Protease ficin which was reported previously as efficient tool to remove bacterial biofilms. After full recovery of the wound the skin samples were taken and microscopic images were analyzed. In the control group the tissue recovery was 48 ± 8%, while in ficin-treated samples the recovery was 78 ± 7% suggesting promising wound-healing activity of ficin. The research was funded by the subsidy allocated to Kazan Federal University for the state assignment in the sphere of scientific activities, project No. 1.12878.2018/12.1.

Multicomponent complexes

P-34-001
Investigations of Dps/DNA crystallization conditions and the structure of biocrystals according to SAXS and cryo-electron microscopy data

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The ability to maintain biological integrity and adapt to changes in the environment during periods of stress is essential for the survival of any organism. Bacteria have evolved a multitude of ways to survive under stressful conditions including the expression of specific stress mediation proteins. One of such proteins vital in stress survival is the DNA-binding protein from starved cells (Dps), which is conserved in more than 300 bacterial species. Dps is highly expressed in stationary phase, it is also involved in the cellular response to oxidative, UV, thermal, and pH shocks. Dps binds both to supercoiled and linear DNA to form a dense biocrystal structure. Although the crystal structure of the Dps dodecamer has been solved, no atomic-scale structure of Dps-DNA assemblies currently exists, and little is known about complex formation and biocrystallization processes. Not resolved in the E. coli Dps crystal structure were the disordered N-terminal regions of each Dps monomer, which extend outward from the dodecamer and contain several lysines proposed to contribute to DNA binding. Removal of large sections of the N-terminal region reduces DNA condensation by Dps, however the exact molecular interactions that cause DNA binding remain unclear. Our investigations report new insight into Dps/DNA crystallization processes from SAXS and cryo-electron microscopy data. Using SAXS data we obtained the full-length structure of Dps protein and analyzed the processes of Dps/DNA complex formation depending on buffer conditions. During our investigations it was found that Dps formed with DNA several types of crystals. Utilizing cryo-electron microscopy, we report here structural models of these Dps/DNA biocrystals. Obtained results can help us to advance understanding of the mechanisms of protection of the bacterial genome from stress factors. This work was supported by the Russian Science Foundation (project No. 18-74-10071).

P-34-002
Surface characterization of domains for anchoring to the surface of Lactococcus lactis

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Display of recombinant proteins on the bacterial surface is an emerging research area with wide range of biotechnological applications. Because of its recognized safety, lactic acid bacterium (LAB) Lactococcus lactis represents an attractive host for surface display and a promising vector for in situ delivery of bioactive proteins. To achieve surface display, recombinant proteins are usually anchored to the cell wall of LAB through anchoring domains. Different types of surface anchoring domains have been described for LAB, with LPXTG-type domains and lysin motif (LysM) domains being among the most frequently applied in Lactococcus lactis. Regardless of the available options, alternative surface display approaches are being sought. The goal of the present study was to characterize new anchoring domains for surface display on Lactococcus lactis, and to evaluate their applicability as an alternative to established anchoring domains, especially to the LysM repeats-containing non-covalent anchor of AcmA. We prepared genetic constructs consisting of secretion signal, reporter protein (B domain or DARPin) and surface anchoring domain of lactococcal or phage origin. 13 non-covalent and 2 covalent anchoring domains were tested with flow cytometry to evaluate surface display of fusion proteins via their ability to bind the Fc region of staphylococcal protein A (B domain-containing fusion proteins) or the Fc region of human IgGs (DARPin-containing fusion proteins). One of the anchoring domains, AM12, demonstrated comparable extent of surface display to that achieved with AcmA. In further studies, AM12 enabled display of CXCL8-binding evasin-3 on Lactococcus lactis, as well as heterologous display, to a level similar to that achieved with AcmA. To conclude, we have demonstrated effective use of different protein anchors in Lactococcus lactis. AM12, in particular, represents a promising alternative to established approaches for surface display on Lactococcus lactis.

P-34-003
The impact of F0F1-ATPase on H2 producing hydrogenase activity in Escherichia coli during mixed carbon sources fermentation

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P.34-003. Escherichia coli has four membrane-bound [Ni-Fe]-hydrogenases (Hyd), which reversibly oxidize H2 to H+. In this work, the role of the F0F1-ATPase in H2 production was first investigated during mixed carbon sources (glucose, glycerol, formate) fermentation by E. coli at different pHs (7.5, 6.5, 5.5) using wild type and mutant strains with defects in various Hyd and/or formate dehydrogenase (FDH) enzymes. F0F1-ATPase specific inhibitor N,N',N'-tricyclohexylcarbodiimide (DCCD) had an inhibitory effect on the duration, but not on the rate of H2 production in wild type at pH 7.5. The external pH of growth medium of selC (lacking formate dehydrogenases) single and hyaB hybC wdC (lacking Hyd-1 and Hyd-2 large subunits and formate dehydrogenases) triple mutants was acidified more by ~ 0.3 units compared to wild type. No effect of DCCD on H2 production at pH 6.5 was determined. But DCCD had a negative influence on Hyd enzymes activity at pH 5.5. The external pH was acidified by 0.7–0.8 units in selC mutant growth medium compared with wild type. The
overwhelming effect of the DCCD on the specific growth rate was detected at pH 7.5 and 6.5, and the effect of DCCD at pH 5.5 was almost excluded, except for selC mutant (reduction by 25%). It was shown that specific growth rate was increased by ~65% and ~50% in wild type and hypF (lacking all Hyd enzymes) mutant with DCCD at pH 6.5. Taken together, it is suggested that FOV1-ATPase has metabolic cross talk with FDH and Hyd enzymes and has a great contribution in H2 production at pH 7.5 and 5.5. It was shown that the main role of FDH in the neutralization of both external and intracellular formate during fermentation. Acidification of Hyd deficient mutant’s external medium and the change of FOV1-ATPase activity indicate an interaction between FOV1-ATPase, Hyd and FDH enzymes in terms of H2 metabolism or H+ transport in the membrane, which function to regulate intracellular pH and thus proton motive force. *The authors marked with an asterisk equally contributed to the work.

P-34-004
The effect of the mixture acetate and glycerol on E. coli growth and H2 production during fermentation
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It is well known that Escherichia coli is able to ferment sugars and/or glycerol for producing molecular hydrogen (H2). H2 produces via four multiple and reversible [Ni-Fe] Hyd enzymes. This study describes growth and the total H2 production in batch cultures during utilization of the mixture of acetate (5 g/L) and glycerol (10 g/L) at various pHs (7.5, 6.5, 5.5) in E. coli wild type and different mutant strains with defects in Hyd 1 or Hyd-2. It was shown that specific growth rate (µ) of E. coli wild type was 0.289 h⁻¹, which was similar for mutant strains at pH 7.5 and 6.5. Only in hybC (lacking large subunit of Hyd-2) mutant µ was increased ~1.3 fold compared to wild type at pH 7.5. It has been determined that in batch tests at pH 7.5 and 6.5 wild type strain evolved H2 during long time ~168 h. No H2 production was observed in all strains at pH 5.5. Interestingly, hyaB (lacking large subunit of Hyd-1) and hybC mutants have exhibited the same results at pH 7.5 and 6.5, especially H2 generation was ~150 h. This is a prolonged period compared to acetate alone fermentation. In hyaB or hybC mutants, H2 production was detected earlier than in wild type at pH 7.5. But at pH 6.5 only in hybC mutant earlier H2 production was detected suggesting an important role of Hyd-2 under these conditions. Taken together, it can be concluded that cell growth and H2 generation depends on external pH and carbon sources. Particularly, Hyd-1 and Hyd-2 work towards H2 oxidation which is in contrast to glyc-