



Doctoral Thesis

## Ueber den Einfluss von Spurenelementen auf Wachstum und Enzyymbildung von *Aspergillus oryzae*(Ahlburg) Cohn

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Über den Einfluß  
von Spurenelementen auf Wachstum  
und Enzyymbildung von *Aspergillus*  
*oryzae* (Ahlburg) Cohn

Von der  
EIDGENÖSSISCHEN TECHNISCHEN  
HOCHSCHULE IN ZÜRICH  
zur Erlangung der Würde eines Doktors  
der technischen Wissenschaften  
genehmigte  
PROMOTIONSARBEIT

Vorgelegt von  
  
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## Summary

The influence of Zn, Fe, Cu, Mn, and Mg on the growth of *Aspergillus oryzae* and on the formation of amylolytic and proteolytic enzymes has been studied. The effect of various carbon sources on the formation of dextrinogenic amylase under defined trace element conditions has also been investigated. For these purposes, both preparatory detailed investigations on purification techniques and studies on optimum conditions for amylase formation, with respect to composition and buffering of the medium, were found necessary. The following results have been obtained:

a) Influence of trace elements on growth and enzyme formation

1. For optimal growth in a purified medium containing 15 g maltose, 40 g phosphate buffer pH 6,0, and 1,5 g  $(\text{NH}_4)_2\text{HPO}_4$  per liter (nutrient soln. B), the following amounts of trace elements are required: 200  $\gamma$  — 1 mg Zn, 200  $\gamma$  — 10 mg Fe, 40  $\gamma$  — 200  $\gamma$  Cu and 15 — 100 mg Mg. Slight decrease in the amounts of any of the above mentioned trace elements retards or prevents spore formation. Pronounced deficiency of any of these elements depresses the dry weight of mycelium. Excess of any of these elements also retards or inhibits sporulation and decreases the dry weight, but to a lesser degree than in the case of deficiency.
2. The growth of the fungus in a Cu-deficient medium follows a different pattern from that of media deficient in Zn, Fe or Mg. The patterns of growth in a Cu-deficient and in a complete medium are similar. In both the dry weight reaches a maximum after about 5 days and then gradually falls due to progressive autolysis. If any of the other trace elements is deficient, the mycelial dry weight continues to increase even after 10 days of growth.
3. The formation of amylolytic enzymes is influenced by the trace elements in the following way:

Zn: Maximum formation of amylase was obtained with 60  $\gamma$  — 200  $\gamma$  Zn/liter. Higher concentrations inhibited amylase production. The specific dextrinogenic activity decreases with increasing Zn-concentration.

Fe: With increasing concentration of Fe amylase formation also increases. Highest yields of amylase were obtained with growth inhibiting Fe-concentrations of 10 and 100 mg/liter.

Cu: In a Cu-deficient medium, amylase formation was higher than at optimal Cu-concentrations for growth.

Mn: No influence was observed between 0 and 20 mg/liter. 40 mg/liter inhibited amylase formation.

Mg: Highest amylase formation was observed on media with 30—100 mg/liter. In Mg-deficient medium, the specific dextrino-

genic activity is diminished. Addition of Ca to a Mg-deficient medium increases the inhibition of amylase formation.

4. For the formation of proteolytic enzymes, Zn and Fe are essential. The culture filtrates practically show no proteolytic activity unless the concentrations of Zn and Fe are sufficient for maximum growth. In a medium deficient in Mg, more proteolytic enzymes are produced than at optimal Mg-concentrations for growth. The same applies, though to a lesser degree, to deficiency of Cu or excess of Fe.
- b) Influence of various carbon sources on the formation of dextrinogenic amylase
1. In purified nutrient solution B, with trace elements added, more amylase is produced with dextrin as carbon source than with maltose, starch, or glucose. Maltose and starch gave about equal yields of amylase. Glucose ranged as the poorest carbon source for amylase formation.
  2. In unpurified nutrient solution B, with trace elements added, however, more dextrinogenic amylase was formed with maltose as carbon source than with dextrin.
  3. In comparison to the specific dextrinogenic activity of the corresponding complete media, Cu-deficiency resulted with all carbon sources in a higher specific dextrinogenic activity, Fe-deficiency with glucose and starch in a higher, with dextrin and maltose in a lower, and Zn-deficiency with glucose, maltose and starch in a higher, and with dextrin in a lower specific dextrinogenic activity.
- c) Influence of pH and nitrogen sources on the formation of dextrinogenic amylase
1. The formation of amylase in nutrient solution B is highest at pH 5.5, decreases toward pH 7.0, and slightly increases again toward pH 8.0. Below pH 5.5, the dextrinogenic amylase is unstable at the incubation temperature of 25° C.
  2. With  $(\text{NH}_4)_2\text{SO}_4$  and  $(\text{NH}_4)_2\text{HPO}_4$  higher amylase yields were obtained than with peptone, urea, and glutamic acid, if the N-concentration of the media corresponded in every case to 2.0 g of  $(\text{NH}_4)_2\text{SO}_4$  per liter.
- d) Purification technique
1. With the  $\text{Al}_2\text{O}_3$ -method of DONALD et al. (1952) it was easy in our laboratory to reproduce their low zero values for Zn and Fe, but not those for Cu and Mn. The application of several modifications to this method gave only small reductions of the Cu- and Mn-zero values.
  2. For purification of the heavily buffered nutrient solution B, as especially developed for the enzymatic investigations, an extraction method with dithizon and oxine proved to be better suited than the above mentioned  $\text{Al}_2\text{O}_3$ -method.