REVIEW

Subject: The scientific production of Ch. Assistant Professor, Dr. Nadia Stoycheva Radchenkova for participation in a competition for the academic position "Associate Professor“for the needs of the Laboratory “Extremophilic bacteria ", Department of General Microbiology, IMicB-BAS in professional field 4.3. Biological Sciences. The competition was announced in State Gazette no. 29 of 09.04.2021

by Assoc. Prof. Dr. Zlatka Miltcheva Alexieva, Institute of Microbiology, BAS

Dr. Nadia Stoycheva Radchenkova, Assistant Professor in the Laboratory "Extremophilic Bacteria" at the Department of General Microbiology at the Institute of Microbiology, BAS is the only candidate who has submitted documents for the competition. The documents are presented in accordance with the requirements of the Law on the Development of the Academic Staff in the Republic of Bulgaria and the relevant Rules for its implementation.

Nadia Stoycheva Radchenkova has a master's degree in engineering and graduated with a degree in Information and Management Technologies with specialty "Information and Management Technologies" in 1999 at UCTM-Sofia. Since 1995 she has been working at IMicB, BAS. Since 1999 she has been a specialist, and since 2008 she has been an assistant in the Laboratory of Extremophilic Bacteria, Department of Applied Microbiology (now at the Department of General Microbiology). Since the beginning of 2013 she has been a doctoral student in independent training at IMicB, BAS. In 2014 she defended a dissertation on the topic: "Production and characteristics of exopolysaccharide (s) synthesized from the thermophilic strain Aeribacillus pallidus 418" and received the Educational and Scientific degree "PhD". Since 2014, after a competition, Dr. Nadia Radchenkova holds the academic position of "Chief. assistant ”at IMicB-BAS.

Her research interests include exopolysaccharides, isolation of productive strains-producers, determination of morphological, physiological, and biochemical properties of microorganisms, purification and characterization of newly isolated polymers.

MAIN SCIENTIFIC INDICATORS

The analysis of the data related to the indicators under the National Minimum Requirements of the Regulations for application of the Law on the Development of the Academic Staff in the Republic of Bulgaria shows the following:

50 points have been determined for the possession of Educational and Scientific degree "PhD". According to indicators from group B, 10 publications are presented: 2 with

Q1; 2 - with Q2; and 6 - others, which given in points gives a sum of 126 points. According to the indicators from group G, within indicator 7 are presented 11 publications, of which 6 - with Q1; 1 publication with Q2; and 4 publications are with Q3. Indicator 8 includes 2 chapters of books. These data give a total of 260 points on indicators G. From the indicator for citations of publications in world-famous databases with scientific information (Web of Science and Scopus) or a total of indicators D, 320 citations without autocitations are presented, as well as 10 in other publications, which collect 650 points. The total number of points included in group E indicators for participation in international and national research projects is 150.

The total number of points from the data filled in the Table for the minimum national requirements is 1236 with a minimum required 430 points.

The conclusion is that Dr. Nadia Radchenkova exceeds the requirements for each of the groups of indicators required for the scientific position of "Associate Professor" and generally scores several times more points than the mandatory national minimum.

Regarding "Additional criteria for the growth of the academic staff in IMicB" it can also be noted that the criteria are overfulfilled. For the competition, Dr. Nadia Radchenkova has submitted a total of 23 scientific papers, of which 21 scientific publications (15 referenced in WoS / Scopus and 6 in other refereed journals) and 2 chapters from books that are outside the dissertation, as well as 9 research projects. The impact factor of the publications submitted for this competition is 30,732.

SCIENTIFIC CONTRIBUTIONS

The scientific papers presented for the competition are works in the field of biodiversity among extremophile microorganisms (Bacteria and Achaea) and the synthesis of valuable biologically active substances (microbial polysaccharides and extremozymes), which leads to new original scientific contributions. The objects of special attention are the thermophiles inhabiting Bulgarian thermal springs and the halophiles from niches connected with the Black Sea salt pans. The results of these studies are the main contributions to the scientific activity of Ch. Assistant Professor Dr. Nadia Radchenkova.

For the first time a study was conducted and halophile bacteria were isolated in two Black Sea salt marshes (Pomorie and Burgas). The results show high taxonomic and metabolic bacterial diversity. All isolates (20 different moderately halophile and two halotolerant strains, grouped into 8 genera) were found to be alkalotolerant and 41% of them were psychrotolerant. More than half of the strains show antimicrobial activity, and nine of

them produce exopolysaccharides (EPS). The highest level of EPS was observed in strain 28 *Chromohalobacter canadensis*. **Xanthan lyase, gelanlyase, arabinase and phytase were first detected in halophile bacteria.** It is shown that the studied area has a high resource of halophile bacteria with biotechnological potential (Boyadzhieva et al, 2018 - G7).

The bacterial community in P18, the largest salt lake in the Pomorie salt pans (34% salinity), was studied in detail. Original results have been obtained that demonstrate great diversity with dominance of different genera that have not previously been reported as dominant. New 16S rRNA sequences have been identified, some of which form individual clones with a similarity of less than 85%. The sequences were assigned to 15 bacterial genera of four types and one candidate type. Representatives of the Firmicutes type predominate (47.5%) (Kambourova et al, 2017 - G7).

The halophile bacterium *Chromohalobacter canadensis* 28 (Pomorie salt marshes), which synthesizes a polymer containing polyglutamic acid (PGA), has been described for the first time. The optimum temperature for the synthesis of the biopolymer is 30 °C and pH 7.3 at a concentration of 15% NaCl. The dispersed composition and characteristics of model emulsions containing sunflower oil and different concentrations of biopolymer synthesized from the halophile strain *Chromohalbacter canandesis* were studied. **Dr. Radchenkova has a significant contribution to the development of engineering aspects of fermentation processes for EP synthesis.**

In order to efficiently scale the bioreactors in order to accurately estimate the oxygen transfer, the mass transfer parameters and the lowest values of Kla (mass transfer coefficient) suitable for the fermentation with the highest synthesis are determined. It was found that the continuous cultivation regime with low dilution rates. Optimization of the culture medium and physicochemical culture conditions led to a twofold increase in the production of the extracellular polymeric substance from the strain and a further increase to 3.08 mg / ml in the presence of surfactants. **The functional analysis of the properties of the produced polymer clearly outlines its potential for application in the cosmetics industry** (Radchenkova et al, 2018 - B; Radchenkova et al, 2020 - B; Kisov et al, 2018; - B).

Biotechnological interest stimulates the research of thermophile microorganisms. Exopolysaccharides (EPSs) from thermophile microorganisms are of special interest due to the advantages of thermophile processes and the non-pathogenic nature of polymer molecules. However, their industrial application is hampered by the relatively low biomass and correspondingly low yield of EPS.

Several thermophile EPS producers were isolated from Bulgarian hot springs and three of them belonging to different genera were selected for more detailed studies: *Geobacillus tepidamans* V264, *Aeribacillus pallidus* 418 and *Brevibacillus thermoruber* 423. The yield of EPS for strain *B. thermoruber* 42 is comparable to that of mesophilic bacteria. **Studies of their molecules show an unknown composition, high molecular weight and thermal stability.** EPS from *G. tepidamans* V264 demonstrated biological activity against cytotoxic compounds, while that from*B. thermoruber* 423 show good compatibility with monkey fibroblast cell lines. **The established functional properties and biological activity determined the possibility for their future application in cosmetics and medicine** (Kambourova et al, 2018 - B).

The ability of *Aeribacillus pallidus* 418 strain to synthesize higher EPS production in a continuous cultivation regime compared to batch cultures was eliminated, eliminating the time for cleaning and sterilization of the vessel. The synthesized EPS exhibits non-Newtonian pseudoplastic and thixotropic behavior, which determines its valuable physicochemical properties and commercial advantages. **The proposed continuous approach to exopolymer synthesis can have huge economic potential for industrial production of thermophilic polysaccharides.**

An original mathematical model of the process of synthesis of exopolysaccharides (EPZ) was created on the basis of experimental data with *Aeribacillus pallidus* 418, obtained from the process of periodic fermentation and modeling ideas borrowed from enzyme kinetics. The main feature of the proposed reaction schemes, resp. models, it is assumed that cellular biomass consists of two dynamically interacting cellular fractions. The applied mathematical model allows drawing conclusions about the basic biological mechanisms, formulating the latter in the form of simple steps of biochemical reaction. (Radchenkova et al, 2014 - B; Radchenkova et al, 2015 - B).

By comparative sequence analysis of genes for 16S rRNA and glycosyl hydrolase from family 57 (GH-57), archaeological and bacterial diversity has been determined in two Bulgarian hot springs, of different tectonic origin and different water temperatures (Levunovo spring - 82 ° C and Vetren Dol spring - 68 ° C). Analysis of the GH - 57 gene allows for increased resolution of biodiversity assessment and in - depth analysis of specific taxonomic groups. Phylogenetic analyzes revealed that more than 1/3 of the archaeological and 1/2 of the bacterial sequences showed less than 97% similarity to other known sequences. The analysis of the created branch libraries from the Levunovo spring shows that the sequences belong to five archeological groups from Crenarchaeota and Euryarchaeota, and

most of the Vetren Dol spring belongs to the Crenarchaeotic hot water group. The formation of a thermophilic group in Mehtanosarcinales is suggested (Stefanova et al, 2015 - G).

A high-yielding strain of *Brevibacillus thermoruber* 423 was isolated during screening for thermophilic exopolysaccharide producers. This bacterium is among the limited number of reported thermophilic EPS producers and shows the highest level of polymer synthesis. **For the first time, the entire bacterial genome of the thermophilic species Brevibacillus was analyzed. Major genes associated with EPS biosynthesis have been identified and, in combination with experimental evidence, a hypothetical mechanism for EPS biosynthesis has been developed.**

Studies in different culture conditions have shown optimal synthesis conditions. The chemical, rheological and biological characteristics of the EPS have been established. It has been established that in a bioreactor twice higher yields and three times higher synthesis are achieved than those described so far in the scientific literature.

B. thermoruber 423 has many potential applications in biotechnology and industry due to its ability to use xylose and synthesize EPS, isoprenoids, ethanol / butanol, lipases, proteases, cellulase and glucoamylase enzymes, as well as its resistance to arsenic. The strain combines the advantage of being non-pathogenic with rapid synthesis and has the potential of a model microorganism for the synthesis of EPS (Yasar Yildiz et al, 2014 - D; Yildiz et al, 2015 - G).

The research is a contribution to the establishment of the biological mechanisms and the overall genomic organization of the thermophilic producers of EPZ and provides an opportunity for the development of rational strategies for genetic and metabolic optimization of the production of biopolymers.

Extracellular bacterial lipases are promising biocatalysts for industry because they are stable and active enzymes from readily available sources.

Dr. Radchenkova participated in the process of about 20-fold purification of a thermostable enzyme produced by a strain of *B. stearothermophilus* MC 7. The molecular weight of the enzyme, the conditions for maximum activity, basic kinetic parameters (Km and Vmax) and affinity for substrates were determined.Based on these studies, the enzyme is classified as lipase, which also has esterase activity. The effect of nonionic surfactants on the enzyme has been studied. The highest activity (up to 2.3-fold magnification) was measured with PEG6000. Lipase preparations retain more than 60% of their activity after a 30-minute incubation at 75 ° C in the presence of Tween 60 or PEG4000. The thermostable lipase studied shows potential for the synthesis of structural lipids, such as dioleoylpalmitoyl

glycerol, used in healthy foods. The immobilized enzyme showed good operative thermal stability (half-life 50 days at 60°C) and a high degree of conversion (50% after 48 h in a solvent-free system) (Kambourova, M. et al, 2003; Guncheva, M. et al, 2007; Guncheva, M. et al, 2008 - G).

Lipase from *B. stearothermophilus* MC7 was immobilized on four polymeric carriers by physical adsorption: chitosan, DEAE-cellulose, polypropylene and polyurethane. The control reaction of the acidolysis of triolein with caprylic acid has shown that all four carriers are suitable for use. The highest degree of conversion of the original triolein was achieved in the presence of PP-MC7. The synthesis of the target di-substituted product (dicapryloyl-oleoylglycerol) was achieved by a selective mono-substitution reaction in the glycerol skeleton (Guncheva M. et al, 2009 - G).

In conclusion, it can be concluded that the studied thermostable lipase from the strain *B. stearothermophilus* MC 7 is one of the most stable described in the literature and with significant biotechnological potential.

In summary, the scientific contributions of Dr. N. Radchenkova highlighted in this section are distinguished by originality and significance, and the presented scientific developments fully correspond to the scientific field and scientific direction of this competition.

CONCLUSION

The scientific and scientific-applied contributions of Ch. Assistant Professor Dr. Radchenkova are united not only with the desired direct connection between fundamental and applied research but also with the skillful application and logical combination of a number of state-of-the-art research methodological approaches.

In her experimental work, Dr. Radchenkova uses a rich arsenal of classical and modern analytical, microbiological, biotechnological and molecular genetic methods: genetic identification by chromosomal DNA isolation and sequence analysis of 16S rRNA gene of EPZ producers; classical methods for phenotypic characterization; TLC method, GC-MS analyzes, 1H-NMR spectrum determination, IR spectroscopic methods; scanning electron microscopy; chromatographic methods for purification, thermogravimetry, etc.

The obtained results reveal opportunities and perspectives for new research on current issues, focused on the needs of real biotechnological productions.

The achievements of Dr. Nadia Radchenkova have been published in authoritative scientific journals and have found a wide positive response in the scientific community, have

been reflected in the funding of a number of international and national research projects, which clearly confirms the importance of the tasks and the applicability of the results.

The science metric data of Dr. Nadia Stoycheva Radchenkova significantly exceed the minimum national and additional requirements of ZRAS RB, and the relevant Rules of IMicB for the academic position of "Associate Professor" in the Professional field 4.3 Biological Sciences.

All this gives me grounds to support the candidate and to convincingly recommend to the respected members of the Scientific Jury to evaluate positively and to propose to the Scientific council of IMicB to award Ch. Assistant Professor Dr. Nadia StoychevaRadchenkova, academic position "Associate Professor".

Sofia, August 11, 2021 REVIEWER:

(Assoc. Prof. Dr. Zlatka Alexieva)