CHAPTER I

INTRODUCTION

In the latter part of the nineteen century, microbes used in industrial production were grown in pure culture for the first time. This development quickly led to improve understanding of the relationships between specific microbes and their products and activities. Specific industries became active in microbiological research and selected certain microbes for their special qualities. As the knowledge of microbial genetics increased, the ability to select and use desirable strains of microorganism was improved (Tortora et al., 1989).

Ethyl alcohol had been many used in industrials as a solvent and as a substrate for the synthesis of numerous organic compounds. Prior to the 1940s most industrial alcohol was produced by yeast fermentation of molasses. As a result of increasing cost of molasses and the relatively low cost of petroleum, for the past several decardes it had been primarily synthesized from petrochemicals. However, the shortage of petroleum and its increased cost had resulted in a return to microbial fermentation (VandDermark and Batzing, 1987). Scientists were developing *Zymomonas* that was able to produce alcohol using cellulose from agriculture wastes rather than from carbohydrates. The

selecting of ethanol-producing microorganisms with an improved tolerance for ethanol might allow the microorganisms to produce the alcohol more efficiently in higher concentrations (Tortora et al. 1989).

Zymomonas mobilis was undoubtedly one of the most unique bacterium within the microbial world. Known since 1912 under the names *Termobacterium mobilis*, *Pseudomonas linderi*, and *Zymomonas mobilis*. The bacterium *Zymomonas mobilis* only exhibits an extraordinary uniqueness in its biochemistry, but also in its growth behavior, energy production, and response to culture conditions, as well as cultivation techniques used. This uniqueness caused great interest in the scientific, biotechnological, and industrial worlds. Its ability to couple and uncouple energy production in favor of product formation, to respond to physical and chemical environment manipulation, as well as its restricted product formation, made it an ideal microorganism for microbial process development (Doelle et al., 1993).

Zymomonas mobilis was a plump rod-sharped cell with rounded ends (1.4-2,0 by 4.0-5.0 um), occurring singly or in pairs, less frequently in short chains, motile by mean of lophotrichous flagella. No resting stages known. It was Gram-negative bacteria (Buchaman and Gibbon, 1975).

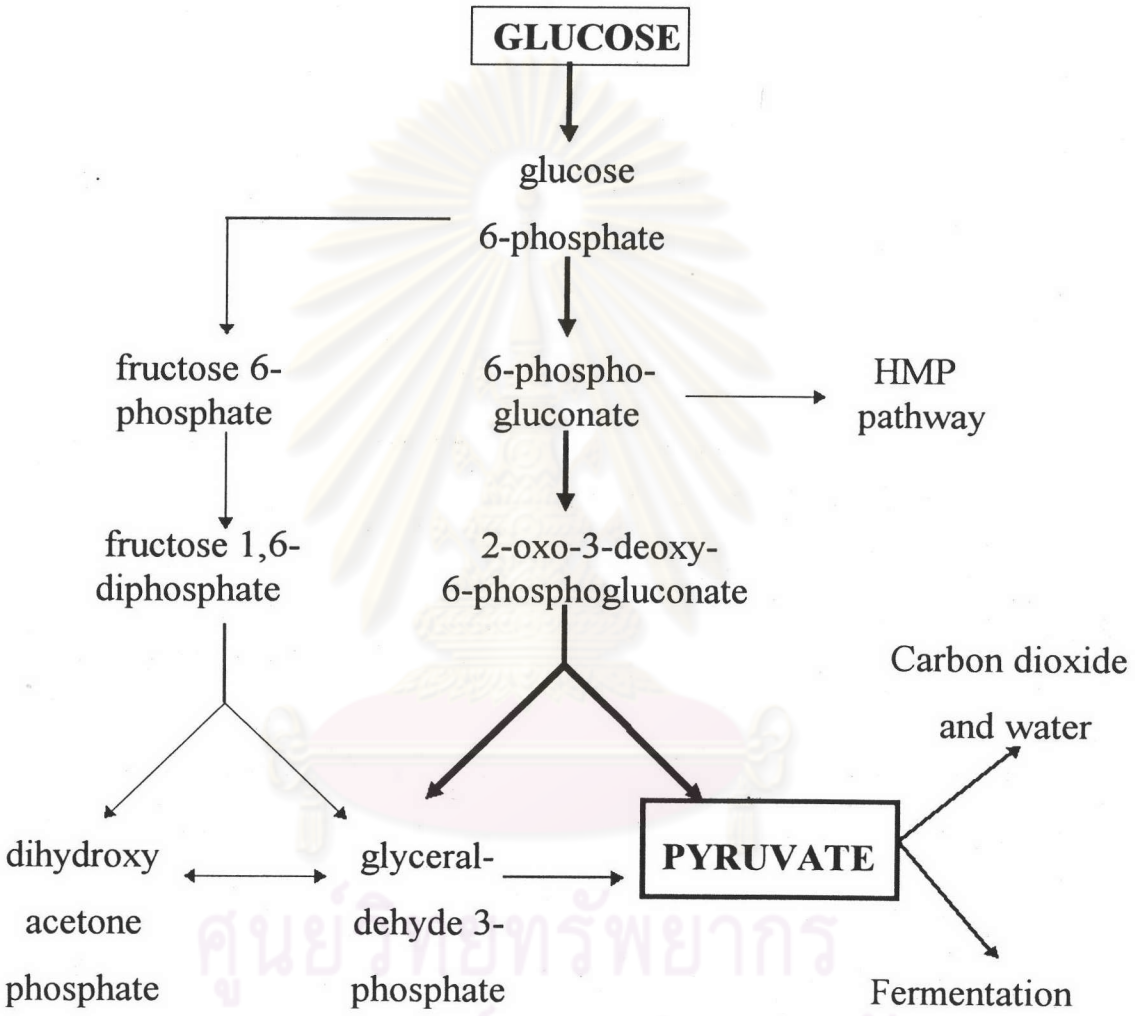
Zymomonas mobilis was originally isolated from pulque, the fermenting sap of *Agave americana*. Also found in the fermentating juice of the Gomuti palm, *Arenga pinnala* (*A.sacchifera*) in central **Java**. Apparently common in fermenting plant juices in tropical countries.

Chemoorganotrophs showing vigorous fermentative metabolism of glucose or fructose which yields nearly equimolar quantities of ethanol and CO_2 by the Entner-Doudoroff pathway (scheme 1.).

One mole of glucose is fermented with the production of 1.6 moles of ethyl alcohol and 1.8 moles of CO_2, with traces of lactate. Fructose and sucrose are also fermented; the latter is metabolized more slowly than the monosaccharides and levan is produced. The fermentation of sucrose is the main characteristic distinguishing this speicies from *Zymomonas anaerobia*. Although not an end product, acetaldehyde accumulates in the culture medium. Arabinose, rhamnose, xylose, galactose, lactose, maltose, raffinose, manitol and dulcitol are not fermented. Growth but no gas production from sorbitol. Ethyl alcohol is metabolized to acetate in aerated cultures.

Panthothenate is the only growth factor required. Growth occurs in a synthetic medium plus panthothenate but is about half of that obtained in yeast extract medium. Good growth is obtained with ammonium ion as the sole source of nitrogen. cell grown aerobically contain cytochromes of the *c* and a_2 type; those grown anaerobically also contain a cytochrome of the *b* type.

Zymomonas mobilis was classified as anaerobes, but able to tolerate some oxygen.Cultured at optimum temperature 30° C.



Scheme 1. Enter-Doudoroff pathway and its relationship to other glycolytic pathways. (Tortora et al., 1989).

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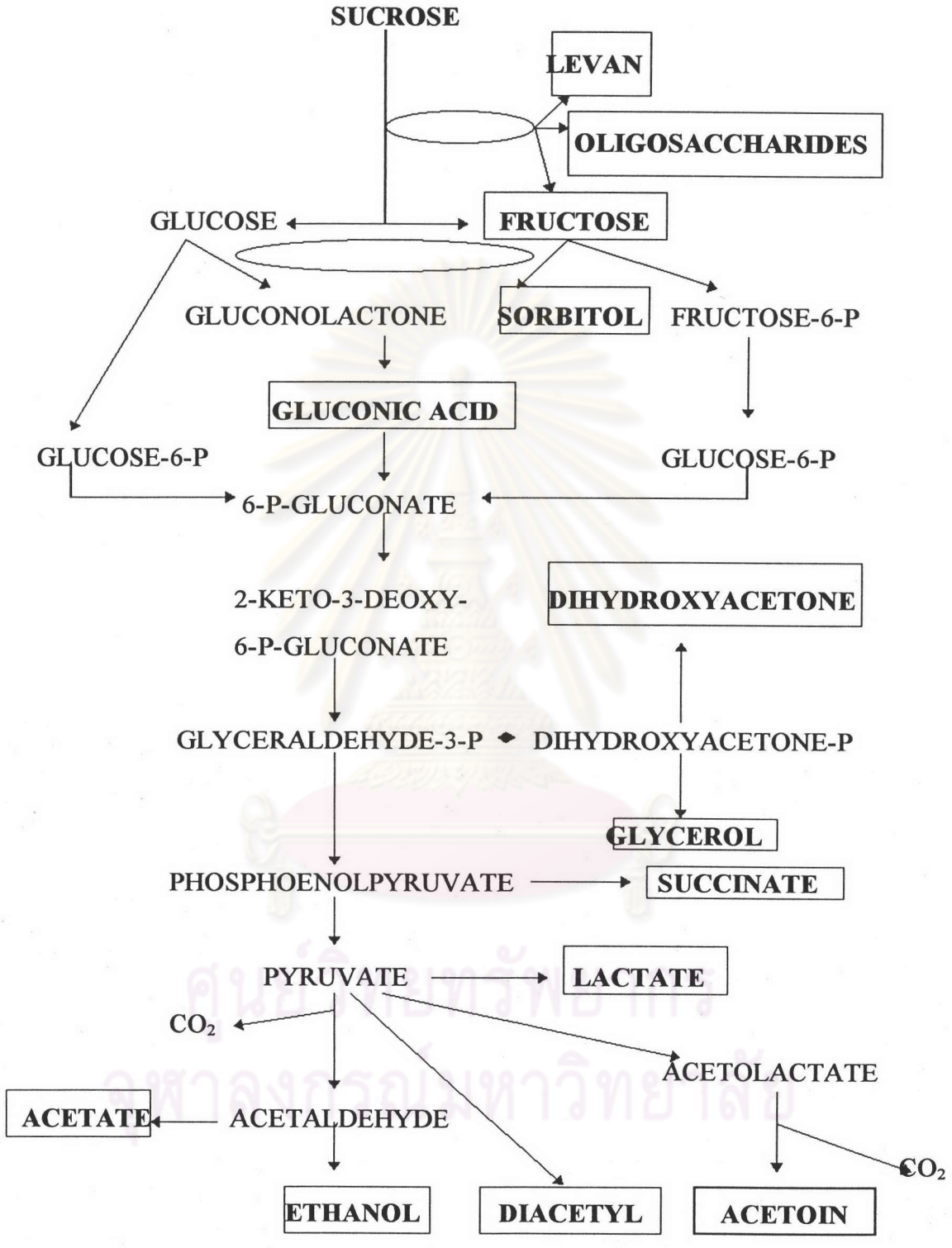
Zymomonas mobilis was an obligately fermentative organism and utilizes sucrose, glucose, and fructose by the Entner-Doudoroff (ED) pathway, which was used mostly by strictly aerobic organisms such as *Pseudomonas* . Although it was classified as an anaerobic microorganism, *Zymomonas* grew well under aerobic conditions. It appeared to lack an oxidative electron transport system, however, and produced a single net ATP molecule per molecule of glucose metabolized under both conditions. Furthermore, it had replaced the active transport system with a facilitated diffusion system. The taxonomic position of *Zymomonas* had not been fully established, therefore, mainly because of the uncertainties about the aerobic metabolism of these either anaerobic aerotolerant or facultative anaerobic bacteria.

Zymomonas mobilis was an anaerobic Gram-negative bacterium that tolerated oxygen had been observed to change its metabolism during the transition from anaerobiosis to aerobiosis. The growth rate was lowered, but the molar growth yield did not decrease in the presence of oxygen. However, a switch from ethanol plus carbon dioxide to the formation of acetaldehyde, acetoin, and other by products occurs. Another product formed under aerobic conditions was acetate, which was produced via the oxidation of acetaldehyde. The production of acetic acid increased in the presence of oxygen. In the presence of oxygen, ethanol and lactate could also serve as sources of reductant being oxidized in the process, suggesting that the enzymatic process involved in the formation of products in reversible.

Switch in metabolism observed in *Zymomonas mobilis* in transition from anaerobic to aerobic condition was not merely due to the diversion of reduced cofactors from the fermentative pathway, but a series of responsed effected at the enzyme level and possible at the transcription and translation level, that was, induction and repression, as observed in facultative anaerobes, in spite of an incomplete tricarboxylic acid cycle.

Zymomonas mobilis appeared to be a metabolically unique microorganism that actively consumes oxygen without an aerobic respiration as indicated by a lack of increase in CO_2 production under aerobic conditions and all end products were of an organic nature, indicating a fermentative activity in the presence of oxygen (Scheme 2.) (Doelle et al., 1993).

Various attempts had been made to prepare *Zymomonas mobilis* for replacing yeast in the industrial ethanol industry using renewable resources such as corn/maize, potato, maltrin, wheat, milo, cassava, and sago. Many mutation experiments were performed. Today, one was constantly looking for viable processes; therefore, the value of the product must be closely examined. A number of different genes have been transffered to *Zymomonas* in an affort to produce highly valued products such as β-carotene, D-alanine, or other claim product preference for acetaldehyde over ethanol because of its ease in product separation. This had not been a widely explored area and appeared to be a specialized market.



Scheme 2. Metabolic pathways to major byproducts in *Zymomonas mobilis*. (Doelle et al., 1993).

Mutation experiments were performed to reduce levan formation of *Zymomonas mobilis* CM141 (Chavapan, 1986), a high ethanol tolerance strain isolated in Chiang Mai, using hydroxylamine. A mutant expected to produce no levan was found to produce red pigment under aerobic conditions. Large quantities of the pigment were secreted on to the medium after the growth phase. This preoperty was proved to be stable by performing ten successive transfers. Preliminary study, by TLC showed that the pigment contains three components; purple, red and pink with the maximum absorption at 544, 534, and 536 nm respectively. When two doses of 2 ml of the crude pigment were fed to white mice, no immediate effect was observed (Supanwong and Chavapan, 1987).

Bacteria pigments are found insides the cells in small amounts and have no prospect for commercial production. The pigments produced by the mutant are secreted on to the medium in large quantity and have potential for commercial production. Possibility of using the pigment in food, cosmetic and pharmacetical industries were expected. On the other hand the pigments which were the low molecular weight secondary metabolic compounds from microorganism may be used in medicinal purposes as antibiotic (Primrose, 1987) and immunomodurating substances (Kohama et al., 1992). Because of the lack of information in the literature on the nature of the red pigments produced by *Zymmomonas mobilis,* this research was aimed to characterize the red pigments and increase the red pigments production.

The study was initiated with the separation, purification and elucidation the chemical structure of the red pigment. And to enchance yield of pigment production the mutation experiments were performed by random mutation and selective procedure.

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