Streszczenie w języku angielskim

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pt. „Wyspecjalizowane rybosomy u sporulującej laseczki siennej *Bacillus subtilis*"

Bacillus subtilis is a very well-studied Gram-positive bacterium and a frequently used model organism. It is widely distributed in nature and its main habitat is the soil. A characteristic feature of *B. subtilis* is the ability to form endospores, highly resistant spore forms that allow it to wait out unfavorable environmental conditions. They are formed as a result of a process closely coordinated by the cell, called sporulation, initiated mainly by an amino acid starvation and an appropriate culture density.

The aim of this study was to characterize the triple deletion mutant *ΔrpmEBΔrpmGCΔrpsNB* of *B. subtilis* (herein referred to as the 3KO strain) in the context of its sporulation process, in order to analyze the role that specialized ribosomes may play during this process.

Specialized ribosomes are hypothetical subpopulations of ribosomes that are supposed to differ structurally and functionally from each other and thus influence the outcome of translation, e.g. by preferentially binding to a specific subset of transcripts. Therefore, the ribosome gains an additional role, becoming an active element in managing the flow of genetic information, which adds a new level to the regulation of gene expression. The number of evidence confirming the existence of specialized ribosomes is increasing, but this hypothesis remains a controversial issue, mainly due to the difficulties in proving their different functionality. It is tested and verified in both eukaryotic and prokaryotic cells.

Firstly, the phenotypic characterization of the tested strains: WT and 3KO of *B. subtilis* was performed. Both strains showed similar growth in both nutrient-rich (LB, CH) and poor (BMM) media as indicated by their growth curves. They also did not differ in sensitivity to selected chemical compounds, including lysozyme, antibiotics (ampicillin, fusidic acid, spectinomycin, thiostrepton), detergents (SDS, Tween-20) and salts (Na_2SO_4, $MnSO_4$, $ZnCl_2$). In addition, no differences in biofilm formation were found, since both strains

forming highly developed and durable biofilms. For additional verification of the correctness of the results, an additional strain of *B. subtilis*, BGSC, was used, which produced much weaker and unstable structures. Nonetheless, a difference in the mobility of the strains was observed, with 3KO showing significantly lower mobility in semi-liquid medium with an agar content of ≤ 0.33%.

Then, the process of the sporulation of *B. subtilis* 3KO and WT strains was investigated by RNA sequencing (RNA-seq) and ribosome profiling (RIBO-seq) as well as microscopic observations. The process was monitored for seven hours after sporulation induction, with hourly sampling. The difference in translation efficiency was detected only in the seventh hour of the process, for 65 genes, of which 38 genes had a higher level of translation in the WT strain, and for 27 genes it was higher in the 3KO strain. Among the 65 identified genes, the largest group with a known function were genes related to the formation of the coat, the outer layer protecting the spore (15 genes), most of which concerned the outermost layer, called the crust (11 genes) and almost all (13 genes) showed more efficient translation in WT strain. The second largest group of genes were general stress proteins (6 genes) and all showed more efficient translation in the 3KO strain. The genes with the greatest difference in translation efficiency (from 7.5 to 5.5) were *yurS* (sporulation protein of unknown function), *ylqC* (putative RNA-binding protein), *gyrA* (DNA gyrase A subunit), *yloS* (thiamine pyrophosphokinase) and *mazF* (a toxin that causes programmed cell death) and all showed more efficient translation in the WT strain.

The following dyes were used for microscopic observations: SynaptoRed to visualize cell membranes; DAPI to visualize the bacterial chromosome and OPP-Alexa to visualize active translation sites. Also, the molecular marker GFP fused to the ribosomal protein RpsB was used to detect the location of ribosomes. Microscopic observation revealed a subpopulation of 3KO strain cells of the six hours after induction of sporulation that were less efficient in translation.

A statistically significant difference in spore germination efficiency was detected. The average efficiency of spore germination of the WT strain was 61.5% ± 2.98, while for the spores of the 3KO strain was almost twice lower and amounted to 30.2% ± 12.78. This difference was also confirmed in culture growth curves initiated from spores.

Due to the observation of significant differences in spore germination, microscopic observations were also carried out for this process. Mature, overnight spores were observed before and after incubation at a high temperature of 90°C for 40 minutes, and at 30, 60, 90, 120 and 180 minutes after germination induction. Analysis of microscopic images revealed, that after exposing the spores to high temperature, the percentage of 3KO spores, that expressed GFP-RpsB fluorescence, decreased dramatically. The measurement of fluorescence intensity also confirmed a statistically significant difference between the 3KO and WT spores for all time points examined, except for the first one, which corresponded to the moment before incubation at high temperature. This indicates that 3KO spores are sensitive to high temperatures, which can denature proteins in their core, including RpsB. At the same time, the spores percentage expressing blue DAPI fluorescence before and at the time of induction of germination differed between the strains and was almost half as low for the WT strain, but at subsequent time points it was similar in both strains and showed an upward trend. This may be due to the difference in spore permeability, with 3KO spores being more permeable to the DAPI dye. Equating the spores percentage of the 3KO and WT strains expressing DAPI fluorescence at 30 minutes after germination induction may be due to the germination initiation, since germinating spores have a similar permeability to those killed by heat (Black & Gerhardt, 1962; Mtimet et al., 2017; Trunet et al., 2019). This also explains the increasing trend in the percentage of spores expressing DAPI fluorescence with the progress of germination.

As a result of microscopic observations of the OPP-Alexa fluorescence intensity, which represents active translation, spores of the 3KO strain were found to show statistically significantly lower levels of translation at 30 minutes and 2 hours after germination induction. In addition, there is generally an upward trend in OPP-Alexa fluorescence intensity as germination progresses. A difference in the length of spores between the strains was also detected, which becomes statistically significant 60 minutes after germination induction. The correlation between the spore length and the difference in translation level, represented by OPP-Alexa fluorescence, is observed.