REVIEW

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of the materials submitted for participation in a competition for the academic position "Professor" in the field of higher education 4. Natural sciences, mathematics and informatics; professional field 4.3. Biological sciences; scientific specialty Microbiology - genomics and regulation of gene expression in prokaryotes

Common part

The competition for "Professor", published in SG no. 47 / 22.05.2020 is in the field of higher education 4. Natural sciences, mathematics and informatics, professional field 4.3. Biological sciences, scientific specialty Microbiology - genomics and regulation of gene expression in prokaryotes and has been announced for the needs of Laboratory "Microbial Genetics", Department of General Microbiology, IMiB - BAS. The only candidate is Assoc. Prof. Penka Mladenova Petrova from the same institute. The review of the documents shows that the procedure for opening and announcing the competition has been followed. The presented set of materials in paper and electronic form is in accordance with the Regulations for the development of the academic staff of the Bulgarian Academy of Sciences, BAS and fulfills the criteria for acquiring the academic position of "professor".

Brief biographical data

Penka Petrova graduated from the Faculty of Science at Sofia University “Kl. Ohridski ”, specialty Biotechnological Processes, Genetic and Cell Engineering in 1994. In 2003 she defended her dissertation on“ Creation of a gene cloning system for Streptococcus thermophilus ”and obtained the scientific degree“ PhD ”in scientific specialty 01.06.12 - Microbiology . From 2003 to 2009 she worked at IMiB successively as a research associate, III, II, I degree, and from 2011 she was elected associate professor. From 2013 to 2020 she was Head of the Laboratory of Gene Expression, IMiB; from 2015 to 2020 she was chairman of the commission for work with GMOs (in IMiB) at the Ministry of Environment and Water; since 2018 she is the head of the Department of General Microbiology and the head of the Laboratory of Metagenomics and Gene Expression. In 2019 she was elected director of IMiB. In 2020 she defended her dissertation on "Molecular biological research of new bacterial glycoside hydrolases with industrial application" and obtained the degree of "Doctor of Science" in Microbiology.

General presentation of scientific papers

Assoc. Prof. Petrova has published a total of 68 scientific papers in the field of the competition. 38 of the publications are in prestigious IF journals and have a total IF of 54,785. Data from Scopus show that her works have been cited more than 400 times, with an h-index of 11. She has participated in 46 national and international scientific forums.

In the current competition, apart from the dissertation for the acquisition of PhD and the competition for associate professor, Assoc. Prof. Petrova has presented 33 scientific papers. Of these, 17 are publications in refereed journals with IF, one is a publication with SJR, two are without IF / SJR but with quartiles (Q4) and two are book chapters in prestigious publishers such as Taylor & Francis Group and Elsevier. One patent is also presented. The materials for participation in the competition include 10 more articles and reports from proceedings of international conferences without IF / SJR and quartiles. In more than half of the publications, Assoc. Prof. Petrova is the first or corresponding author. The publications submitted for the competition have a total IF of 31,078 and are cited 156 times according to data from Scopus. Assoc. Prof. Petroa has participated in 19 scientific forums since taking up the academic position of "Associate Professor"

Of the presented publications, 5 are distinguished as habilitation work. According to the quartiles in which the Journal of Citation Reports (JCR) of the Web of Science groups the scientific journals, three are with Q1, one is with Q3 and one is with Q4, with which Assoc. Prof. Petrova has 105 points and covers the required 100 points according to the Regulations for application. The indicators from group G are as follows: three publications with Q1; three publications with Q2; two publications with Q3; one publication with Q4; two chapters of books and one patent, collecting a total of 245 points (minimum 200 points required). According to the citations indicator for the period 2012-2020, 200 citations are presented, which corresponds to 400 points (minimum 100 points required).

Scientific contributions

The contributions of Assoc. Prof. Petrova's research are in three main areas: proving new enzymes through genomic, transcriptional and enzymological approaches; sequencing of genomes and metagenomas and application of genetic engineering methods to construct new bacterial producers of acids and fuels.

Applying modern methods in the field of genomics and transcriptomics, Assoc. Prof. Petrova conducts in-depth research on enzymes responsible for the breakdown or synthesis of prebiotic carbohydrates (inulin, fructooligosaccharides, galactooligosaccharides and starch) from lactic acid bacteria. The genetic approaches used to identify and strain the LAB, as well as a detailed analysis of the geographical biodiversity of strains, are described in papers 4, 24 (reference B) and 1 (reference G). A study of the Bulgarian LABs revealed some of the world's first producers of amylase, β-fructosidase and β-galactosidase. For the first time, genes responsible for the hydrolysis of α-glucans in some LAB species have been identified and amylolytic members of Lactobacillus sakei and Enterococcus have been isolated. A transcription analysis was performed and the regulation of the gene expression of starch-modifying enzymes was studied (5, 6, 9, 12, 14, 15, 20, 21 and 25 - reference G).

It has been shown that Bulgarian LAB strains isolated from fermented cereals and beverages can grow in an environment containing prebiotic carbohydrates as the only carbon source (15 and 20). Phylogenetic analysis reveals that the amylolytic LABs belong to 4 main groups of bacteria: L. casei / paracasei, L. plantarum / pentosus, P. acidilactici / pentosaceus and E. faecium / durans. Amylolytic representatives of L. fermentum species, Str. bovis, L. sakei and

Lc. lactis and the expression of the genes: amy1, glgB, agl, malL, treC and dexC has been demonstrated (15). Bioinformatics analysis of genes and enzymes shows that the protein encoded by treC in L. paracasei is an ortholog of the malL gene. Boza strains isolated from amylolytic and probiotic properties have been described for the first time, combining amylolytic and probiotic properties and having antimicrobial efficacy against pathogenic species E. coli, K. pneumoniae, V. cholerae and Listeria monocytogenes (6 and 12).

Examination of 115 ICDs revealed four strains capable of degrading long-chain inulin: L. paracasei B41, LC1 and 7, and P. acidilactici PD3 (20). The inu gene of L. paracasei B41 has been sequenced and has been shown to encode a new cell-associated fructan-β-fructosidase. The enzyme was successfully purified from the cell wall and its biochemical parameters were determined. Wall-localized enzymes have been shown to have a different number of cell wall binding domains, ranging from 4 to 6. For the first time in L. paracasei, the key effect of manganese ions (Mn2 +) on the activity of the enzyme inulinase has been described and demonstrated. expression of the inulinase gene (demonstrated by RT-Real time PCR), transport of fructose through the cell wall and enzymes from the glycolytic pathway. A new biotechnological process for microbial production of fructose from inulin has been developed (4 and 5 of reference B).

The ability of Rhodope strains of L. delbrueckii ssp. bulgaricus to synthesize galactooligosaccharides (GOS) with a proven bifidogenic effect. An atypical binding of β- (1 → 4) to the last galactose unit has been demonstrated and this is probably related to the unique beneficial properties of Bulgarian yoghurt (4 - reference D). Strains of the genus Bacillus have been shown to produce extracellular enzymes involved in the degradation of starch and lignocellulosic substrates (1, 3, 10, 11 - reference G). The first experimental evidence for the ability of B. velezensis, B. toyonensis and B. safensis to break down lignocellulosic carbohydrates is presented. It has been shown for the first time that they produce 2,3-butanediol, not only from the substrate glucose, but also from sugars in the composition of lignocellulose.

A new cgt gene and the enzyme cyclodextrin glucanotransferase have been identified in Bacillus pseudalcalophilus, and the amino acid sequence homology with previously known enzymes is below 87%. For the first time, a recombinant enzyme cyclodextrin glucanotransferase was immobilized in magnetically modified carriers to produce cyclodextrins. The reuse of CGT-ase magnetic biocatalyst shows the possibility of obtaining three to four times more cyclodextrins than the yield of single-use enzyme preparations (papers №10, 11, 18 and 23).

A gene encoding the enzyme neuraminidase from the non-toxigenic strain Vibio cholerae has been sequenced. Sequencing and characterization of the nanH gene and the enzyme encoded by it have made it possible to develop the safe production of sialidase (7).

For the first time, a metagenomic sequencing of a cellulose degrading community has been performed with application for waste degradation in manned space missions. The direction is new for IMikB and marks the beginning of metagenomic research of communities for space purposes (2 - reference B).

The genome of B. velezensis 5RB (NZ_QXJL01000001.1) (1 and 3 - D) was completely sequenced. The genome consists of 3.9 MB, contains 4,605 genes, of which 3,745 encode proteins, 81 - mRNA and 8 - rRNA. Gene analysis reveals the ability of B. velezensis to convert cellulose, lignocellulose, starch and inulin directly into valuable low molecular weight products. Seven complete clusters for the synthesis of antibiotics (macrolactin, bacillary, deficidin, fengicin, bacilbactin, bacillisin and surfactin) were also found in its genome. Due to its diverse metabolic capabilities, B. velezensis is a species with huge potential for new applications in biotechnology.

Part of the research of Assoc. Prof. Petrova is aimed at constructing new strains-producers. For the first time, a recombinant strain of Klebsiella pneumoniae G31 – A was constructed with the introduced α-amylase gene from Bacillus licheniformis 44MB82 / G. The recombinant strain is able to fully convert highly concentrated starch solutions to 2,3-butanediol. (8). The extracellular α-amylase gene was introduced for the first time in Zymomonas mobilis DSM 424. The Bacillus licheniformis gene was cloned into shuttle vectors pZT1, pZT2 and pZT3, under the control of the Plac (Escherichia coli) and SacC (Zymomonas mobilis) promoters, and its heterologous expression was demonstrated (16 and 17). Part of the studies (10, 11, 18 and 23) are dedicated to the successful heterologous expression in E. coli of the enzyme cyclodextring glucanotransferase (CGTase) of Bacillus pseudakaliphilus 8SB. The results presented in this section make a significant contribution as they are related to the formation of one of the first recombinant CGTases. Sequence analysis of the cgt gene revealed that the CGTase of strain 8SB was a new enzyme, with low similarity to those known until then (11). Successful extracellular secretion of the enzyme from E. coli strain BL21 (DE3) and the original purification steps allow the production of large amounts of recombinant enzyme (10). The construction of a strain of Klebsiella pneumoniae capable of producing 2,3-butanediol from starch is shown. The amylase amylase gene from strain B. licheniformis 44MB82 / G was cloned into the successful producer of 2,3-DB glycerol K.-pneumoniae G31 from patent (13). under the control of various promoters - Plac (Escherichia coli) and SacC (Zymomonas mobilis).

Assoc. Prof. Petrova also presented a vision for her future research. Guarantee for the success of the planned genetic studies of lactic acid bacteria and construction of new producers of valuable metabolites from renewable substrates, in addition to indisputable scientific achievements, experimental and administrative experience is the financial security provided by 7 ongoing projects.

Project activity

According to the completed report on the implementation of minimum national requirements (indicator E), Assoc. Prof. Petrova is a leader or participant in the development of 20 research projects funded mainly by MES, contracts with foreign companies, national and European scientific, operational and framework programs. The total value of the funds raised from projects under her leadership amounts to BGN 252,000, as BGN 225,000 are under a project financed by the Chinese company Bright Dairy & Food Co. Ltd.

Pedagogical activity

Under the supervision of Assoc. Prof. Petrova, two PhD thesis have been successfully defended. She was the scientific supervisor of 5 successfully defended graduates from Sofia University and of 42 trainees in Bulgaria and abroad. She is a holder of courses and training programs: Genetic engineering and gene expression in bacteria, Genetic engineering and recombinant DNA technologies, Genetically modified microorganisms, Molecular studies of lactic acid bacteria.

Expert activity

Assoc. Prof. Petrova has participated and is participating in various scientific councils and scientific expert commissions, scientific juries for awarding ONS "Doctor" and competitions for academic positions, examination commissions. She is a member of the Union of Scientists in Bulgaria, Section of Microbiology, Immunology and Virology, the European Federation of Microbiological Societies (FEMS), the editorial board of the Journal of Investigative Genomics, MedCrave Group, USA

Conclusion:

The documents and materials submitted by Assoc. Prof. Petrova meet all the requirements of the Academic Staff Development Act in the Republic of Bulgaria (RASRB), the Regulations for its implementation, the Regulations for the development of the academic staff of IMiB, BAS and exceed the minimum national requirements for acquiring academic position of "professor". The presented materials give me give me a reason to express my positive opinion regarding the candidacy of Assoc. Prof. Penka Petrova for the academic position of "professor". She is an established specialist with authority in the scientific community. As a member of the Scientific Jury for the announced competition, I give a positive assessment and recommend to the members of the esteemed Scientific Council of IMiB, BAS to elect Assoc. Prof. Penka Mladenova Petrova to the academic position "Professor in Professional Field 4.3. Biological sciences; scientific specialty Microbiology - genomics and regulation of gene expression in prokaryotes

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