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A novel PGA/TiO2 nanocomposite prepared with poly(γ-glutamic acid) from the newly isolated *Bacillus subtilis* 17B strain

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Abstract: Poly(γ-glutamic acid) (PGA), naturally produced by *Bacillus* species, is a biodegradable, non-toxic, biocompatible, and non-immunogenic negatively charged polymer. Due to its properties, it has found various applications in the food, cosmetic and pharmaceutical industries. In this work, *Bacillus subtilis* 17B was selected as the best PGA producer among fifty wild-types *Bacillus* strains tested and characterized as a glutamate-independent producer. The production of PGA by the newly identified strain was optimized and increased tenfold using the Box-Behnken experimental design. The purity of PGA after recovery and purification from the fermentation broth was confirmed by SDS-PAGE followed by Methylene Blue staining. PGA was characterized by ESI MS and used for the preparation of a new nanocomposite with TiO_2. The synthesis of PGA/TiO_2 nanocomposite, its structural analysis, and cytotoxic effect on the cervical cancer cell line (HeLa cell) was investigated to determine the potential anti-cancer usage of this newly prepared material. Encouraging, PGA/TiO_2 nanocomposite showed an increased cytotoxic effect compared to TiO_2 alone.

Keywords: PGA production; wild-type *Bacillus* strain; PGA characterization; cytotoxicity.

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INTRODUCTION

Polyglutamic acid (PGA) is a biodegradable naturally occurring biopolymer that consists of L- and D-glutamic acid. PGA is a negatively charged water-soluble polymer. Unlike amino acids in polypeptides, glutamic acid monomers in PGA create amide bonds between α-amino and γ-carboxyl groups.[1] PGA was first discovered in 1937. as a major component of the *B. anthracis* capsule.[2] Owing to its biodegradability, PGA has various current and potential applications including drug carrier, anti-cancer drug delivery treatment of rheumatoid arthritis, genes, protein and peptide delivery, water treatment polluted by heavy metal and basic dye adsorber, thickener, and bitterness relieving agent, cryoprotectant, etc.[3] PGA proved to be an efficient drug delivery matrix as a nontoxic, biodegradable, biocompatible, and non-immunogenic polymeric material for water-soluble and insoluble drugs and other molecules as nanoparticles.[3] Negatively charged serum proteins and erythrocytes were reported to not aggregate with anionic polymeric carriers (e.g. PGA), which overcame serum inhibitory effects.[4] It is also known that polymeric nanoparticles can collect specifically in cancer tissues,[5] making them effective anticancer drug carriers. Studies of titanium dioxide nanoparticles and their various organic compound hybrid materials are becoming increasingly important due to their potential use in novel medical therapies. Furthermore, to improve efficient anticancer and antimicrobial therapies, many other approaches utilizing TiO_2 have been tested.[6] The importance of the development of new formulations is also indicated by the fact that increasing the therapeutic efficiency of TiO_2 can be achieved by using nanocomposites.

Currently, the majority of commercial γ-PGA is made by cost-effective microbial fermentation from biomass instead of chemical synthesis, peptide synthesis, or biotransformation. Polyglutamic acid is mainly produced by bacteria of the genus *Bacillus*. Most common species include *B. licheniformis*, *B. subtilis*, *B. megaterium*, *B. pumilis*, *B. mojavensis*, and *B. amyloliquefaciens*.[1] A few unusual PGA producers include the halophilic archaebacterium *Natrialba aegyptiaca*[7] and the Gram-negative bacterium *Fusobacterium nucleatum.*[8] These microorganisms produce PGA as an extracellular viscous material during fermentation that can then be isolated and purified.[9]

Bacillus species are widely used industrial organisms due to their high growth rates, short fermentation cycle times, and relatively inexpensive nutritional requirements.[10] The types and characteristics of a PGA-producing bacterial strain dictate the medium composition. Firstly, all PGA-producing bacteria can be divided into glutamic acid-dependent and independent.[11] Glutamic acid-dependent strains require the presence of L-glutamic acid in the fermentation broth, usually, in the range of 20-30 g L^{-1}.[12] For successful PGA production, it is necessary to take into account the individual but also synergistic impact of the above factors for the design of bacterial growth and production medium. According to the literature, the

DoE approach has successfully been used for carbon and nitrogen source selection for PGA production.[13] To find optimal production conditions, it is necessary to use a statistical method that takes into account the analysis of the synergistic effect of at least three important factors, such as Box-Behnken Design (BBD). There are examples in the literature where a high level of production of a significant product was obtained by using BBD to optimize the microbiological process.[14]

As the possibility of preparation of PGA/TiO_2 nanocomposite and the potential of its anticancer activity has not been investigated so far, this was set as the goal of this work.

In this study, the best PGA producer among 50 different natural *Bacillus* isolates originating from the soil, and different locations in Serbia, was selected and identified. In order to achieve the highest level of PGA production influence of carbon source was analyzed using the DoE approach (BBD) where concentrations of glucose, glycerol and glutamate were varied. Purified PGA was used for the preparation of nanocomposite with TiO_2. Both, PGA and PGA/TiO_2 were structurally characterized by ESI MS. PGA/TiO_2 cytotoxicity was examined using cervical cancer (HeLa) cells.

EXPERIMENTAL

Chemicals

Unless otherwise stated, all reagents and solvents were purchased from Merck (Darmstadt, Germany) and Sigma-Aldrich (St. Louis, MO, USA). All chemicals were of the best available purity and used without further purification.

Screening of γ-PGA producers

PGA production was investigated in 50 isolated wild-type strains of *Bacillus* sp. An overnight culture of each strain was prepared by incubation of a single colony in 5 mL of sterilized Luria–Bertani (LB) medium and grown for 18 h at 37 °C and 150 rpm. The screening medium was prepared according to the medium most commonly used for PGA production and contains per liter:[15] glucose, 100 g; sodium glutamate, 0.5 g; citric acid, 0.5 g; NH_4Cl, 10 g, KH_2PO_4, 0.34 g; Na_2HPO_4 x $12H_2O$, 0.895 g; MgSO4 x $7H_2O$, 0.5 g; $FeCl_3$ x $6H_2O$, 0.04 g; $CaCl_2$ x $2H_2O$, 0.15 g; $MnCl_2$ x $4H_2O$, 0.26 g. The fermentation broth was prepared by mixing 2 mL of an overnight culture of each strain with 48 mL of the screening medium. Fermentation was carried out for 5 days at 37 °C and 150 rpm. The best producer strain was selected and used in further experiments.

Identification of selected strain

The selected *Bacillus* 17B strain was identified by 16S rRNA gene sequence analysis. Total DNA from *Bacillus* 17B strain was isolated using the phenol-chloroform extraction method previously described by Hopwood and co-authors with minor modifications.[16] Logarithmic phase cells were treated with lysozyme (4 mg mL^{-1}, for 15 min at 37°C) prior to treatment with 2 % SDS. The 16S rRNA gene was amplified using 27F (5'- AGA GTT TGA TCC TGG CTC AG - 3') and 1492R (5'-GGT TAC CTT GTT ACG ACT T-3') universal primers.[17] The amplified PCR fragment was sequenced by a Macrogen sequencing service (Macrogen Europe, Amsterdam, Netherlands). Sequence annotation and a database search for

sequence similarities were completed using the BLAST program of the National Center for Biotechnology Information – NCBI.[18]

Optimization of PGA production media

BBD (Box Behnken Design) was used to identify optimal concentrations of three different carbon sources using Design-Expert software (Version 11, Stat-Ease, Inc, USA). Three analyzed factors were glucose (A), glycerol (B), and glutamate (C). The evaluated response Y was the level of γ-PGA production (g L^{-1}). All three factors (A, B and C) were studied at three different levels (concentrations) (Table S-I) through sets of 14 experiments (Table S-II). The other components of the medium were constant (citric acid, 0.5 g; NH_4Cl, 10 g, KH_2PO_4, 0.34 g; Na_2HPO_4 x $12H_2O$, 0.895 g; $MgSO_4$ x $7H_2O$, 0.5 g; $FeCl_3$ x $6H_2O$, 0.04 g; $CaCl_2$ x $2H_2O$, 0.15 g; $MnCl_2$ x $4H_2O$, 0.26 g). The fermentation broth was prepared by mixing 2 mL of an overnight culture with 48 mL of medium. Production fermentations were carried out for 5 days at 37 °C and 150 rpm in a thermostat shaker (KS 4000i, IKA).

The experimental data of the BBD was represented in the general form of the two-factor interaction (2FI) model as shown in Equation 1, to develop an empirical model which will be used to analyze the effect of factor interactions.

$$y = \beta_0 + \sum_{i=1}^{q} \beta_i x_i + \sum_{1 \le i \le j}^{q} \beta_{ij} x_i x_j + \varepsilon \quad (1)$$

where Y is the predicted response (PGA yield), β_0 is a constant coefficient, q is the number of variables, β_i is the linear coefficient, β_{ij} represents the interaction coefficient and x_i and x_j are the process variables and ε is the residual. Analysis of variance (ANOVA) was used to assess the significance of the model and the impact of coefficients in regression analysis.

Isolation of γ-PGA from the culture broth

The culture broth was centrifuged at 10000 *g* for 20 minutes at 25 °C. The supernatant was adjusted to pH 3 using 1 M H_2SO_4 and stored at 4 °C for 12 h. The precipitate was removed by centrifugation at 10000 *g* for 20 minutes at 25 °C. The resulting supernatant was mixed with ice-cold ethanol in a ratio of 1:3 (v:v). The PGA precipitate formed was separated after centrifugation at 5000 *g* for 20 minutes at 4 °C. The obtained PGA precipitate was dissolved in distilled water and purified using a Sephadex G-25 column. The remaining proteins in the PGA solution were hydrolyzed with 50 μg mL^{-1} Proteinase K,[19] and the resulting mixture was ultrafiltered through a Microcon membrane with a 30 kDa cut-off to protein hydrolysis products. The retentate (PGA) was air-dried and used for further analysis.

To confirm the purity of the isolated PGA, SDS PAGE was performed using a Hoefer™ Mighty Small™ II Mini Vertical Electrophoresis System with a 10 % polyacrylamide gel.[20] The gel was stained by the modified method of Yamaguchi *et al.*[21] To verify the presence of proteins, the gel was first stained with Coomassie Brilliant Blue (G-250) and rinsed with 7 % acetic acid/ 5 % ethanol. After a short rinse with distilled water, PGA in the gel was stained with 0.5 % methylene blue dissolved in 3 % acetic acid. The gel was rinsed with distilled water.

Fourier-transform infrared spectroscopy

Fourier-transform infrared spectroscopy (FTIR) of PGA and TiO_2 was done using Thermo Electron Corporation Nicolet 380 Spectrometer in attenuated total reflection (ATR) mode. Spectra resolution was 4 cm^{-1} in the range of 4000-400 cm^{-1}.

Electrospray ionization mass spectrometry

An isolated polymer of glutamic acid and acidic hydrolyzed PGA was analyzed by electrospray ionization mass spectrometry (ESI MS) on a Waters Quattro micro API mass spectrometer. All samples were previously diluted with 0.1 % water solution of formic acid to the concentration of 0.1 mg mL^{-1}, whereas the hydrolysate was diluted 1000 times, to avoid the influence of the high concentration of HCl used for its hydrolysis.

PGA was hydrolyzed by using a microwave digestor (Milestone START D, SK-10T) in the presence of 6 M HCl. The process was performed according to the manufacturer´s instructions and it was finished within 20 minutes.

The spectra acquisition was done in the positive ion mode in the mass range from 50 to 4000 Da. The temperature of the ionization source was 125 °C, of a gas carrier 380 °C, the capillary voltage was 3 kV, whereas the conus voltage was in the range from 20 to 90 V, depending on the sample.

PGA/TiO2 nanocomposite

Formation and characterization of nanocomposite

Colloidal spherical TiO_2 nanoparticles (average diameter, d~5 nm) were synthesized by the modified method of Rajh *et al.*[22] Nanoparticle concentration was determined after dissolving the particles in concentrated H_2SO_4 by the concentration of the peroxide complex, as previously described.[23]

PGA and TiO_2 were mixed in different ratios (present in Table S-III) and incubated for 2 hours at 25 °C with agitation at 150 rpm. [24] A formed white PGA/TiO_2 nanocomposite (NC) precipitate was recovered by centrifugation at 10000 *g* for 30 minutes and air-dry.

Cytotoxic effect of nanocomposite

HeLa cells were cultured in Dulbecco's Modified Eagle's Medium supplemented with glucose, L-glutamine, fetal bovine serum, penicillin, and streptomycin solution, according to the supplier's instructions. Cells were seeded in flat-bottomed 96-well microtiter plates (2000 per well) and incubated overnight with varying concentrations of PGA, TiO2 nanoparticles and an NC. Samples were prepared in distilled water and incubation terminated after 48 h. Cell viability was determined using the sulphorhodamine B (SRB) assay.[25] The absorbance was measured at 550 nm with a reference wavelength of 690 nm in a microplate reader (Wallac, VICTOR2 1420 Multilabel counter, PerkinElmer, Turku, Finland). The results were presented as a percent of cell viability determined according to the following equation:

$$Cell\ viability\ (\%) = \frac{Absorbance\ of\ sample}{Absorbance\ of\ control} \times 100 \qquad (2)$$

The measurements were made in quintuplicate and the results were presented as the mean ± standard deviation.

RESULTS AND DISCUSSION

PGA producer screening

PGA is an extracellular polymer produced by certain *Bacillus* species including *Bacillus paralicheniformis* ATCC 9945a,[26] which was used as a standard PGA producer strain for comparisons with wild-type isolates. The PGA-producing ability of 50 different *Bacillus* sp. strains originating from Serbia, deposited in a laboratory bank UB483, was investigated. Screened *Bacillus* isolates produced PGA in the range of 0.40 to 4.07 mg mL^{-1}. The strain indicated as 17B exhibited

the highest yield of PGA (4.07 mg mL^{-1}), higher even than *B. paralicheniformis* ATCC 9945a (2.27 mg mL^{-1}) under the conditions used for fast selection of strains. Selected isolate (17B) was identified as *B. subtilis* according to the highest identity (99 %) with the 16S rRNA gene of the species *Bacillus subtilis* from the NCBI database. Strain *B. subtilis* 17B was selected for medium optimization, as it showed the highest PGA-producing capabilities.

Optimization of culturing conditions for PGA production

The Box-Behnken experimental design was set using the concentrations of carbon sources (glucose, glutamate, and glycerol) as variables and PGA yield was measured as a response (Table I). Response surface methodology (RSM) was used to determine the synergistic effect of glucose, glycerol, and glutamate concentrations on PGA yield, and the results are given in Table I.

Table I. Box-Behnken experimental design PGA yield.

Run	$c_{glucose}$ /g L^{-1} (A)	$c_{glycerol}$ /g L^{-1} (B)	$c_{glutamate}$ /g L^{-1} (C)	$Yield_{PGA}$, mgmL^{-1} (y)	
				Actual value*	Predicted value
1	10	0	2.75	13.9	13.7
2	80	0	2.75	28.0	28.3
3	10	50	2.75	38.6	37.0
4	80	50	2.75	34.0	32.9
5	10	25	0.5	18.8	23.6
6	80	25	0.5	28.4	32.6
7	10	25	5	28.0	27.1
8	80	25	5	30.0	28.6
9	45	0	0.5	20.6	17.7
10	45	50	0.5	40.0	38.5
11	45	0	5	21.6	24.3
12	45	50	5	27.3	31.4
13	45	25	2.75	37.4	27.9
14	45	25	2.75	25.2	27.9

*All data are significantly different from each other according to Tukey's test ($p<0.05$).

The regression analysis has produced a two-factor interaction (2FI) model that describes PGA yield as a function of independent variables and their interactions in terms of coded factors:

$$PGA\ yield\ (mgmL^{-1}) = 27.99 + 2.64A + 6.98B - 0.113C - 4.68AB - 1.90AC - 3.42B \quad (3)$$

The coefficients are obtained based on the least-squares method in such a way that the sum of the squares of the errors, ε, is minimized. A positive coefficient value indicates that this variable has a positive effect on PGA yield, while a minus indicates a negative impact. Statistical analysis was performed to assess the significance of the model used and the coefficients in the 2FI regression equation. ANOVA parameters are shown in Table S-IV. Models F and p values, 3.88 and

0.049 respectively, indicate that the model is significant. Both glucose and glycerol have a positive impact on PGA production, while glutamate has a negative impact. Based on ANOVA, only glycerol shows the most significant impact on the increase of PGA yield. The two-factor interactions of these components have insignificant negative impacts. Response surface 3D graphs show the two-interactive effects of independent variables on PGA yield (Figure S-1). Figure S-1a represents the combined effects of glucose and glycerol concentrations, while Figure S-1b shows the effects of glycerol and glutamate concentrations on PGA production. Glycerol has a higher linear coefficient and steeper rise than glucose (Figure S-1a), thus positively impacting PGA production. Even though glutamate has a negative effect on PGA yield with its linear coefficient being -0.113, its impact is barely detectable. Neither graph reveals a finite plateau, implicating a possibility of further medium and yield improvement. According to the results, a minimal concentration of glutamate (0.5 g L^{-1}) is optimal. Also, it is necessary to provide a high concentration of glucose and glycerol (80 g L^{-1} and 50 g L^{-1}, respectively) for the production of 38 g $L^{-}1$ of PGA.

The obtained result indicates that *B. subtilis* 17B used in this study is a glutamate-independent strain, which in the literature implies a potentially lower production cost. [27] In recent years, studies on the synthesis of PGA have mostly concentrated on glutamate-dependent strains.[28,29] However, the main limitation for large-scale production of PGA is the high production costs due to the addition of significant amounts of expensive exogenous L-glutamate.[30] Since glutamate-independent strains could significantly lower the cost of PGA synthesis and simplify the fermentation process, more attention is given to them nowadays.[31] Glutamate-independent strains were considered ineligible for industrial γ-PGA production, limited by their low γ-PGA productivity,[31] but based on the results shown in this study this state could be modified. In a screening study, *B. subtilis* 17B strain showed two times higher productivity of PGA than *B. paralicheniformis* ATCC 9945a and after optimization of the cultivation, productivity was improved tenfold.

Isolation and purification of PGA

PGA was purified from the fermentation broth from accompanying small molecules including yellow pigment and proteins (using Proteinase K) and analyzed by SDS-PAGE after standard CBB staining and Methylene Blue staining.[32] SDS PAGE electropherogram is given in Figure 1. Samples before Proteinase K treatment are rich in proteins (line 1). After the treatment with Proteinase K, most proteins were removed from samples (line 2). Detection of PGA in SDS PAGE was not possible by staining with CBB, therefore the basic dye Methylene Blue was used for PGA's visualization (Figure 1, MB). This is most likely because polyglutamic acid is a negatively charged polymer with no hydrophobic regions that could be stained using CBB.[33] The polymer (1-2 MB)

showed multiple wide blue bands. Methylene blue staining of PGA was better after proteinase treatment, presumably because of the stronger interaction of PGA and methylene blue in absence of proteins. This phenomenon could be due to the formation of electrostatic interactions between PGA and proteins from the sample, thus reducing the number of free functional groups available for interaction with Methylene Blue. Furthermore, successful basic dye staining proved the presence of a negatively charged polymer.

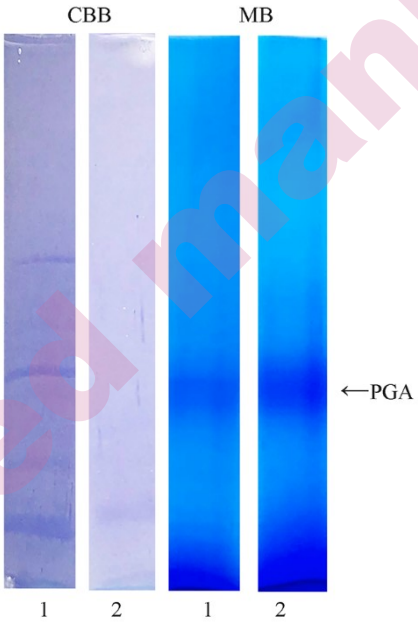

Fig. 1. SDS PAGE electropherograms of PGA produced by *B. subtilis* 17B stained with CBB and Methylene Blue (MB). 1 – before; 2 - after Proteinase K treatment.

Mass spectrometry of isolated PGA

Detection of the number of Glu units in the PGA was determined by ESI MS, and the identity of individual signals was confirmed by comparison with hydrolyzed polymer and standard (Glu). The sample ionization has been assisted by the addition of formic acid, in order to increase the ion yield, and the positive ion ESI mass spectra are given in Figure S-2, whereas the signal identity is listed in Table S-V. ESI MS is, a so-called, “soft” ionization technique,[34] and the extent of fragmentation are low. Mostly, the loss of H_2O, CO or CO_2 from the -COOH group could be detectable.

In summary, all detected ions imply that isolated polymer contains Glu and that the highest number of Glu units is 15 which is in agreement with the literature.[35]

Characterization of PGA-TiO_2 nanocomposite systems and potential application

The potential application of PGA produced by *B. subtilis* 17B was tested by the formation of PGA/TiO_2 NC for the possibility to increase the cytotoxic effect of TiO_2 nanoparticles against HeLa cells. To examine nanocomposite formation, we recorded the spectra of purified PGA, PGA/TiO_2 nanocomposite, and TiO_2 nanoparticles (Figure S-3).

The band at 1650-1660 cm^{-1} in the PGA FTIR spectrum can be identified as the amide I band. The signal at 1079-1116 cm^{-1} is the result of a C-N bond. The low-intensity signal at 1450-1457 cm^{-1} originates from weak carbonyl absorption and is consistent with the literature.[36] The over 3000 cm^{-1} region is of lower intensity, presumably because the sample was isolated from the basic medium making it mostly deprotonated. This reduces the number of hydrogen bonds that otherwise amplify the O-H and N-H bond signals. To this broad region belong signals of aliphatic N-H stretching that have no clear bands. However, region 3409-3442 cm^{-1} can be distinguished and may correspond to OH group absorption.[37] All the assigned signals indicate a glutamic acid polymer, while the absence of a C-O bond confirms the absence of polysaccharide and that PGA was successfully purified.

Although the PGA/TiO_2 nanocomposite FTIR spectrum shows a significant difference compared to the PGA spectrum, the problem might arise from the concentration ratios, as the TiO2 signals dominate. It seems that all of the main signals from the PGA spectrum disappear in the NC spectrum, implying the existence of PGA-TiO_2 interaction. The high-intensity signals at 2321-2365 cm^{-1} for the PGA spectrum and 2312-2355 cm^{-1} for the nanocomposite spectrum originate from the asymmetric stretching of CO_2 that was present in both samples and it is stemming from the air.[38] However, the interaction between these two components is demonstrated by slower sedimentation of TiO_2 nanoparticles in the presence of PGA in the solution. The experiment that was performed by centrifugation of suspensions containing varying PGA: TiO_2 mass ratio at 12 000 × g was done. Whereas only a short impulse was required to sediment TiO_2 nanoparticles from a physiological solution without PGA (pH 7), at the PGA: TiO_2 ratios at 1:25 to 1:2, more than 30 minutes of centrifugation was necessary, which indicates stabilization of TiO_2 in the physiological solution. Since the electrostatic interactions between an organic polymer (alginate) and TiO_2 nanoparticles resulting in their stabilization has already been demonstrated,[39] we assume that in a similar manner, a -COO- group interacts with the surface of TiO_2 nanoparticles.

In the next step, we preliminarily tested the cytotoxicity of all components and the NC system against HeLa cells. Namely, colloidal TiO_2 nanoparticles form a

stable colloidal solution at pH 2, which is not in a physiological range and limit their application in biological systems.[40] Therefore, our hypothesis was that the addition of PGA will stabilize the system and enable its application in the physiological range.

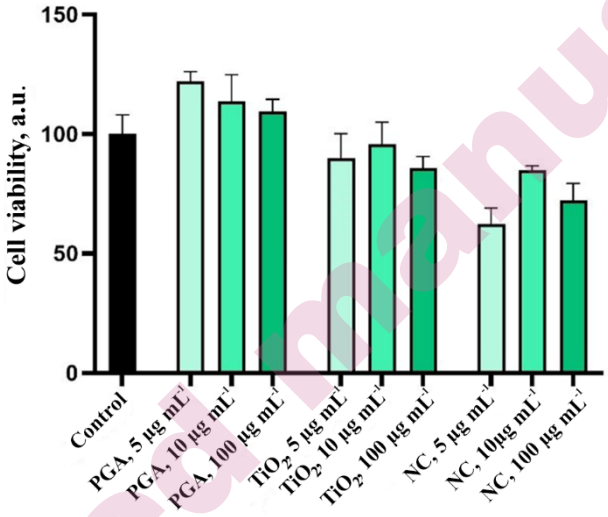

Fig. 2. HeLa cells viability after treatment with three different concentrations of PGA isolated from *B. subtilis* 17B, TiO_2 nanoparticles, and PGA/TiO2 NCs.

As presented in Figure 2, the addition of PGA produced by *Bacillus* sp. 17B increased the cytotoxic effect of TiO_2, whereas PGA alone showed no cytotoxicity, as compared to control/untreated cells. Although the TiO_2 nanoparticles demonstrated a light-induced cytotoxic effect, there are also data that show that these nanoparticles can also be cytotoxic in the dark,[41] and our results are in line with these findings.

These preliminary results demonstrated the potential application of PGA as stabilizers for TiO_2 nanoparticles that have various biological applications,[42] but the mechanism of its action and other physicochemical properties of the system need to be further investigated in more detail.

CONCLUSION

The newly isolated strain of *B. subtilis* 17B, a natural isolate from the soil, a promising PGA producer (initially showing twice the PGA production compared to the commercially used strain *B. paralicheniformis* ATCC 9945a) produced a high level of PGA after applying statistical optimization methods (PGA production increased tenfold). *B. subtilis* 17B was characterized as glutamate-independent

which candidate it as a promising strain for PGA production due to the cost-effectiveness process. The PGA-TiO_2 nanocomposite showed better cytotoxicity toward HeLa cells than TiO_2, which opened up the possibility of application in biological systems considering that TiO_2 nanoparticles are not stable in a physiological pH range.

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SUPPLEMENTARY MATERIAL

Additional data are available electronically at the pages of journal website: https://www.shd-pub.org.rs/index.php/JSCS/article/view/12132, or from the corresponding author on request.

И З В О Д

НОВИ ПГК/TiO_2 НАНОКОМПОЗИТ ДОБИЈЕН ОД ПОЛИ(γ -ГЛУТАМИНСКЕ КИСЕЛИНЕ) ИЗ НОВОИЗОЛОВАНОГ СОЈА *BACILLUS SUBTILIS* 17Б

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Поли(γ-глутаминска киселина) (ПГК), коју производе бактерије рода *Bacillus*, је биоразградив, нетоксичан, биокомпатибилан и неимуноген негативно наелектрисани полимер. Због својих својстава нашао је разноврсну примену у прехрамбеној, козметичкој и фармацеутској индустрији. У овом раду, *Bacillus subtilis* 17Б је изабран као најбољи ПГК продуцер међу педесетак тестираних природних изолата бактерија из овог рода и окарактерисан као глутамат независтан продуцер. Производња ПГК овим новоидентификованим сојем је оптимизована и десетоструко увећана коришћењем Box-Behnken експерименталног дизајна. Чистоћа ПГК након изоловања и пречишћавања из ферметационе течности је потврђена електрофорезом (SDS-PAGE) након бојења метиленским плавим. ПГК је окарактерисана масеном спекроскопијом (ESI MS) и коришћена за добијање новог нанокомпозита са TiO_2. Синтеза ПГК/TiO_2 нанокомпозита, његова структурна анализа и цитотоксични ефекат на ћелијску линију рака грлића материце (HeLa ћелије) је испитан да би се утврдила потенцијална употреба овог новодобијеног материјала у борби против ћелија рака. Нанокомпозит ПГК/TiO_2 показао је повећан цитотоксични ефекат на поменуте ћелије рака у поређењу са самим TiO2.

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