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The Incredible Impact of the *In-vitro* Enzymatic Cascade

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INTRODUCTION

Although *in-vitro* enzymatic cascade is a helpful biomanufacturing method, its economic viability is constrained by the need for expensive exogenous cofactors. In this study, we created an expensive cofactor-free enzymatic cascade to convert glucose into pyruvate, a crucial platform substance widely utilised in several industries. Only five enzymes involving glucose oxidase from *Aspergillus niger*, mutant alditol oxidase from *Streptomyces coelicolor*, 2-keto-3-deoxy-d-gluconate aldolase from *Sulfolobus acidocaldarius*, catalase from *Aspergillus niger*, dihydroxy acid dehydratase from *paralcaligenes ureilyticus* and Mg^{2+} were needed for pyruvate production from glucose. In 8 hours, 30.82 mm of pyruvate was produced with a yield of 74.27% of the theoretical value under the ideal reaction conditions. In addition, by further including pyruvate decarboxylase from *Zymomonas mobilis*, another significant platform chemical, acetoin, may also be generated from glucose. As well as thiamine pyrophosphate into the chain of enzymes. In 10 hours, acetoin was created from glucose with an 84.71% theoretical yield at a concentration of 17.79 mm.

DESCRIPTION

For the manufacture of value-added compounds or building blocks, *in vitro* biosynthetic systems offer a promising alternative to conventional chemical synthesis and microbial fermentation methods. *In-vitro* biosynthetic systems can provide quick reaction times, high product yields, straightforward process control and simple target product purification whereas compared to chemical synthesis and microbial fermentation. Most of reported *in-vitro* enzymatic cascades, however, involve reactions that necessitate costly cofactors like NAD(P)H, $NAD(P)^+$ or ATP, which prevents their use on an industrial scale. Coupling with a substrate intended for sacrifice and an enzyme for regeneration can be used to acquire cofactor regeneration. However, the inclusion of cofactor regeneration also provides to the complexity of the *in-vitro* biosynthetic system, necessitating the insertion of little amount of an expensive cofactor at the start of the reaction.

In the food, pharmaceutical, chemical and agrochemical industries, pyruvate is a key platform compound that is frequently used. It may be converted into a number of very valuable compounds, including 2, 4-dihydroxybutyric acid, l-tyrosine, acetoin and n-butanol. The biological process for producing pyruvate that has been looking into the most thoroughly in microbial fermentation. Pyruvate, however, is located at the key node where microbial metabolic networks branch out. It is exceedingly challenging to create bacteria that accumulate pyruvate under natural circumstances. In order to synthesize pyruvate from various substrates, numerous *in-vitro* enzymatic cascades have been developed. As an illustration, Honda et al., created a synthetic route incorporating. For instance, Honda et al., created an artificial pathway to transform chitin into pyruvate by combining 12 enzymes from thermophilic bacteria. The enzymatic cascade involves processes which need expensive cofactors like NAD^+ and ATP, making this method of pyruvate synthesis commercially unviable.

CONCLUSION

In this study, we designed a costly cofactor-free enzymatic cascade composed of glucose oxidase from *Aspergillus niger* (AnGOX), mutant alditol oxidase from *Streptomyces coelicolor* (ScALDO), 2-keto-3-deoxy-d-gluconate aldolase from *Sulfolobus acidocaldarius* (SaKDGA), catalase from *A. niger* (AnCAT), dihydroxy acid dehydratase from *Paralcaligenes*

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ureilyticus (PuDHT) and Mg^{2+} for pyruvate production from glucose. Acetoin was created from glucose by incorporating *Zymomonas mobilis*' (ZmPDC) pyruvate decarboxylase further into the enzymatic cascade, making it one of the 30 platform chemicals given priority by the U.S. department of energy for development and utilization.