INDIAN INSTITUTE OF TECHNOLOGY GUWAHATI
SHORT ABSTRACT OF THESIS

Name of the Student	:	Payel Sarkar
Roll Number	:	126106023
Programme of Study	:	Ph.D.

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Name of Thesis Supervisor(s)	:	Prof. Debasish Das
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SHORT ABSTRACT

Rapid expansion and development of industries along with exponential growth in world population have led to a surge in the global energy demand in recent times. An escalating increase in dependence on conventional non-replenishing fossil-based fuels have triggered concerns on the reduction in their reserves and subsequent increase in global carbon footprint with its detrimental effects on climate change. To that end, extensive efforts and culmination of ideas prompted development of sustainable and renewable fuels. Biofuels, especially bioethanol and biodiesel, have emerged as prospective alternative renewable energy source with the potential to significantly reduce the thrust on the depleting fossil fuel reserve as well as carbon dioxide emissions. Bioethanol through microbial fermentation of organic matter or wastes has been considered as a redoubtable competitor of the existing petroleum centric transportation fuels, owing to its desirable properties such as easy blendability with gasoline, reduced greenhouse gas emission, carbon neutral etc., to mention a few. Conventional use of food crops, as substrates, for bioethanol production rendered assailable socio-economic conditions, which led to the exploitation of inexpensive, sustainable and abundant alternative substrate e.g., lignocellulose, for bioethanol production through microbial fermentation. Lignocellulose is majorly composed of cellulose, hemi-cellulose and lignin, which upon hydrolysis yields a mixture of sugars comprising majorly of hexose (D-glucose) and pentoses (D-xylose and L-arabinose). The typical composition of lignocellulosic hydrolysates necessitates the development an ethanologenic microbial platform with the ability to effectively utilize both hexose as well as pentose sugars, which would also mark its significance in commercial bioethanol fermentation process. Zymomonas mobilis, owing to its higher ethanol yield, productivity, and ethanol-tolerance, proved to be an exceptional candidate bioethanol producer, when compared to commercially acknowledged ethanol producing S. cerevisiae. However, owing to lack of a complete pentose phosphate pathway, wild type Z. mobilis cannot metabolize xylose or arabinose. The present study has been designed and implemented with the primary rationale to overcome the challenges existing towards efficacious pentose fermentation, by development of engineered Z. mobilis strains adept in utilizing hexose and pentose sugars with comparable efficiencies, for commercially realizable production of bioethanol. To that end, two Z. mobilis strains, i) Zymomonas mobilis ATCC 31821 or ZM4 (a wild type Z. mobilis strain) and ii) Zymomonas mobilis ATCC ZW658 (a recombinant

Z. mobilis strain), were characterized in terms of growth, substrate utilization, and product formation. Based on their performance, Z. mobilis ATCC 31821 or ZM4 was initially selected for genetic manipulation towards simultaneous utilization of glucose and xylose. Directed metabolic engineering was employed to modify ZM4 with heterologous xylose utilizing genes unresponsive to glucose inhibition (Carbon catabolite repression). However, the engineered ZM4 strain did not manifest the desired traits as expected. Thereafter, Zymomonas mobilis ATCC ZW658 (ZW658) endowed with heterologous xylose metabolizing genes integrated in its genome was selected for genetic engineering using a systematic Adaptive Laboratory Evolution (ALE) strategy. ZW658 was subjected to extended ALE involving 50 transfers, carried out over a period of 200 days. The strain was grown under strict selection pressure of increasing xylose concentration from 30 g L-1 to 100 g L-1 to obtain an adapted strain designated as AD50. Process engineering strategies were designed to enhance the phenotypic response of AD50 in terms of xylose utilization and ethanol formation. However, since the primary objective of this study was to develop a Z. mobilis strain that could simultaneously utilize glucose & xylose and produce high amounts of ethanol, AD50 was further subjected to adaptation in presence of glucose under two different strategies. In the first strategy undertaken, AD50 was alternatively subcultured in media containing 10% (w/v) xylose or 2% (w/v) glucose. In the second strategy, AD50 was serially subcultured in media containing 8% (w/v) xylose in the initial 48 h of growth followed by the addition of 2% (w/v) glucose in the same cultivation media and the culture was allowed to grow further for 24 h. After 72 h of cultivation, the serial subculture steps were repeated. After 6 serial transfers, single isolated colony was obtained, one each from the first and second strategy of ALE in glucose and xylose-based media. The adapted strains were designated as AS1-6 and AS2-6, respectively. The adapted strains AD50, AS1-6 and AS2-6 were further characterized in media containing 5% (w/v) glucose and 5% (w/v) xylose. It was observed that AD50 outperformed AS1-6 and AS2-6 in terms of specific xylose uptake rate in presence of glucose. The evolved strain (AD50) exhibited 1.65 times increase in the overall specific xylose utilization rate when compared with the parent strain (ZW658) and other developed strains reported till date. AD50 also displayed enhanced performance in terms of co-fermentation of xylose in presence of glucose with specific xylose utilization rate of 1.34 g g−1 h −1 , as compared to that of the parent strain ZW658 (0.13 g g−1 h −1). High throughput (Next-gen) sequencing revealed novel mutations in xylose assimilating, metabolizing, and crucial regulatory pathway genes, which substantiate the improved phenotypic response of AD50 in terms of co-utilization of glucose and xylose, higher ethanol and reduced xylitol production. Enzyme activity assays were carried out to validate the performance of the strain with high confidence. Although, directed ALE proved to be an efficient strategy for development of a Z. mobilis strain, which can co-utilize glucose and xylose at comparable rate, however, there was scope for improvement in terms of simultaneous utilization of xylose in presence of glucose by AD50. Previous research endeavours have highlighted bottlenecks associated with xylose metabolism in Z. mobilis. Hence, our second objective was to investigate ‘transport’ as a possible obstacle for simultaneous of glucose and xylose. In particular, we hypothesized that the slow uptake of xylose through the promiscuous Glf transporter may limit the efficiency of xylose metabolism in Z. mobilis. To test this hypothesis, an array of constructs with xylose specific transporters were developed using AD50 as the host organism. XylE, the low-affinity xylose transporter from Escherichia coli, XylE* (a mutant variant of XylE), XylFGH, the ABC type transporter from E. coli, was expressed in AD50. Introduction of the xylose transporters, especially an ABC type transporter system, manifested into increased rate of xylose utilization by 48.9%, in presence of glucose by the novel engineered Z. mobilis strain, as compared to that of the host strain (AD50), leading to notably reduced fermentation time and co-utilization of glucose and xylose coupled with comparable ethanol production to that of AD50. The specific utilization rate of xylose in presence glucose was observed to be 2.04 g g-1 h -1 , which is comparable to that of increased glucose (2.49 g g-1 h -1). Thus, in the ensued study an improvement of 14.7-fold was observed in term of specific utilization rate of xylose in presence glucose as compared to that of the parent strain ZW658, used as the initial platform strain for genetic manipulation. The ethanol titer was observed to be 47.4 g L-1 , with productivity and yield

of 1.97 g L-1 h -1 and 0.472 g g-1 , respectively. This study confers an apprehension of the effect of different xylose transporters in Z. mobilis, which have not been explored till date. The phenotypic response of the best performing strain, Zm-Ppdc-XFGH was observed to be consistent under scale up conditions in a bioreactor. Hence, the ensued study successfully demonstrates development of an efficient bioethanol producing Z. mobilis strain with a potential to co-utilize glucose and xylose, which might aid towards commercial realization of ethanol biosynthesis.