The Melibiose Operon of *Escherichia Coli*: Mechanism of Catabolite Repression and Strategies for Overcoming it

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by

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Dedicated to

Indian Soldiers
&
Indian Farmers

CERTIFICATE

This is to certify that the thesis entitled **"The Melibiose Operon of *Escherichia coli*: Mechanism of Catabolite Repression and Strategies for Overcoming it"** being submitted by **Ms. Shilpi Jain** to the **Indian Institute of Technology Delhi**, for the award of the degree of **'Doctor of Philosophy'**, is a record of the bonafide research work carried out by her, which has been prepared under my supervision in conformity with the rules and regulations of the 'Indian Institute of Technology Delhi'. The research reports and the results presented in this thesis have not been submitted for any degree or diploma in any other University or Institute.

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ABSTRACT

In *Escherichia coli (E. coli)*, the expression of the catabolic operons is suppressed in the presence of glucose. This phenomenon is called glucose effect and is usually explained by two regulatory mechanisms namely, cAMP effect and inducer exclusion. The data suggests that both these mechanisms cannot explain the 600-fold effect of catabolite repression. A mechanism called 'positive feedback' has been hypothesized to explain the huge effect of glucose on catabolic operons by Narang and Pilyugin (2007). Since positive feedback can amplify the small effects of inducer exclusion, cAMP effect, and dilution, it seems to be the most plausible mechanism for catabolite repression. In this study, positive feedback mechanism was checked by an indirect method, i.e., by testing for the presence of bistability as it is the definite proof of the existence of positive feedback. Bistability has been observed frequently with non-metabolizable inducers. But to date, studies investigating bistability with metabolizable inducer have produced ambiguous results. The main aim of this study is to check bistability under various conditions in the melibiose operon. In this work, we studied bistability in the strains having varying activities of α-galactosidase, keeping the permease level constant. We observed bistability in all the strains, importantly, wild-type strain (*lacY*) for melibiose operon. In *E. coli mel* operon, catabolite repression was abolished by over-expressing the melibiose permease by four-fold under the control of an inducible promoter. These two observations strongly suggest the existence of positive feedback in the melibiose operon.

We proceeded to investigate the effect of overcoming of catabolite repression on carbon flux. For this, we used various constitutively expressed melibiose permease mutants and measured the glucose and melibiose consumption rates. We found that as we increase the uptake rate of melibiose, carbon throughput increased by more than 50 % which has important implications in

biotechnology. We also observed that on increasing the permease level, growth rate increases on melibiose as the sole carbon source. It suggests that melibiose uptake is the limiting factor for growth on pure melibiose.

सार

एस्चेरीचिया कोली (ई. कोलाई) में, ग्लूकोज की उपस्थिति में catabolic operons (अपाचिक ओपेरॉन) की अभिव्यक्ति दब गई है। इस घटना को 'ग्लूकोज प्रभाव' कहा जाता है और इसे आमतौर पर दो नियामक तंत्रों द्वारा समझाया जाता है, cAMP effect (cAMP प्रभाव) और Inducer exclusion (Inducer अपवर्जन)। आंकड़ों से पता चलता है कि दोनों तंत्र इस प्रकार के पेटी दमन (catabolite repression) के 600 गुना प्रभाव की व्याख्या नहीं कर सकते हैं। नारंग और पीलीगुइन (2007) द्वारा catabolic operons पर ग्लूकोज के विशाल प्रभाव की व्याख्या करने के लिए 'सकारात्मक प्रतिक्रिया' (Positive feedback) नामक एक तंत्र की परिकल्पना की गई है। चूंकि 'सकारात्मक प्रतिक्रिया' Inducer अपवर्जन, cAMP प्रभाव, और कमजोर पड़ने के छोटे प्रभावों को बढ़ा सकती है, इसलिए यह पेटी दमन के लिए सबसे अधिक सक्षम तंत्र लगता है। इस अध्ययन में, सकारात्मक प्रतिक्रिया तंत्र की अप्रत्यक्ष विधि द्वारा जांच की गई, अर्थात्, द्विस्तरीयता (Bistability) की उपस्थिति के परीक्षण के लिए, क्योंकि यह सकारात्मक प्रतिक्रिया के अस्तित्व का निश्चित प्रमाण है। गैर-मेटाबोलाइज़ेबल Inducers के साथ बार-बार द्विस्तरीयता देखी गई है। लेकिन आज तक, metabolizable inducer के साथ द्विस्तरीयता की जांच अध्ययन ने अस्पष्ट परिणाम दीये है। इस अध्ययन का मुख्य उद्देश्य मेलीबियोज़ ऑपरोन में विभिन्न स्थितियों के तहत द्विस्तरीयता की जांच करना है। इस काम में, हमने सीमा-स्तर को स्थिरांक रखते हुए, α-galactosidase की अलग-अलग गतिविधियों वाले प्रवाह में द्विस्तरीयता का अध्ययन किया। हमने सभी उपभेदों में द्विस्तरीयता देखी,

महत्वपूर्ण बात, मेलीबियोज़ ऑपरोन के लिए जंगली प्रकार के जीव में भी। ई. कोली मेल ओपेरॉन में, अपुष्ट प्रमोटर के नियंत्रण में चार गुना द्वारा मेलीबियोज़ की अनुमति अभिव्यक्त करके 'पेटी दमन' को समाप्त कर दिया गया। इन दोनों टिप्पणियों ने मेलीबियोज़ ऑपरोन में 'सकारात्मक प्रतिक्रिया' के अस्तित्व का जोरदार सुझाव दिया है।

हमने कार्बन फ्लक्स पर पेटी दमन का सामना करने के प्रभाव की जांच की । इसके लिए, हमने विभिन्न गठित रूप से व्यक्त किए गए मेलीबियोज़ सारभूत म्यूटेंट का इस्तेमाल किया और ग्लूकोज और मेलीबियोज़ खपत दर को मापा। हमने पाया है कि जब तक हम मेलीबियोज़ की 'तेज' गति को बढ़ाते हैं, कार्बन कार्यक्षमता 50% से अधिक की वृद्धि करते हैं, जो जैव प्रौद्योगिकी में महत्वपूर्ण निहितार्थ हैं। हम यह भी मानते हैं कि सीमा के स्तर को बढ़ाने पर, एकमात्र कार्बन स्रोत के रूप में मेलीबियोज़ पर विकास दर बढ़ जाती है। यह बताता है कि मेलीबियोज़ 'तेज', मेलीबियोज़ पर विकास के लिए सीमित कारक है।

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF ABBREVIATIONS

cAMP	Adenosine 3'-5' Phosphate (Cyclic 3', 5'-AMP)
CAP	Catabolite Activator Protein
IPTG	Isopropyl-β-D-1-Thiogalactopyranoside
LacI	Lactose repressor
LacY	Lactose permease
LacZ	β-galactosidase
MelA	α-galactosidase
MelB	Melibiose permease
MelR	Melibiose regulator (activator)
$MgSO_4$	Magnesium Sulfate
$MnCl_2$	Manganese Chloride
NAD^+	Nicotinamide Adenine Dinucleotide
NaOH	Sodium Hydroxide
OD	Optical Density
α-PNPG	Para-Nitro-Phenyl-α-Galactopyronside
α-PNP	Para-Nitro-Phenol
PEP:PTS	Phosphoenolpyruvate-Carbohydrate Phosphotransferase System
SDS	Sodium Dodecyl Sulfate
TMG	Methyl-β-D-Thiogalactoside