glutamic acid) nanoparticles elicits an anti-tumor effect against mouse liver tumor," Cancer Immunol Immunother, 2010, 59: 759–767.
144. Uto T, Akashi M, Wang X, Sato K and Baba M: "Targeting of Antigen to Dendritic Cells with Poly (γ-Glutamic Acid) Nanoparticles induces antigen-specific humoral and cellular immunity," The Journal of Immunology 2007, 178: 2979–2986.
145. Uto T, Akagi T, Yoshinaga K, Toyama M, Akashi M and Baba M; "The induction of innate and adaptive immunity by biodegradable poly (γ-glutamic acid) nanoparticles via a TLR4 and MyD88 signalling pathway," Biomaterials 2011, 32: 5206-5212.
146. Mao HQ, Roy K, Troung-Le VL, Janes KA, Lin KY, Wang Y, August JT and Leong KW: "Chitosan-DNA nanoparticles as gene carriers: synthesis, characterization and transfection efficiency," J Control Release 2001, 70, 399-421.
147. Kim TH, Park IK, Nah JW, Choi YJ and Cho CS: "Galactosylated chitosan/DNA nanoparticles prepared using water-soluble chitosan as a gene carrier," Biomaterials 2004, 25: 3783-3792.
148. Morille M, Passirani C, Vonarbourg A, Clavreul A and Benoit JP: "Progress in developing cationic vectors for non-viral systemic gene therapy against cancer," Biomaterials 2008, 29: 3477-3496.
149. Kim TH, Jiang HL, Jere D, Park IK, Cho MH, Nah JW, Choi YJ, Akaike T and Cho CS: "Chemical modification of chitosan as a gene carrier in vitro and in vivo," Prog Polym Sci 2007, 32: 726-753.
150. Peng SF, Yang MJ, Su CJ, Chen HL, Lee PW, Wei MC, Sung HW: "Effects of incorporation of poly (γ-glutamic acid) in chitosan/DNA complex nanoparticles on cellular uptake and transfection efficiency," Biomaterials 2009, 30, 1797-1808.
151. Peng SF, Tseng MT, Ho YC, Wei MC, Liao ZX and Sung HW: "Mechanisms of cellular uptake and intracellular trafficking with chitosan/DNA/poly (γ-glutamic acid) complexes as a gene delivery vector," Biomaterials 2011, 32, 239-248.
152. Heeswijk WAR, Hoes CJT, Stoffer T, Eenink MJD, Potman W and Feijen J: "The synthesis and characterization of polypeptide–adriamycin conjugates and its complexes with Adriamycin," Part 1, J. Control. Release 1985, 1: 301–315.
153. Hoes CJT, Potman W, van Heeswijk WAR, Mud J, de Grooth BG, Greve J and Feijen J: "Optimization of macromolecular pro drugs of the antitumor antibiotic Adriamycin," J. Control. Release 1985, 2, 205–213.
154. Hoes CJT, Grootoonk J, Duncan R, Hume IC, Bhakoo M, Bouma JMW and Feijen J: "Biological properties of Adriamycin bound to biodegradable polymeric carriers," J. Control. Release 1993, 23, 37–54.
155. Zunino F, Pratesi G and Micheloni A: "Poly (carboxylic acid) polymers as carriers for anthracyclines," J. Control. Release 1989, 10: 65–73.
156. Moromoto Y, Sugibayashi K, Sugihara S, Hosoya KI, Nozake S and Ogawa Y: "Antitumor agent poly (amino acid) conjugate system as a drug carrier in chemotherapy," J. Pharm. Dyn. 1984, 7: 688–698.
157. Roos CF, Matsumoto S, Takarura Y, Hashida M and Sezaki H: "Physicochemical and antitumor characteristics of some poly amino acid pro drugs of Mitomycin C," Int. J. Pharm. 1984, 22: 75–87.
158. Kato Y, Saito M, Fukushima H, Takeda Y and Hara T: "Antitumor activity of 1--D-arabinofurano sylcytosine conjugated with poly glutamic acid and its derivative," Cancer Res. 1984, 44: 25–30.
159. Tansey W, Ke S, Cao XY, Pasuelo MJ, Wallace S and Li C: "Synthesis and characterization of branched poly (L-

glutamic acid) as a biodegradable drug carrier," Journal of Controlled Release 2004, 94: 39–51.
160. Sonaje K, Chen YJ, Chen HL, Wey SP, Juang JH, Nguyen HN, Hsu CW, Lin KJ and Sung HW: "Enteric-coated capsules filled with freeze-dried chitosan/poly (γ-glutamic acid) nanoparticles for oral insulin delivery," Biomaterials 2010, 31: 3384–3394.
161. Su FY, Lin KJ, Sonaje K, Wey SP, Yen TC, Panda N, Chuang EY, Maiti B and Sung HW: "Protease inhibition and absorption enhancement by functional nanoparticles for effective oral insulin delivery," Biomaterials 2012, 33: 2801-2811.
162. Taniguchi M, Kato K, Shimauchi A, Ping X, Fujita KI, Tanaka T, Tarui Y and Hirasawa E: "Physicochemical properties of cross-linked poly-γ-glutamic acid and its flocculating activity against kaolin suspension," J. Biosci. Bioeng. 2005, 99(2), 130-135.
163. Taniguchi M, Kato K, Matsui O, Ping X, Nakayama H, Usuki Y, Ichimura A, Fujita K, Tanaka T, Tarui Y and Hirasawa E: "Flocculating activity of cross-linked Poly-γ-Glutamic acid against Bentonite and *Escherichia coli* suspension pre-treated with $FeCl_3$ and its interaction with Fe^{3+}," Journal of Bioscience and Bioengineering. 2005, 100(2): 207-211.
164. Zheng H, Gao Z, Yin J, Tang X, Ji X and Huang H: "Harvesting of microalgae by flocculation with poly (γ-glutamic acid)," Bioresource Technology 2012, 112: 212–220.
165. Mark SS, Crusberg TC, Da Cunha CM and Di Iorio AA: "A heavy metal biotrap for waste water remediation using poly γ -glutamic acid," Biotechnol. Prog. 2006, 22: 523-531.
166. Bodnar M, Kjonikse AL, Molnar RM, Hartmann JF, Daroczi L, Nystromb B and Borbely J: "Nanoparticles formed by complexation of poly-gamma Glutamic acid with lead ions," J. Hazard. Matter. 2008, 153, 1185–1192.
167. Inbaraj BS and Chen BH: "In vitro removal of toxic heavy metals by poly (γ-glutamic acid)-coated super paramagnetic nanoparticles," International Journal of Nanomedicine. 2012, 7: 4419–4432.
168. Ben-Zur N and Goldman DM: "γ-Poly glutamic acid: a novel peptide for skin care," Cosmetics Toiletries Mag. 2007, 122 (4): 64–72.
169. Zhiqiang Y, Zhang Y, Markland P and Yang VC: "Poly (glutamic acid) poly (ethylene glycol) hydrogels prepared by photo induced polymerization: Synthesis, characterization, and preliminary release studies of protein drugs," J Biomed Mater Res 2002, 62: 14–21.
170. Kroft LJ and de Roos A: "Blood pool contrast agents for cardiovascular MR imaging," J. Magn. Reson. Imaging 1999, 10: 395-403.
171. Bogdanov AA, Lewin M and Weissleder R: "Approaches and agents for imaging the vascular system," Adv. Drug Delivery Rev. 1999, 37: 279-293.
172. Lu ZR, Wang X, Parker DL, Goodrich KC and Buswell HR: "Poly (L-glutamic acid) Gd (III)-DOTA conjugate with a degradable spacer for magnetic resonance imaging," Bioconjugate Chem. 2003, 14 (4), 715–719.
173. Li C, Wen X, Jackson E and Wallace S: "Poly (L-glutamic acid) paramagnetic material complex and use as biodegradable MRI contrast agent," WO 063304; 2005.
174. Wen X, Jackson EF, Price REE, Kim WE, Wallace QSC, Charnsangavej JG, and Li, C: "Synthesis and Characterization of Poly (L-glutamic acid) Gadolinium Chelate: A New Biodegradable MRI pool Contrast Agent. Bioconjugate Chem. 2004, 15: 1408-1415.
175. Chung S, Gentilini C, Callanan A, Hedegaard M, Hassing S and Stevens MM: "Responsive Poly (γ-Glutamic acid) fibres for biomedical applications," J. Mater. Chem. B. 2013, 1: 1397-1401.
176. Brasch RC: "New directions in the development of MR imaging contrast media," Radiology 1992, 183: 1-11.
177. Bajaj IB and Singhal RS: "Enhanced production of poly (γ-glutamic acid) from *Bacillus licheniformis* NCIM 2324 by using metabolic precursors," Appl. Biochem. Biotechnol. 2009, 159: 133–141.
178. Yao J, Xu H, Shi N, Cao X, Feng X, Li S and Ouyang P: "Analysis of carbon metabolism and improvement of γ-polyglutamic acid production from *Bacillus subtilis* NX-2," Appl. Biochem. Biotechnol. 2010, 160(8), 2332–2341
179. Wu Q, Xu H, Shi N, Yao J, Li S and Ouyang P: "Improvement of poly (γ-glutamic acid) biosynthesis and redistribution of metabolic flux with the presence of different additives in *Bacillus subtilis* CGMCC 0833," Appl. Microbiol. Biotechnol. 2008, 79(4): 527–535.
180. Wu Q, Xu H, Liang J and Yao J: "Contribution of glycerol on production of Poly (γ-glutamic acid) in *B. subtilis* NX-2," Appl. Biochem. Biotechnol. 2010, 160, 386-392.
181. Richard A and Margaritis A: "Rheology, oxygen transfer, and molecular weight characteristics of poly (glutamic acid) fermentation by *Bacillus subtilis*," Biotechnol. Bioeng. 2003, 82, 299-305.
182. Gross RA: "Bacterial γ-poly (glutamic acid)," In: Kaplan, D.L. (ed) Biopolymers from renewable resources. Springer, Berlin Heidelberg, New York, 1998: 195-219.
183. Roelants G and Goodman J: "Immunochemical studies on the poly-δ-d-glutamyl capsule of *Bacillus anthracis*. IV. The association peritoneal exudates cell ribonucleic acid of the polypeptide in immunogenic and non-immunogenic forms," Biochemistry 1968, 7: 1432-1440.
184. Strobel RJ and Sullivan GR: "Experimetal design for improvement of fermentations," In: Demain, A, L.; Davies, J.E. (eds) Manual of industrial microbiology and biotechnology. Washington. ASM Press, 1999: 80-93.
185. Kambourova M, Tagney M and Priest F: "Regulation of polyglutamic acid synthesis by glutamate in *Bacillus licheniformis* and *Bacillus subtilis*," Appl. Environ. Microbiol. 2001, 67(2): 1004-1007
186. Tomalia DA, Baker H and Dewald J: "A new class of polymers: starburst-dendritic macromolecules," Polymer Journal. 1985, 17: 117–132.
187. Panyam J and Labhasetwar V: "Biodegradable nanoparticles for drug and gene delivery to cells and tissue," Adv Drug Deliv Rev. 2003, 55: 329–347.
188. Sahoo SK and Labhasetwar V: "Nanotech approaches to drug delivery and imaging," Drug Discov Today. 2003, 8: 1112–1120.
189. Li C: "Poly (γ-glutamic acid) - anticancer drug conjugates," Adv Drug Deliv Rev. 2002, 54: 695-713.
190. Ringsdorf H: "Structure and properties of pharmacologically active polymers," J Polym Sci Symp. 1975, 51: 135–153.

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