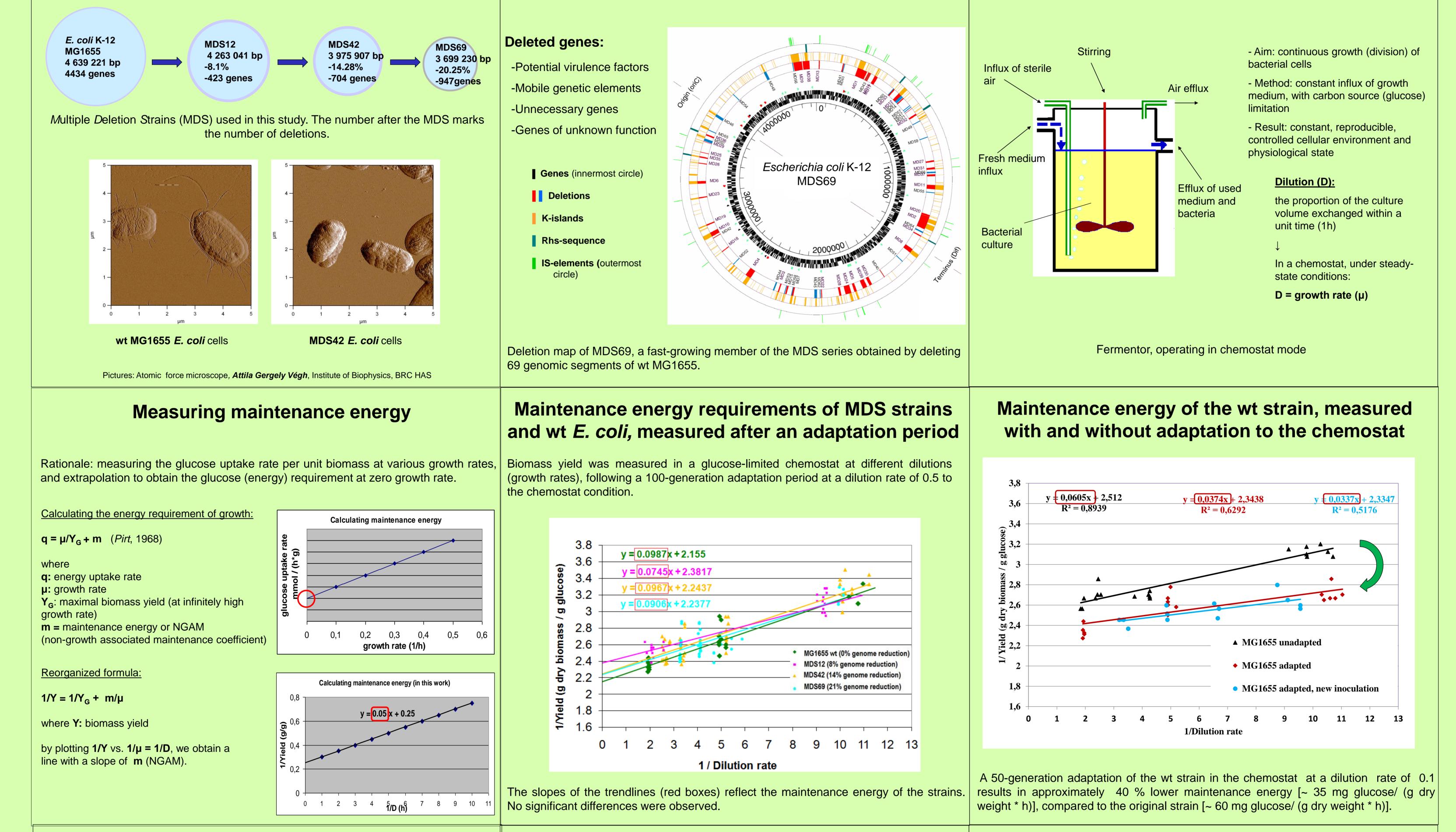
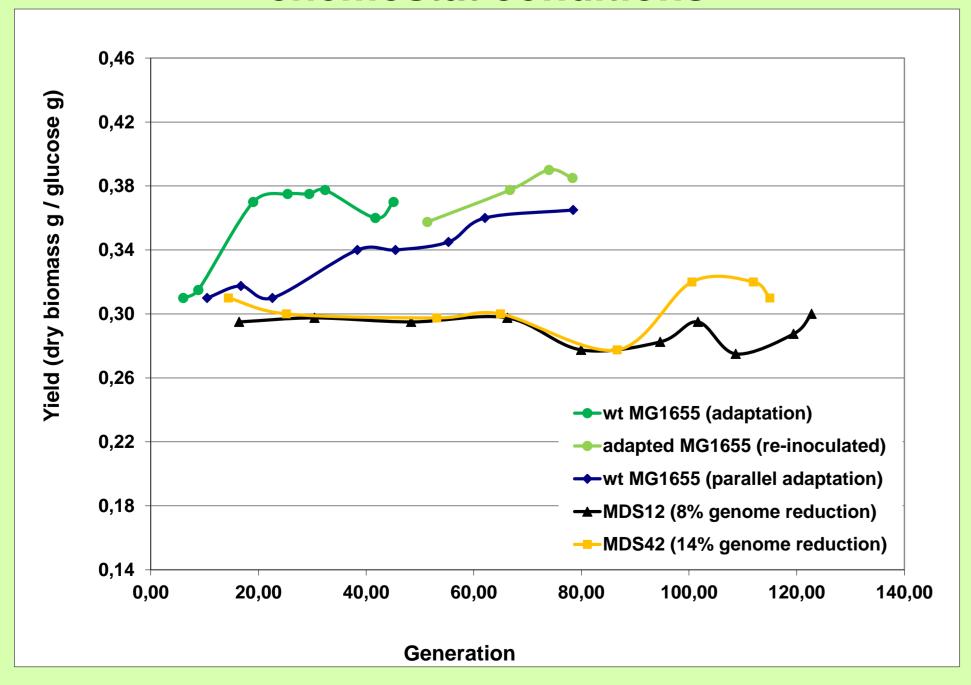
# Maintenance energy requirement and genetic adaptation of streamlined-genome *Escherichia coli*

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Genome Engineering Group, Institute of Biochemistry, Biological Research Centre, Hungarian Academy of Sciences, Szeged, Hungary Using approaches of synthetic biology, we are focusing on the rational large-scale remodeling of the genome of *E. coli* K-12 (Pósfai et al., 2006, Science). A cell with a streamlined, semisynthetic genome could serve as an improved model organism, and as a programmable cellular chassis for industrial applications. It was hypothesized that deletion of the mobile genetic elements and other unneeded genomic islands would result in a cell displaying lower complexity, higher genetic stability, and lower energy consumption. Lower complexity is evident, higher genetic stability has been previously demonstrated (Umenhoffer et al., 2010, Microb Cell Fact; Csörgő et al., 2012, Microb Cell Fact). Regarding cellular energetics, we hypothesized that due to the elimination of unnecessary, energyconsuming processes (e.g., lack of flagella synthesis), streamlined-genome strains use resources more economically than wild-type cells. This might be manifested in enhanced growth properties or in higher recombinant protein production. To accurately quantitate the energy consumption of the cellular machinery, we measured the maintenance energy (energy needed to maintain the cell at zero growth) requirement of the various cells in a series of controlled steady-state growth experiments in a chemostat.



# Changes in biomass yield during adaptation to chemostat conditions



### Conclusions

Contrary to expectations, elimination of unnecessary, energyconsuming processes did not seem to result in significant reduction of the maintenance energy (with the possible exception of MDS12). We currently hypothesize that the potential gain was off-set by the loss of adaptation capabilities and by some structural disturbances of the chromosome, resulting in suboptimal working of the transcriptional machinery (preliminary data).

Paralleling the streamlining process, a tendency of reduced

## References

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Wt *E. coli* MG1655 produced a significantly elevated biomass yield after 40-60 generations of growth at a dilution rate of 0.1. Re-inoculated wt cells continue to produce elevated biomass, indicating a genetic basis for adaptation. Spreading on a plate, wt adapted cells show phenotypically heterogeneous colonies. In contrast, the yields of MDS strains remain constant after 100 generation, and cells remain phenotypically homogeneous. MDS cells have a lower capacity for genetic adaptation.

genetic adaptability was observed. Wild-type cells, displaying a certain initial maintenance energy requirement in the chemostat, quickly develop genetic changes that result in a phenotypically heterogeneous population and in a decrease of energy consumption. In contrast, the initial maintenance energy requirement of the streamlined-genome cells (especially those with more deletions) remains basically unchanged for a hundred generations, and the cells remain phenotypically and genetically uniform. Whole-genome sequencing of the adapted wild-type cells is underway to elucidate the genetic basis for this difference in the adaptation capabilities of the wild-type and streamlined-genome strains.

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