

2014

BioTechnology

An Indian Journal

FULL PAPER

BTAIJ, 10(24), 2014 [15184-15190]

Review in the biosynthesis and applications of poly-glutamic acid

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ABSTRACT

γ -Poly glutamic acid has been of interest in recent years due to its excellent performance and widely application in various fields. This paper reviews the properties, producing bacterium, production process and mechanism of microbial synthesis of γ - poly glutamic acid, and applications in food, agricultural and cosmetic fields, and summarizes currently production of γ - poly glutamic acid.

KEYWORDS

γ -Poly glutamic acid; Biosynthesis; Application; Review.



INTRODUCTION

γ -poly-glutamic acid (γ -PGA) is a biomacromolecule materials, which is biodegradable and can retain water well. In 1973, γ -poly glutamic acid is founded to be one main content of some bacillus capsule structure and water-soluble euron compound for biological natural synthesis^[1]. The poly-glutamic acid is composed of L-type and D-type glutamic acid, which is linked by the amido bond. The molecule structure is shown as the figure 1.

Microbes such as bacillus anthracis, *Bacillus subtilis* and *Bacillus licheniformis* can produce γ -PGA. Generally its molecular weight is between 100-1000KDa, which is equivalent to 500-5000 glutamic acid monomers. The glutamic acid with different molecular weights can be obtained by using different methods, e.g. acid and alkali hydrolysis, ultrasonic degradation^[2] and enzyme degradation^[3], even content change of the culture medium^[4] may affect distribution of its molecular weights. γ -PGA is secure and innocuous to human being and environment and is an environment-friendly polymer material^[5]. γ -PGA and its made hydrogel are extensively applied in different fields due to its powerful water absorption time and water retaining performance^[6,7], e.g. degradable bio-fertilizers in the agriculture field, flocculant for processing waste water in the environment protection field and downstream processing in the food industry^[8], support material, skin humectant, skin factor retarder, hairspray and natural beauty mask in the cosmetics field^[9]. It can also be used as the antifreeze, thickener, low-temperature protective agent^[10], humectant, drug carrier and osteoporosis prevention factor^[11].

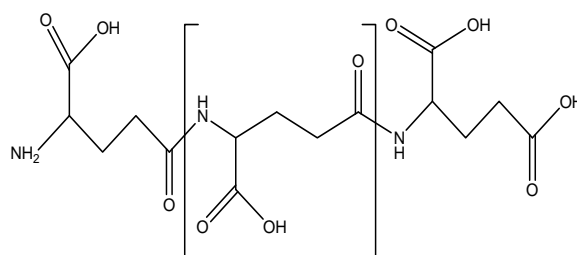


Figure 1 Formula of γ -poly glutamic acid

BIOSYNTHESIS OF POLY GLUTAMIC ACID

Produce bacterial strain

The bacterial strain, which can produce massive poly glutamic acids, has potential industrial value and is also focused by researchers in recent years. The producing strain of the ploy glutamic acid is divided into the glutamic acid dependent bacteria and non- glutamic acid dependent bacteria. The glutamic acid dependent bacteria (e.g. *B. subtilis* IFO3335 and *B. licheniformis* ATCC 9945a) indicates the bacillus, which will produce γ -PGA by adding glutamic acid into the culture medium. Other bacteria are called as non-glutamic acid dependent bacteria (e.g. *B.subtilis* TAM-4 and *B.licheniformis* A35). Although the glutamic acid non-dependent bacteria can synthesize the glutamic acid by using the cheap raw materials without glutamic acid, the productivity is lower. The table compares several representative bacterial strains, which are thoroughly studied:

TABLE 1: The confrontation of the representative strains

Class	Glutamic dependent bacteria		Non glutamic dependent bacteria	
Bacterial strain	<i>B. subtilis</i> IFO3335 ^[12]	<i>B. licheniformis</i> ATCC 9945a ^[13-14]	<i>B.subtilis</i> TAM-4 ^[15]	<i>B.licheniformis</i> A35 ^[16-17]
Prescription of culture medium (g/L)	30ml glutamic acid, 20-50ml citric acid, 5-10ml ammonia sulfate, 1ml dipotassium hydrogen phosphate, 0.5ml epsom salt, 0.02ml manganese sulfate, 0.05ml ferric chloride hexahydrate and 50ml biotin	20ml glutamic acid, 12 citric acid, 80ml glycerol, 7ml ammonia chloride, 0.5ml dipotassium hydrogen phosphate, 0.5ml epsom salt, 0.04ml ferric chloride hexahydrate, 0.1ml manganese sulfate and 0.15ml calcium chloride dehydrate	75ml amylaceum, 18ml ammonia chloride, 1.5ml dipotassium hydrogen phosphate, 0.35ml epsom salt, 0.05ml manganese sulfate and 30ml calcium carbonate	75ml amylaceum, 18ml ammonia chloride and inorganic salt
Relative molecular weight	1 million	0.2-0.8 million	0.2 million (change in culture)	-
L/D percent	80:20	(45-85) : (55-15)	78:22	(50-20)
Output(g/L)	10	20.5	10	8

The above table indicates the external glutamic acid is the required precursor for γ -PGA synthesized by the bacterial strain and can affect output, molecular and configuration of γ -PGA. In 1954, Thorne^[18] found that multiple factors will affect output of γ -PGA, e.g. glutamic acid, inorganic salt, carbon source density and ventilation condition. Yoon^[19] controlled supply and culture of the citric acid and L-glutamic acid and established γ -PGA high-productivity process. Based on the past research achievements, Qiao Changcheng^[20] optimized the culture medium and conditions of *Bacillus.licheniformis*CGMCC3336 and got a production process for the poly glutamic acid, which can produce the poly glutamic acid and reduce generation of other impurities in fermentation.

Synthesis principle

It is certain that the glutamic acid is the direct precursor of γ -PGA synthesized by glutamic dependent bacteria, but γ -PGA is synthesized by L-type and D-type glutamic acid monomer, how is the D-type glutamic acid synthesized^[21]. The biosynthesis mechanism of γ -PGA is not clear, but the cell wall of bacteria includes D-alanine and the cell includes alanine racemase, so the cytoplasm will include D-alanine instantaneously. The D-type alanine results from D- alanine, which is transformed to ammonia under catalysis of D-aminopherase^[22], the possible biological synthesis route of γ -PGA is shown as the following figure 2.

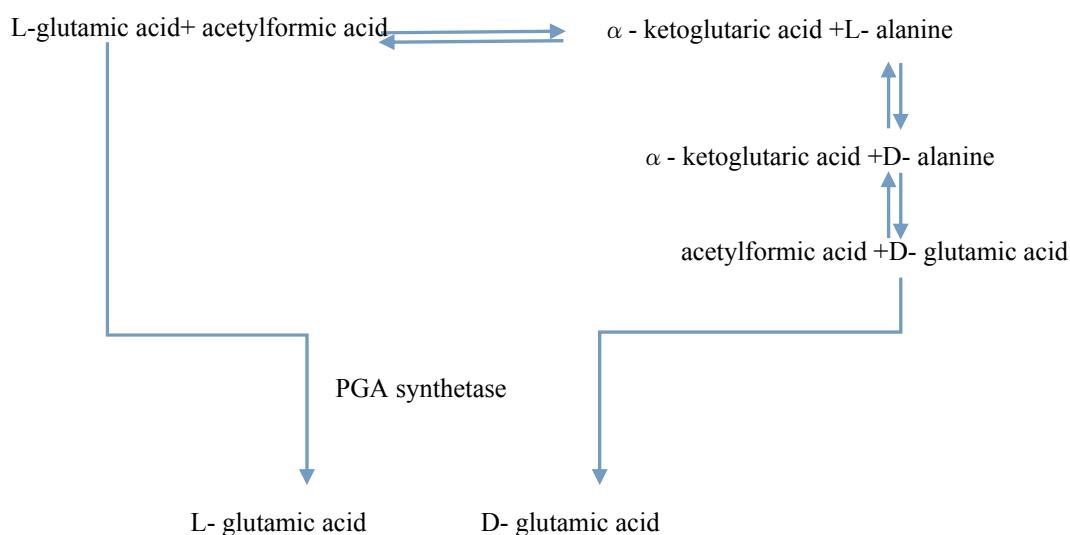


Figure 2 Possible route of γ -PGA biosynthesis

Synthesis method

The poly glutamic acid can be synthesized by using chemical synthesis method, extraction method and microbe fermentation method. Now many chemical synthesis methods for the poly glutamic acid are available. The molecular bond mode and space chemical structure are not restricted, but the molecular weight of the artificial synthesized poly glutamic acid (<9000) is significantly smaller than it of the natural poly glutamic acid and the artificial synthesized poly glutamic acid has no features of the natural poly glutamic acid. The extraction method targets at the poly glutamic acid in the natto mucus as one traditional fermented good in Japan^[23]. The microbe fermentation method is the unique promising production technology for massive production of the poly glutamic acid, which facilitates selection of the best culture conditions according to the requirements of the producing bacteria to metabolic nutrients, ventilation, mixing, temperature and pH requirements and features easy massive industrialization, standardization, automated production and high productivity^[24].

Fermentation process and its influence factors

The liquid fermentation process of the poly glutamic acid not only depends on the productivity of the bacterial strain, but also depends on the fermentation environment condition. The oxygen dissolution level, pH value control and nutrient concentration are also very important. With growth of output, the culture medium will become viscous in culture, which will change the oxygen content in the fermentation liquid and affect growth of the bacterial strain. Su^[25] first integrates the hemoglobin gene of the bacteria into the chromosome of the bacillus subtilis in a homologous recombination manner. The mutated bacillus subtilis S18-3-vgb+ can normally express VHB and enhance the oxygen uptake capability, so it successfully overcomes insufficient oxygen dissolution capability caused by increased viscosity in case of fermentation and makes γ -PGA output increase to 60.5g/L. Yoo^[19] gets the method for high γ -PGA yield via batch feeding and culture, which can keep 40% oxygen dissolution level in fermentation. When the γ -PGA yield reaches a certain level, the viscosity of the culture medium is too high, which will reduce oxygen dissolution level, so the pure oxygen is supplied. Huang^[25] from Zhejiang University can make the maximum concentration of γ -PGA reach 83g/L by feeding amyloaceum with 3-10g/L concentration and controlling the oxygen dissolution level to be over 10%. PH value will affect growth of bacteria strains and

product synthesis. if pH value is too low, the bacteria will not survive because the bacteria cells will generate the stress mechanism at a lower pH value, which will change the permeability of the cell membrane and make the materials inside the cells leak^[27]. The fermentation process can keep under the required level in fermentation at any time by controlling pH value of the fermentation liquid, which can be manually intervened. Wu Qun^[28] produces γ -PGA by using bacillus subtilis CGMCC0833 and controls pH value by intervals. pH value is controlled to be 7.0 in the initial 24 hours to get the maximum biomass. Next pH value reduces to 6.5 to get the high transformation rate of the glutamine and high yield of γ -PGA.

The mixed culture indicates to use the metabolin of one microbe as the substrate of another microbe. To avoid use of external glutamic acid, Zhejiang University cultivates the glutamic acid corynebacterium as the producing bacteria of the glutamic acid together with the γ -PGA producing bacteria to produce γ -PGA^[29]. Two bacteria are pre-cultivated and are put into the fermentation tank at a proper proportion. In the initial fermentation period, the glutamic acid corynebacterium will generate L-glutamic acid. L-glutamic acid generated in the middle and late period will be used as the substrate for γ -PGA producing bacterial to synthesize γ -PGA. It can produce 33g/L γ -PGA under the optimal culture condition^[30].

γ -PGA can also be produced by using the gene engineering bacteria. Some persons have used the colibacillus and glutamic acid corynebacterium as host bacteria to produce γ -PGA. the colibacillus provides three genes of γ -PGA synthetase (pgsB, pgsC and pgsA) and IPTG-induced promoter, but the yield is very low. With feeding culture, the final concentration is 3.7g/L^[31]. The glutamic acid synthesized by the glutamic acid corynebacterium can replace the glutamic acid fed by external source and can be directly used for poly γ -PGA. Compared to the external glutamic acid, it reduces the mass transfer resistance, so it is suitable for use as the host bacteria. In fact, the yield of γ -PGA is only 0.5g/L in the glutamic acid corynebacterium with the γ -PGA synthase gene^[32].

On the whole, the production process of the glutamic acid is not stable. The productivity of excellent bacterial strain can be further improved. Parameters should be repeatedly validated in fermentation. In future, the difficulties in oxygen and mass transfer in the highly viscous solution should be overcome in research on the glutamic acid. If so, this method can be enhanced much.

Separation and extraction process

γ -PGA extraction methods include organic solvent sedimentation method, chemical sedimentation method and film separation and sedimentation method^法[33-36]. For the organic solvent sedimentation method, the low-concentration inferior alcohols are added to the supernatant (e.g. carbinol and alcohol). γ -PGA is obtained via sedimentation. γ -PGA is dissolved with water again. Small molecules are removed via dialysis. The filtered liquid is frozen and dried to get the white crystal, which is the pure γ -PGA. For the chemical sedimentation method, the saturated CuSO₄ solution is used to replace the inferior alcohols in order to salt out and sediment γ -PGA[37]. For the film separation sedimentation method, the ultrafiltration separation unit is introduced, γ -PGA is intercepted, the pigment and salt impurities are filtered, γ -PGA solution is concentrated to some extent, and the alcohol sedimentation is used to get γ -PGA. This method [18] should overcome the high fermentation liquid viscosity and difficult centrifugation problem and reduce the pH value of the fermentation liquid to 2-4. The viscosity will reduce with decrease of pH value. When pH value is 3, the viscosity of the fermentation liquid is only 1/6 of the old viscosity. If pH value is less than 2, the bacteria will degrade. To adjust pH value, the charge on the cell surface will reduce, the bacteria will agglutinate, and the centrifugation effect will become better [39]. The white crystals obtained via the low-pressure freezing and drying is only rough γ -PGA. The rough γ -PGA will be dissolved in the distilled water again and the dissolved impurities will be removed by using the centrifugation method. The γ -PGA water solution will be obtained by using the dialysis or electroosmosis salt removal method. After low-pressure freezing, the refined γ -PGA can be obtained, shown as the figure 3.

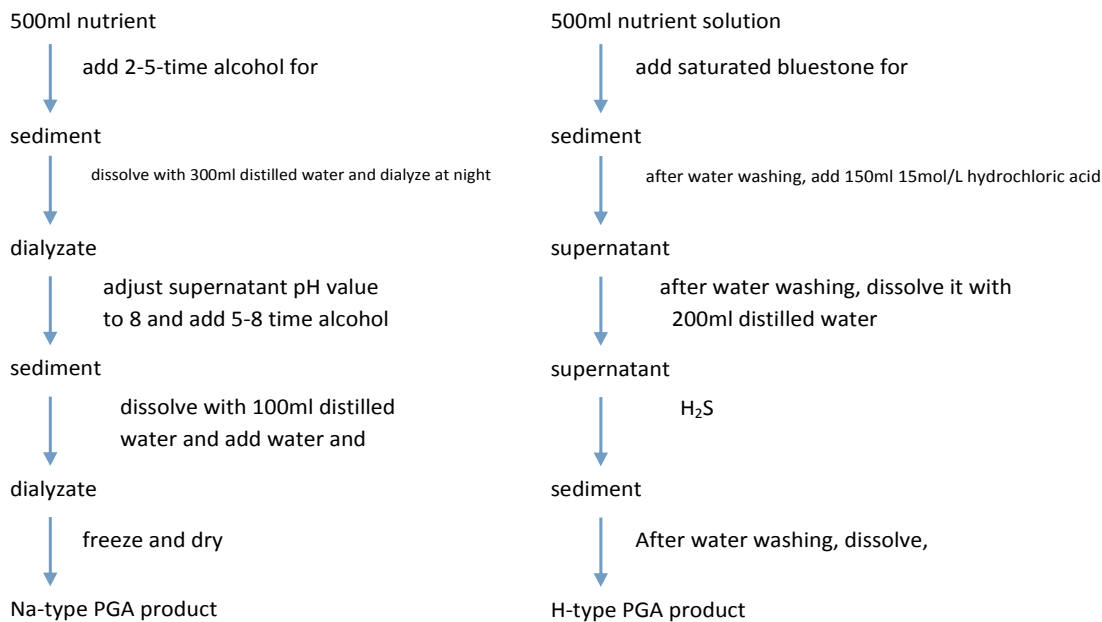


Figure 3 Separation and purification of γ -PGA

The above studied extraction processes of the poly glutamic acid are used to remove the impurities in the fermentation liquid. The process for refining the poly glutamic acid includes too many steps and is complicated, so the contents of the culture medium can be simplified and the fermentation conditions can be controlled on the fermentation phase, which can reduce impurities and strain metabolites in extraction, simplify extraction steps and improve yield.

APPLICATION OF POLY GLUTAMIC ACID

The microbe-synthesized poly glutamic acid is the biodegradable, edible and environment-friendly polymer with a high molecular weight. Its molecular chain includes massive free carboxyl, so it is high-performance, e.g. stronger hygroscopicity, chelate heavy metal and moisture retention, so it is extensively applied.

Food

As one traditional fermentation bean product in Japan, natto mainly includes the poly glutamic acid, which is accepted by human being as a food. The poly glutamic acid developed in recent several years is mainly used as the additive and protective agent in foods. The poly glutamic acid features efficient anti-freezing activity and slight odor, so it is suitable for the food freezing protective agent^[40]. It can promote mineral absorption due to its excellent hygroscopicity and biodegradability^[41]. Some researches indicate that the poly glutamic acid can improve the anti-freezing performance of the yeast^[44], reduce frozen storage death rate of the yeast cells^[43]. When the fermentation duration is long, it can improve the fermentation capability of the yeast in the late period^[44]. Ding Shanshan^[45] feeds 1% poly glutamic acid into the frozen sweet dough and bread and finds that it is a better protective agent for the frozen dough, which can reduce the content of frozen water and improve specific volume, quality structure and sense quality of the sweet dough after constant-temperature frozen storage and freezing and thawing circulation.

New agriculture

Research on agricultural production of the poly glutamic acid was launched from 90s of 20th century and mainly covers influence of the poly glutamic acid on the crop's seed activity. Later, the poly glutamic acid can improve the soil damage due to long-term use of the fertilizer in the agriculture, so it is gradually accepted by the market. Now the poly glutamic acid is mainly used as the agricultural water retention agent. The commercial organic fertilizer made of the poly glutamic acid is in implementation. The poly glutamic acid can be used as the fertilizer due to its degradation role. The poly glutamic acid can alleviate the damage of the fertilizer and pesticide and enhance their efficiency, so the poly glutamic acid is quickly developing. The gel made of the poly glutamic acid can exert better water retention effect due to its higher water absorbency. Li Zheng from Tianjin Industry University^[46] makes the aquogel by using the poly glutamic acid, which can absorb 42.5-time water in dissolution and expansion balance of the normal saline. After improvement, a composite aquogel of the poly glutamic acid and pullulan^[47], which can absorb 260-time distilled water. Zhang Chao^[48] studies and finds that the amount of retained water in the soil will significantly increase with growth of the content of the high absorbent resin of the poly glutamic acid. The water retained in the soil with the poly glutamic acid high-absorbent resin will be significantly higher than that of the contrast soil. It indicates that the poly glutamic acid not only significantly improves the water amount and retention capability of the soil, but also suppresses water evaporation in the soil and extends the water retention duration of the soil. Zhang Shigen^[49] tests growth of the pakchoi by using the γ -glutamic acid product. The test results indicate that γ -glutamic acid can increase the pakchoi output by 30% and reduce 30% of used fertilizers, so it has significant output growth and fertilizer saving effect. The glutamic acid can absorb, chelate and store the nutrients and store excessive nutrients. With growth of the plants, the glutamic acid can slowly release nutrients and avoid early attenuation and fertilizer lack. Cai Zhijian^[50] finds that γ -glutamic acid can promote phosphor release of the ground phosphate rock and can activate the ground phosphate rock. With growth of concentration of γ -glutamic acid, the released phosphor in the water-dissolved phosphor of the ground phosphate rock will gradually increase. γ -quarter bend active ground phosphate rock can enhance effect of the yield of the pakchoi compared to ground phosphate rock. The growth rate reaches 23.6%.

Cosmetics

γ -PGA has stronger moisture retention capability. When it is added to the cosmetics or maintenance products, it can effectively increase the moisture retention capability and promote skin health. Now the hyaluronic acid is publically recognized to have the strongest moisture retention capability. The moisture retention effect of γ -PGA is 2-3 time higher than it of the hyaluronic acid, so γ -PGA is new-generation biological technology moisture retention content. The γ -poly glutamic acid glue is a colorless, tasteless, transparent, and soft gel and features super water absorption and slow release capability, better film formation capability and better soft and smooth function. γ -PGA can improve the long-term effect and high moisture retention effect of the skin, can effectively reduce the water loss due to the skin, promote skin elasticity, improve the natural moisture retention content of the skin, can whiten skin^[51], suppress the melanin and maintain pH value of the skin.

CONCLUSIONS

The poly glutamic acid is extensively focused by persons due to its excellent performance and extensive application, so massive manpower and finance resources are invested to study it. Now the large-scale producers of the poly glutamic acid

mainly include Taiwan Vedan Group, Japan AJINOMOTO and America ADM. Vedan branch was established in 1991 in Ho Chi Minh City, Vietnam, which occupied 120 ha. The poly glutamic acid plant is in formal operation. The poly glutamic acid evolves late in China, the fermentation level is low and the production cost of the products is higher, so it extremely restricts large-scale industrial production of γ -PGA and its application in different fields. Taiwan Vedan has successfully developed out γ -PGA by using the glutamic acid as the foundational raw materials and natto bacteria fermentation technology, so Vedan becomes a representative company in the industry. Vedan has applied multiple patents in Taiwan, Japan, America, China mainland and Europe. On Nov 25, 2013, Vedan held the new product promotion meeting in Nanjing together with the Yantai Hongyuan Bio-Fertilizer Technology Co., Ltd. as the copartner of Vedan in agricultural material, which further promotes the market and position of the poly glutamic acid in China mainland. With application promotion and process maturity of the poly glutamic acid, poly glutamic acid will play a more important role in different fields.

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