Chapter 11
Genomics Perspectives of Bioethanol Producing *Zymomonas Mobilis*

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ABSTRACT

In recent years, there has been continuous increase in demand for fossil fuels that has led to the need for new potential fuel sources. Biofuels, in particular ethanol, are of high interest because of dwindling fossil fuels. Among the ethanol producers, Zymomonas mobilis has acquired greater interest because it is a renewable source of bioethanol. Zymomonas mobilis is an aerotolerant, gram-negative, ethanol producing bacterium that shows high ethanol yield, tolerance, and greater productivity. This chapter focuses on recent efforts made to engineer Z. mobilis, transcriptomic, genome-based metabolomic studies, and bioinformatics exploitation of the available genomic data for the production of bioethanol. Recently, several bioinformatics tools have been used to predict the functional properties of the carbohydrate active ethanologenic enzymes in Z. mobilis. A number of processes were used to study the functional properties of the ethanologenic enzymes of Z. mobilis. Thus, functional genomics seeks to apply technologies that would help to improve the production of bioethanol by Z. mobilis.

INTRODUCTION

The theme of this chapter is based upon the recent developments in post-genomic perspective of ethanologenic *Z. mobilis*. During the pre-genomic period, much of the research was focused upon biochemical and molecular aspects, fermentation studies, heterologous expression of key ethanologenic enzymes, process development, saccharification, co-culture studies and strain improvement. Post-genomic studies after 2004 primarily focused upon key areas such as data mining of the genome to look out for possible enzymes involved in lactose, maltose, and cel-

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lobiose metabolism that would play role in the ethanol production. The genome-level modeling, *in silico*, transcriptomic, metabolomic, and proteomic studies of ethanologenic enzymes of *Z. mobilis* will be discussed in this chapter.

BACKGROUND

In the present 21st century, there has been ever-increasing demand for the fuel in particularly bioethanol. The various causes for the increase in demand for the fuel are as follows: 1) The depletion of the available energy resources such as fossil fuel, lignite, coal; 2) increase in pollution level and global warming; 3) rapid urbanization; 4) lack of manpower and technologies in the developing countries to tap solar, wind and tidal energy. Thus, these sources of energy are non-renewable when compared to bioethanol. Ethanol production from food crops like corn is mostly dependent upon fuel subsidies and it consumes food crop to produce biofuel. Moreover, the retail price for commercial microbial ethanol is 2.62 US $ /gallon compared to a gallon of gasoline priced at 3.03 dollar. Bioethanol is an ideal candidate in the current scenario because of its potential application as fuel, beverage, feedstock, antiseptic and industrial solvent. The major steps for large-scale microbial production of ethanol are fermentation of sugars, distillation, and dehydration. *Z. mobilis* has been known to have favourable traits like limited growth requirements, genetically amenable, higher sugar, and ethanol tolerance. The primary focus of this chapter is to review the current developments in the field of functional genomics and bioinformatics analysis of the genome of *Z. mobilis*.

During the pregenomic era, there was considerable amount of research focused upon substrates optimization for ethanol fermentation process, genetic engineering of strains. Gunasekaran et al. (1986) demonstrated production of bioethanol by 4 different strains of *Z. mobilis* by using natural substrates like cane juice and molasses. Kamini and Gunasekaran (1987, 1989) has shown that synchronized ethanol production from lactose by strains of *Z. mobilis* 1960, B 4286 by coculturing with *K. fragilis* 665. Gunasekaran and Kamini (1991) demonstrated elevated ethanol production from lactose by immobilized cells of *Z. mobilis* B 4286 cocultured with *K. fragilis* 665. Nellaiah et al. (1988a) has reported the production of 94.0 g l^{-1}, 76.9 g l^{-1}, and 66.5 g l^{-1} of ethanol at glucose, fructose, and sucrose concentrations of 200 g l^{-1}, respectively by *Z. mobilis* B-4286. Nellaiah et al. (1988b) has reported the production of 80.0 g l^{-1} of ethanol from enzymatically hydrolysed cassava starch at glucose concentrations of 171 g l^{-1}, by *Z. mobilis* B-4286. Nellaiah and Gunasekaran (1992) showed that highest ethanol concentration of 59 g l^{-1} and productivity of 3.57 g l^{-1} could be obtained from cassava starch hydrolysate by immobilized *Z. mobilis*. Amutha and Gunasekaran (1994) showed that concurrent saccharification and fermentation of cassava starch using *Z. mobilis* produced 57 g l^{-1} of ethanol. Baratti et al. (1991) has demonstrated that *Z. mobilis* possess an extracellular sucrase which is mainly responsible for sucrose hydrolysis during fermentation. Kannan et al. (1998) has shown that improved ethanol production of 73.5 g l^{-1} from 150 g l^{-1} sucrose by a mutant of *Z. mobilis* than of its parent strain, B-806 (65.2 g l^{-1}).

The genome sequence of *Z. mobilis* ZM4 was reported by Seo et al. (2005). The genome sequence shows the presence of enzymes that metabolize sucrose, fructose, glucose, mannose, raffinose, and sorbitol. The key enzymes (glucose-6-phosphate dehydrogenase, lactonase, 6-phosphoglucanate dehydratase) necessary for the conversion of glucose to yield ethanol have been reported in this study. More than 54 genes were found to be more expressed when ethanol production is vigorous. NAD(P)H: quinone oxidoreductase and oxidoreductase were found to be highly expressed during ethanol production. It was reported that there was no obvious gene for metabolizing lactose. Our initial sequence analysis and experimental approach led to the study of beta-galactosidase in *Z. mobilis*