The optimization of medium for the growth of *B.subtilis* using different media components

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Backround

B.subtilis (*Bacillus subtilis*) is a gram-positive bacterium. It is one of the most important production organisms in the biotech industry and is used to produce bulk enzymes for detergent-, paper-, and other industries. The popularity of this organism is based on the fact that it is a safe organism and easy to genetically manipulate. Most importantly, it can efficiently secrete enzymes in large quantities. The enzyme secretion in *B.subtilis* takes place through the Sec pathway (Secretory pathway). This is the most important transport channel in *B.subtilis* for the transport of exported proteins. Nevertheless, many enzymes aren't properly secreted by the *B.subtilis*. Preliminary results showed that the transcription, translation, and secretion peaks of different enzymes were different, although they used the same expression system. Besides that, the results also showed that the components in the medium play a major role in the secretion process. Despite these results, it is still not clear why some enzymes are secreted much better than others.

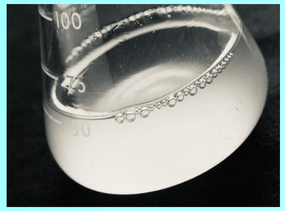
Figure 1. Microscopic view of the B.subtilis with a color-enhanced scanning electron microscope.
The B.subtilis here is a gram-positive and rod-shaped bacteria.

Aim

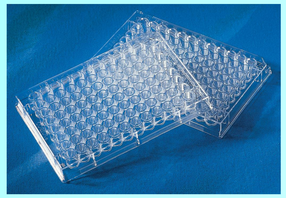
In the background it was said that the results showed that the components in the medium play a major role in the secretion process. Therefore, the purpose of this research is to perform a determination and thereby determine what will happen to the growth rate of the wild-type when it is exposed to media with different components (4 different concentrations of trace element, cas and glucose).

In order to find the best combination of components for the medium to grow the wild-type, to subsequently research in a follow-up study how this combination of components influences the secretion levels of the industrial proteins: XynA and AmyM that are produced by the *B.subtilis.*

Method



The wild-type will first be grown overnight in 10 mL of Minimal Medium at 30 °C.



96-wells-plates will then be prepared with different media (4 different concentrations of trace element, cas, and glucose) and the overnight grown wild-type.

The growth of the wild-type will then be measured with the Multiskan plate reader, at 37 °C with medium shaking for 20h, every 30 min with an absorbance of 595 nm.

Results

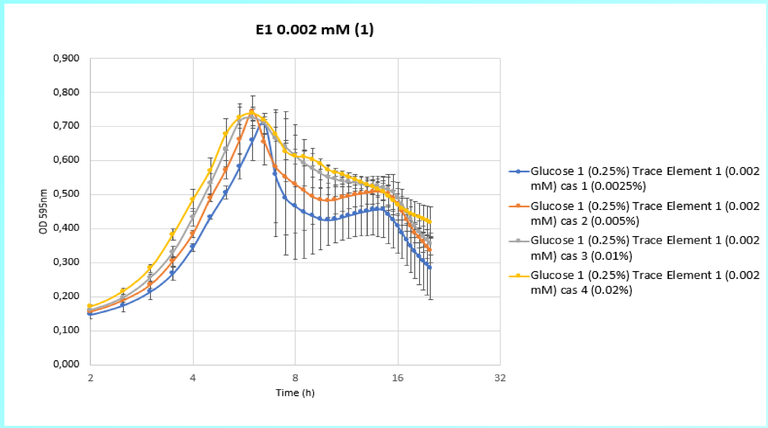


Figure 1. Growth rate of the wild-type grown in different media with 4 different concentrations.

Figure 1 shows that cas 4 is the main element, which ensures rapid growth of the wild-type.

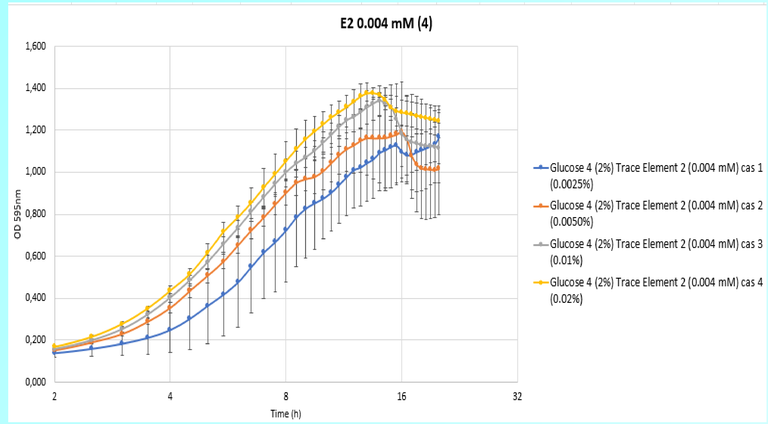


Figure 2. Growth rate of the wild-type grown in different media with 4 different concentrations.

Figure 2 shows that high concentrations of glucose 4, trace element 2 and the main element cas 4 are more beneficial for the growth of the wild-type.

Conclusion

Figures 1 and 2 show that the components: glucose, trace element and cas are very helpfull for the growth of the wild-type. But the best combination of the 3 components for a more beneficial, faster growth and preventing cell lysis are: Glucose 4 (2%), Trace Element 2 (0.004 mM), and cas 4 (0.02%).