Biotechnology beyond the stars: how *Aspergillus niger* forms the industry of the future

Flóra Faggyas

florafaggyas@gmail.com
Tóth Árpád High School, Hungary

1. INTRODUCTION

The microorganisms' resistance to environmental changes is very high. In 1988 fungi were found on the surfaces of Mir space station. By improving the robustness of these fungi, substances could be produced with them in the space, making future missions more sustainable **[1]**.

Citric acid is produced by the fermentation of *Aspergillus niger*. Fermentation means breeding cells such as bacterium and fungus under special circumstances so they produce substances that we need in a higher concentration.

During citric acid production, keeping the concentration of Mn^{2+} under 5 µg/l in the reactor, 90% specific molar yield can be achieved. However, creating a Mn^{2+} free environment is hard because the Mn^{2+} content of stainless steel parts of bioreactor is more than 2% and during corrosion effects, Mn^{2+} leaches out into the medium **[2]**.

Since creating a Mn^{2+} free environment is so hard, developing an *Aspergillus niger* mutant that can produce high concentration of citric acid even in a Mn^{2+} added environment is very important. *CexA* is a gene which codes a transporter protein responsible for getting out citric acid into the medium. A cexA overproducing mutant created by the Vienna University of Technology was also tested by us.

2. AIMS

My goal was to investigate the leaching of Mn^{2+} induced by low pH and hot temperature during sterilization of the reactor. Moreover, to investigate the effect of Mn^{2+} on citric acid production at different times during the fermentation. Third, to test the fermentation yield of the cE cexA mutant strain.

3. METHODS AND MATERIALS

During our fermentations we used three *Aspergillus niger* citric acid overproducing strains: NRRL 2270, ATCC 1015 and cE cexA. **[Fig. 1]**

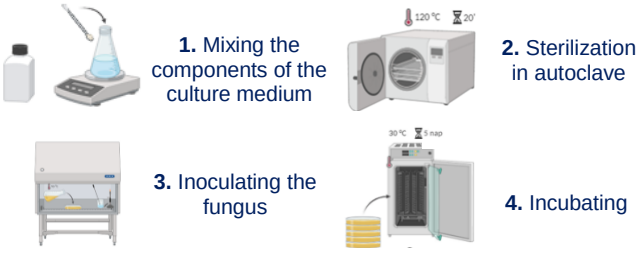

Figure 1. Strain maintenance

The concentration of the carbon source (glucose) and citric acid was measured by HPLC and the concentration of Mn^{2+} was measured by ICP-MS. We carried out the fermentations in an older bioreactor and a newly purchased one.

4. RESULTS AND DISCUSSION

We discovered that the leaching of Mn^{2+} induced by hot temperature during sterilization was more significant in the older bioreactor (B) **[Table 1].** Investigating the effect of Mn^{2+} on citric acid production at different times during the fermentation, we could determine that the presence of Mn^{2+} is critical at the early stage of fermentation **[Table 2].**

We also determined that the decrease of pH affects the leaching of Mn^{2+} in a significant way.

We compared the fermentation of ATCC 1015 and cE cexA strains in Mn^{2+} added environment. The cE cexA mutant strain produced much more citric acid.

Table 1. Mn^{2+} concentration (µg/l) after sterilization in autoclave

	Before sterilization	After sterilization	
		30 min	60 min
Bioreactor A	2.05 ± 0.10	2.90 ± 0.5	2.95 ± 0.4
Bioreactor B	2.01 ± 0.18	**20.30 ± 2.48**	**25.98 ± 3.02**

Table 2. Effect of Mn^{2+} addition at different time points on yield.

Mn^{2+} ion addition (h)	5 µg/l
	$Y_{p/s}$
0	**0.73 ± 0.05**
6	0.71 ± 0.04
72	0.83 ± 0.05
172	**0.88 ± 0.04**

5. CONCLUSIONS

During our research we investigated the leaching of Mn^{2+} caused by low pH and hot temperature during sterilization. We also discovered a direct connection between Mn^{2+} concentration and cexA gene expression. If we could map the interactions of the genes in *Aspergillus niger* even more thoroughly, we could create an even more robust fungus that could be used in space exploration.

6. ACKNOWLEDGEMENT

I would like to thank the Department of Biochemical Engineering at the University of Debrecen and the support of the Count István Tisza University of Debrecen Foundation.

7. REFERENCES

[1] Cortesão et al. (2020), Fungal Biotechnology in Space: Why and How? In Grand Challenges in Biology and Biotechnology (p.: 501–535).

[2] Fekete et al. (2022), Bioreactor as the root cause of the "manganese effect" during *Aspergillus niger* citric acid fermentations. Frontiers in Bioengineering and Biotechnology, (10) 935902.