Chapter 33
Date Palm as a Source of Bioethanol Producing Microorganisms

N. Gupta and H. Kushwaha

Abstract Expanding world population, increasing energy demand, depleting reserves of fossil fuels and increasing effects of pollution from these fuels demand more ecofriendly alternatives which can substitute for fossil fuel (petrol, diesel, coal etc.). Ethanol derived from biomass has the potential to be a substitute of fossil fuel which is renewable, non-toxic, biodegradable and more ecofriendly. The three major classes of feedstocks for ethanol production are sugars, starches and lignocelluloses. Date palm (*Phoenix dactylifera*) sap is highly nutritive and has high sugar content which varies from 60% to 70%; it is also a very good source of fermentation microorganisms. Palm-wine fermentation is always alcoholic-lactic-acetic acid fermentation, involving mainly yeasts and lactic acid bacteria. Currently research is being directed to develop metabolically and genetically engineered *Saccharomyces* strains and other ethanol-fermenting microflora that have the potential to utilize a wide range of substrates including pentose and hexose sugars, an ability for direct and efficient ethanol production from cellulosic materials and to tolerate ethanol stress. Thus *Saccharomyces* strains from date palm sap could be genetically modified to overcome the constraints in the path of higher yield ethanol production.

Keywords Biofuels • Bioethanol • Biomass • Fermentation • *Phoenix dactylifera*

N. Gupta (✉)
Department of Microbiology and Biotechnology, Faculty of Science, M S University, Baroda, Gujarat, India

Department of Molecular Biology and Genetic Engineering, College of Basic Sciences and Humanities, G.B. Pant University of Agriculture and Technology, Pantnagar, Uttarakhand 263 145, India
e-mail: nidhigupta_2411@yahoo.co.in

H. Kushwaha
Department of Molecular Biology and Genetic Engineering, College of Basic Sciences and Humanities, G.B. Pant University of Agriculture and Technology, Pantnagar, Uttarakhand 263 145, India

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33.1 Introduction

Rapid depletion of energy resources, based on non-renewable fuels, is of deep concern worldwide, especially in the developing countries. This is mainly due to increased transportation, modernization and industrialization which ultimately lead to environmental pollution resulting in health hazards and ecological imbalances. Hence, there is a dire need to search for an alternative fuel, which is more ecofriendly and enhances quality of life. Global energy usage is projected to nearly double in the next two decades, and biological fuel production might serve as a sustainable, carbon-neutral energy source compatible with current engine technology. Conversion of biomass to biofuels has been the subject of intense research efforts since the 1970s. This work has recently gained significant political and scientific momentum owing to concerns about climate change, global energy security and petroleum supply. Today, biomass covers about 10% of the world's primary energy demand. Plant biomass is an abundant and renewable source of energy-rich carbohydrates which can be efficiently converted into biofuels by microbes. Today bioethanol is one of the major products available commercially.

The world's largest producers of bio-ethanol are Brazil (sugarcane ethanol) and the United States (corn ethanol). Brazil is largest exporter of ethanol; delivering 70% of worldwide supply and the USA is that country's largest client as it imported 1.74 billion liters in 2006, which represents 58% of Brazil's ethanol exports (Wust 2007). In an effort to offset increases in consumption and to limit the fossil fuel-related negative impacts on the environment, the US Department of Energy has established the goal of supplanting 30% gasoline consumption with cellulosic ethanol by 2030 (Herrera 2006). Similarly, a European Union Directive of 2003 aims to replace 5.75% of all gasoline and diesel transport fuels with biofuels by 2010 (Schubert 2006). A few countries are actively involved in generating biofuel from date palm at a very large scale by setting up various companies aimed at ethanol production. *Oman Green Energy Company Makes Ethanol From Date Palm, Plans Large Refinery, 100 Ethanol Pumps By 2010*, reads a news account (Gulf News 2007). In Algeria, Algerian Biotech Company aims at production of biomethanol from dates.

Owing to physical and political limitations on arable land, it is believed that future biofuels will, of necessity, originate from abundantly available lignocellulosic biomass. There are many advantages to using bioresource derived ethanol as a liquid transportation fuel. Bioethanol blended with gasoline extends crude oil utilization, reduces reliance on oil imports and helps to mitigate increasing oil prices. The higher oxygen content of ethanol results in relatively cleaner combustion and has long been used as an additive in gasoline to reduce urban smog and other environmental pollution problems. Therefore, ignition improvers, glow-plug, surface ignition and pilot injection are applied to promote self-ignition by using diesel-bioethanol blended fuel (Kim et al. 2005).

The most popular blend for light-duty vehicles is known as E85, and contains 85% bioethanol and 15% gasoline. In Brazil, bioethanol for fuel is derived from sugarcane and is used either pure or blended with gasoline in a mixture called gasohol (24% bioethanol, 76% gasoline) (de Oliveria et al. 2005). In several states

of the United States, a small amount of bioethanol (10% by volume) is added to gasoline, known as gasohol or E10. Blends having higher concentrations of bioethanol in gasoline are also used, e.g. in flexible-fuel vehicles that can operate on blends of up to 85% bioethanol-E85 (Malca and Freire 2006). Some countries have exercised biofuel program involving both forms: bioethanol-gasoline blend program, e.g. the United States (E10 and for Flexible Fuel Vehicle (FFV) E85), Canada (E10 and for FFV E85), Sweden (E5 and for FFV E85), India (E5), Australia (E10), Thailand (E10), China (E10), Columbia (E10), Peru (E10), Paraguay (E7) and Brazil (E20, E25 and FFV any blend) (Kadiman 2005).

33.2 Major Feedstocks and Bioethanol Production Processes

The three major classes of feedstocks for ethanol production are sugars (e.g., molasses, cane juice), starches (corn, wheat, cassava) and lignocelluloses (rice straw, wheat straw, bagasse, wood, energy crops) (Fig. 33.1). Starch and sugar-based ethanol is often referred to as a first-generation biofuel. Even though the production of ethanol from starch represents the most convenient and technically advanced option for bioenergy in the USA, it would result in severe competition between energy and food supplies. Lignocellulosic feedstock can be acquired from either dedicated biomass crops or forestry and agricultural residuals (Boerjan 2005; Sims et al. 2006). The key obstacle for transitioning from starch-based to lignocellulosic biofuels is the

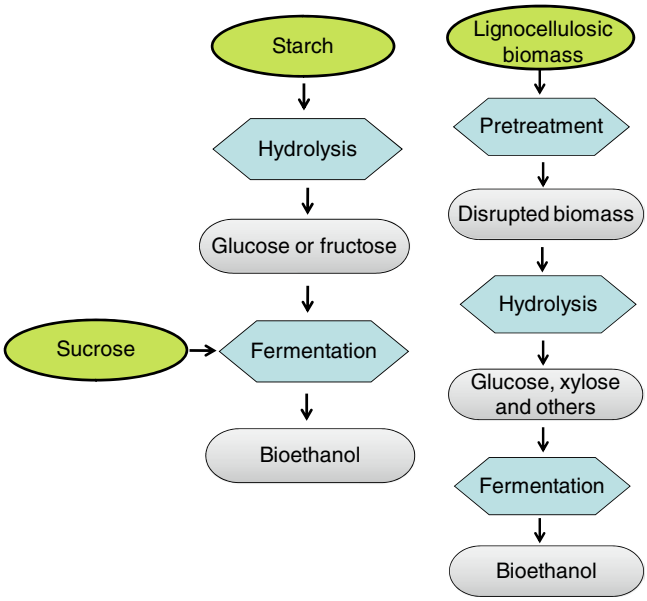

Fig. 33.1 Platform for fermentation process by major feedstocks of ethanol *A* Sucrose, *B* Starch and *C* Lignocellulosic material

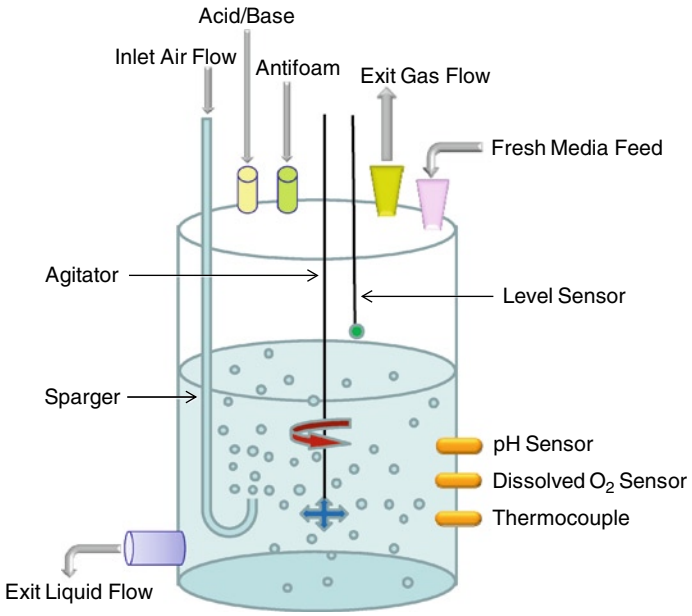

Fig. 33.2 Structure of fermentation tank

complicated structure of the cell wall, which is resistant to breakdown and represents a recalcitrance problem. Current processes for lignocellulosic biomass include pretreatment, saccharification (hydrolysis) and fermentation (Ragauskas et al. 2006). Improvement or replacement of these processes is crucial for increasing efficiency and for decreasing biofuel production costs. Obviating pretreatment, along with simultaneous saccharification and fermentation, are two important factors that would decrease the cost of lignocellulosic ethanol production (Ragauskas et al. 2006).

Ethanol production is a simple process that can be run either as a batch reactor in a confined space or as a continuous process (Fig. 33.2). An entire plant can in fact fit in one's own backyard making ethanol a very attractive fuel source for communities or even countries that wish to be self-sustainable and not reliant on foreign resources.

33.3 Date Palm: An Overview

33.3.1 Date Production

Date palm (*Phoenix dactylifera* L.), a diploid with 2n = 36, is a member of the monocot family Arecaceae classified as a dioecious tall evergreen. Date palm is the most cultivated palm in the arid and semi-arid regions of the world. Dates constitute part of a popular subsistence among the populace of the Middle Eastern peninsula.

There are estimated 90–100 million trees worldwide and mainly concentrated between latitudes 10–30° North, in arid regions of the Middle East and North Africa, where it is thought to have been cultivated for several thousands of years. According to the FAOSTAT database (http://faostat.org), world date fruit production has risen from 2,659,406 mt in 1980 to 7,109,974 mt in 2008. The major producers of date palm are Egypt, Iran, Saudi Arabia, United Arab Emirates, Pakistan, Algeria, Iraq, Sudan, Oman and Libya.

33.3.2 Date Chemical Composition

The development of date fruits is divided into three final stages: khalal, rutab and tamar. Khalal stage dates are immature with hard texture, yellow, red or pink in color, total soluble solids (TSS) of 30–45° brix, astringent and in some cultivars edible; rutab stage dates soften at the tip of the fruit, with TSS of 55–60 brix, are free of astringency and edible; tamar stage dates are fully ripe with TSS of 60–84° brix and edible (Pareek 1985). Dates are generally harvested at the tamar stage, that is after the development of TSS of 60–70° brix. Date fruit at the tamar stage contains moisture ranging of 10–22%; total sugars 62–75%; protein 2.2–2.7%; fiber 5–8%; fat 0.4– 0.7%, ash 3.5–4.2%; total acidity 0.06–0.20% and ascorbic acid of 30.0–50.0 mg%, on a dry weight basis (Baraem et al. 2006; El-Sharnouby et al. 2007; FAO 1962).

The fruit of the date palm is composed of a fleshy pericarp and seed. Pits (seeds) of date palm are a waste product of many date fruit processing plants producing pitted dates, date powders, date syrup, date juice, chocolate-coated dates and date confectionery. Date palm female trees bear fruits at 3–5 years and are fully mature at 12 years. The fruit is a nutritious source of sugar, minerals, and vitamins. Appropriately called the *palm of life*, for over 5,000 years the date palm has provided food, ornament and material for shelter, fiber and fuel in a harsh environment where relatively few other plants are able to grow. Mature date palms are highly desirable landscape subjects because they are plentiful, relatively inexpensive, uniform in size and habit and highly ornamental.

33.3.3 Date Utilization

Dates are known to be rich in carbohydrates (80%) but quite low in protein (2–3%) (Al-Hooti et al. 1997). Dates are an excellent source of simple sugars, minerals and vitamins (El-Shaarawy et al. 1989) and its fiber content reaches about 8% (FAO 1962; Lambiote 1982). The flesh of a fully ripe date (tamar), consist of two-third sugars and one-quarter water, the rest being mainly cellulose, pectin, ash and vitamins (FAO 1962). The date is considered as a nutritious fruit as research has indicated the clear contribution of dates to human health when consumed with other food

constituents (Lambiote 1982). There are at least 15 minerals in dates. The percentage of each mineral in dried dates varies from 0.1 to 916 mg/100 g date depending on the mineral. In many varieties, potassium can be found at a concentration as high as 0.9% in the flesh while it is as high as 0.5% in some seeds. Other minerals and salts that are found in various proportions include boron, calcium, cobalt, copper, fluorine, iron, magnesium, manganese, potassium, phosphorous, sodium and zinc. Additionally, the seeds contain aluminum, cadmium, chloride, lead and sulphur in various proportions. Dates contain elemental fluorine that is useful in protecting teeth against decay. Selenium, another element believed to help prevent cancer and important in immune function, is also found in dates. The protein in dates contains 23 types of amino acids, some of which are not present in the most popular fruits such as oranges, apples and bananas. Dates contain at least six vitamins including a small amount of vitamin C, and vitamins B_1 thiamine, B_2 riboflavin, nicotinic acid (niacin) and vitamin A. The fruits have an important therapeutic role in glycemic and lipid control of diabetic patients. Dates have also been identified as having antioxidant and antimutagenic properties, and were found to reduce heart disease and cancer.

33.4 Indigenous Ethanol Producing Microflora of Date Palm Sap

Since the principal constituent of the date fruit is sugar and its total sugar content at harvest ranges from 70% to 80%. High sugar content also is present in date palm sap and could be used as a good source of fermentative microorganisms. In almost all tropical locations in Asia where palm trees grow, the sap obtained from the decapitated inflorescence of various palm species is fermented to produce an alcoholic beverage called palm wine or toddy. There is an art in binding the flower spathes, pounding them to cause the sap to flow properly by cutting the spathe tip and collecting the sap into the earthen pitchers which contain yeasts and bacteria in the left-over toddy from the previous lots. The fermentation starts as soon as the sap flows into the pitcher.

Palm wine is either consumed fresh as it is brought down from the tree or fermented for up to 24 h. The freshly harvested sap is generally a dirty brown sweet liquid having 10–18% w/w sugar, a pH of 7.0–7.4 and traces of ethanol, which after fermentation results in the formation of a product containing as much as 9% (by volume, v/v) ethanol and pH of 4.0–5.5 (Joshi et al. 1999; Steinkraus 1996).

Palm wine fermentation is always alcoholic-lactic-acetic acid fermentation, involving mainly yeasts and lactic acid bacteria. In the fermenting sap, *Saccharomyces cerevisiae* is invariably present but lactic acid bacteria such as *Lactobacillus plantarum, L. mesenteroides* or other species of bacteria like *Zymomonas mobilis* and *Acetobacter* spp. vary. The other yeast types include *Schizosaccharomyces pombe*, *Saccharomyces chevalieri*, *S. exiguus*, *Candida* spp.; *Saccharomycodes ludwigii, S. pombe*, *Saccharomyces cerevisiae*, *Kodamaea ohmeri* and *Hanseniaspora occidentalis* which are characterized as maximum ethanol producers in toddy

(Joshi et al. 1999). The yeasts, especially *Saccharomyces* spp., are largely responsible for the characteristic aroma of palm wine (Uzochukwu et al. 1999). During fermentation, there is continuous effervescence as a result of the production of carbon dioxide. A yeasty odor develops and after a couple of hours yeasts form sediment at the bottom of the container.

Palm wine is a good source of B vitamins. Recently Gupta et al. (2009) reported the occurrence of high ethanol producing microorganisms (*Saccharomyces* sp.) with faster growth rate in date palm sap. Various indigenous strains of *Saccharomyces* sp. were isolated from date palm sap and were evaluated for alcohol dehydrogenase (ADH) enzyme activity, ethanol production and alcohol tolerance limits. Alcoholic contents in juice samples fermented with different yeast strains varied considerably (8.9–12.5%, v/v) as determined by GLC. Yeast cultures showed varied *in vitro* ethanol tolerance (3–12%). Isolate SCP-1 was found superior showing 12.5% ethanol production, high ADH enzyme activity (4.38 units/ml) and higher alcohol tolerance maintaining cell viability at 12% ethanol in YPD medium up to 48 h (Gupta et al. 2009).

33.5 Constraints in Bioethanol Production

33.5.1 Lack of Proper Substrate Utilization for Biofuel Production

Bioethanol production from plant biomass has received considerable attention recently in order to mitigate global warming and demands for petroleum. Currently, bioethanol is produced mainly from sugar-containing or starchy biomass such as sugarcane and corn as the raw material. As sugar-containing and starchy biomass is used for food and animal feed, there arises competition for its use as both food and fuel. Due to this competition, lignocellulosic bioethanol production has been eagerly researched worldwide. Lignocellulosic biomass, such as woods and agricultural residues, is an attractive feedstock for bioethanol production because of its large amount of potentially-fermentable sugars. The main structural components of lignocellulosic biomass are cellulose, hemicellulose and lignin. Of these, only cellulose and hemicellulose can be used as raw materials to produce ethanol by fermentation of carbohydrates obtained by chemical or enzymatic hydrolysis (saccharification). The main component of lignocellulosic hydrolysates is glucose, a hexose sugar derived from cellulose and hemicellulose. Although the proportion of monosaccharides in hemicellulose hydrolysates varies depending on the raw material and the hydrolysis procedure (Hendriks and Zeeman 2009; Lee 1997), they all contain both pentose sugars, such as D-xylose and L-arabinose and hexose sugars. D-xylose is the second most abundant carbohydrate and its content is particularly high in grass and hardwood. Thus, a substantial number of the hydrolysates obtained from lignocellulosic biomass contain xylose, requiring an economic conversion of biomass into ethanol through xylose utilization.

The microorganisms for hexose sugars including glucose, mannose, and galactose is *Saccharomyces cerevisiae*, yeast with high ethanol productivity, high tolerance to ethanol and tolerance to inhibitory compounds present in the hydrolysate of lignocellulosic biomass (Olsson and Hahn-Hagerdal 1993; Olsson and Nielsen 2000). Although *S. cerevisiae* can grow well even at relatively low pH, which prevents contamination by other bacteria, native strains are unable to utilize xylose for growth or fermentation. Instead, it metabolizes D-xylulose, an isomerization product of D-xylose (Chiang et al. 1981; Wang and Schneider 1980; Wang et al. 1980). Some yeast strains have been reported to ferment xylose into ethanol (Jeffries 1981; Schneider et al. 1981; Slininger et al. 1982), but the rate and yield of ethanol production from xylose in these xylose-utilizing yeast strains are considerably low as compared to their glucose fermentation. Therefore, genetic engineering and/or adaptation may be promising methods to develop sufficient pentose sugar fermentation in *S. cerevisiae*.

For the choice of the fermenting microorganism, complete substrate utilization, inhibitor tolerance and ethanol productivity are important aspects. The yeast *Saccharomyces cerevisiae* satisfies the last two conditions (Almeida et al. 2007; Bettiga et al. 2009; Piskur et al. 2006), while metabolic engineering is required to obtain strains able to ferment lignocellulosic biomass (Olofsson et al. 2008), which mainly consists of pentose and hexose, where the ratio depends on the source of lignocellulose. Since a limited number of microorganisms can ferment pentose, in the cases that pentose is the main sugar such as in corn stover and bagasse, usually a pentose-fermenting microorganism such as *Pichia stipitis* is used for cultivation. Lignocellulose is a complex polymer consisting of fibrous bundles of crystalline cellulose encased in a polymeric matrix of hemicellulose and lignin. Although compositions vary, this material can be generally regarded as being composed of 50% cellulose, 25% hemicellulose and 25% lignin (and extractables). For bioconversion, the carbohydrate portion must be solubilized while the lignin and residues can be used to provide energy for ethanol purification. The glycosidic linkages in hemicellulose are readily hydrolyzed by dilute acids at elevated temperatures to yield syrup containing xylose and arabinose for agricultural residues and hardwoods or mannose, xylose and glucose for softwoods. However, no natural organisms have been found which can efficiently and rapidly convert xylose and arabinose into ethanol. For the complete fermentation of the pentose fraction in lignocellulose hydrolyzate, a strain combining L-arabinose and D-xylose utilization is desired. Fermentation of either L-arabinose or D-xylose by recombinant *S. cerevisiae* has been demonstrated.

33.5.2 Ethanol Fermentation Utilizing Related Substrates and Ethanol Extraction: Lack of Suitable Technology

Lignocellulose is the most plentiful renewable biomass produced by photosynthesis and its annual production was estimated in 1×10^{10} millions tons worldwide (Sanchez and Cardona 2008). The potential for using lignocellulosic materials in

bioethanol production is well recognized. However, the task of hydrolyzing lignocellulose to fermentable monosugars is still technically problematic because the linear polymer has a strong crystallinity and is usually surrounded by lignin, which reduces accessibility to hydrolytic enzymes. Many pretreatment techniques have been used to increase the hydrolysis of lignocellulosic biomass; for instance, dilute acid, ammonia recycle percolation, lime, steam explosion (Hendriks and Zeeman 2009), alkaline and acidic wet oxidation (Varga et al. 2004). Current processes for lignocellulosic biomass include pretreatment, saccharification (hydrolysis) and fermentation. Some options proposed to reduce the cost of the conversion of lignocellulose to ethanol, include: eliminating pretreatment, increasing cellulose hydrolysis yield, enhancing the enzyme activity to reduce its consumption, and improving the fermentation process both in yield and specificity. This conversion includes two processes: (i) hydrolysis of cellulose in the lignocellulosic materials to fermentable reducing sugars and (ii) fermentation of the sugars to ethanol. The hydrolysis is usually catalyzed by cellulase enzymes and the fermentation is carried out by yeast or bacteria. The factors that have been identified to affect the hydrolysis of cellulose include porosity, i.e., accessible surface area of the waste materials, cellulose fiber crystallinity and lignin and hemicellulose content (McMillan 1994). The presence of lignin and hemicellulose makes the access of cellulose enzymes to cellulose difficult, thus reducing the efficiency of the hydrolysis. Removal of lignin and hemicellulose, reduction of cellulose crystallinity and increase of porosity in pretreatment processes can significantly improve the hydrolysis (McMillan 1994).

33.5.3 Increased Ethanol Concentration During Fermentation

One of the most common stresses that yeast cells encounter during fermentation is the increased ethanol concentration. Yeast cells have developed appropriate mechanisms to deal with several types of damages caused by increased ethanol concentration. First, ethanol increases the fluidity of the plasma membrane and destroys the normal membrane structures. In response, yeast cells may change membrane compositions to antagonize membrane fluidization and stabilize plasma membrane. Specifically, it has been shown that the levels of unsaturated fatty acids (UFAs) (You et al. 2003), and ergosterol (Daum et al. 1998; Swan and Watson 1998), increase in response to the high concentration of ethanol. Furthermore, the addition of some types of amino acids (Hu et al. 2005; Takagi et al. 2005), and inositol (Kelley et al. 1988), can enhance ethanol tolerance when provided as a supplement, most likely through enhanced membrane stability. Second, the expression of factors that stabilize and/or repair denatured proteins in yeast cells, such as trehalose and induced heat shock proteins (HSPs), have been revealed to correlate with the capabilities to tolerate alcohol (Swan and Watson 1998; Vianna et al. 2008). Third, some candidate proteins involved in the expression of stress-related genes like the zinc finger protein (MacPherson et al. 2006) and the recently reported alcohol sensitive ring/PHD finger 1 protein (Asr1p) (Betz et al. 2004), also play a role in ethanol tolerance in

Saccharomyces cerevisiae. Lastly, the global transcription machinery engineering (gTME) technology can reprogram gene transcription and then improve glucose/ethanol tolerance of yeast cells (Alper et al. 2006).

33.6 Strain Improvement Through Mutagenesis and Recombinant DNA Technology

A number of genetic and molecular biological mechanisms based advanced technologies could be used for strain improvement for ethanol production. Improvement in the yeast strain should address the following topics.

33.6.1 Utilization of a Wide Range of Substrates

Sustainable and economically viable manufacturing of bioethanol from lignocellulose raw material is dependent on the availability of a robust ethanol producing microorganism, able to ferment all sugars present in the feedstock, including the pentose sugars L-arabinose and D-xylose. *Saccharomyces cerevisiae* is a robust ethanol producer, but needs to be engineered to achieve pentose sugar fermentation. *S accharomyces cerevisiae*, which is one of the most prominent ethanol-producing microorganisms utilizing hexose, has been found unable to utilize xylose due to lack of the key enzymes in the xylose-metabolising pathway (Meinander et al. 1999). Thus, the efficient utilization of xylose in hemicellulose in addition to glucose in cellulose by a recombinant xylose-fermenting *S. cerevisiae* strain would offer an opportunity to reduce the production cost of bioethanol significantly (Chandrakant and Bisaria 2000). To date, numerous studies regarding the metabolic engineering of *S. cerevisiae* for xylose utilization have been reported, and many reviews have already addressed the current advancement in metabolic engineering of xylose-fermenting strains and factors which affect xylose metabolism in yeasts (Almeida and Hahn-Hagerdal 2009; Aristidou and Penttila 2000; Chu and Lee 2007; Dien et al. 2003; Gong et al. 1999; Hahn-Hagerdal et al. 2001, 2007a,b; Jeffries 2006; Jeffries and Jin 2004; Jeffries and Shi 1999; van Maris et al. 2006, 2007).

33.6.2 Direct and Efficient Ethanol Production from Cellulosic Materials

Lignocellulosic biomass shows significant potential as a future substrate for bioethanol production. An optimal microorganism for lignocellulose-based bioethanol production must be able to hydrolyze sugar polymers, utilize all fermentable compounds, and convert them into ethanol at a high rate, yield and productivity.

No single microorganism is known to possess all of these characteristics. For example the traditional ethanol producer, *Saccharomyces cerevisiae*, cannot hydrolyze cellulose and hemicelluloses. Moreover, it cannot utilize many compounds which are generated during hydrolysis of lignocellulosic biomass, such as xylose, arabinose, glucuronic acids and cello-oligosaccharides. Another problem that arises during biomass pretreatment in a lignocellulosic process is the formation of several substances which inhibit yeast growth, such as furans, organic acids, phenols and inorganic salts.

Since the yeast *Saccharomyces cerevisiae* cannot utilize cellulosic materials, these materials must undergo saccharification to glucose before ethanol production can take place. Various cellulose and β-glucosidase genes have been expressed in *S. cerevisiae* with the aim of direct ethanol production from cellulose (Okada et al. 1998). Fermentation of cellulose to ethanol by recombinant yeast cells has, however, not been successful, although some recombinant yeast strains are able to assimilate soluble cellooligosaccharides (2–6 glucose units) as carbon sources (Cho et al. 1999; Murai et al. 1998; van Rensburg et al. 1998). Recently, yeast strains displaying various proteins on the cell surface have been developed by using genetic engineering techniques (Boder and Wittrup 1997; Kondo et al. 2002; Murai et al. 1998; Nakamura et al. 2001; Schreuder et al. 1996; Ueda and Tanaka 2000). Yeast strains which codisplay cellulolytic enzymes on the cell surface through cell surface engineering could mimic cellulosome, which is a multienzyme complex consisting of cellulase and hemicellulase organized on the bacterial cell surface (Bayer et al. 1994; Shoham et al. 1999). The most promising strategy for converting cellulose to ethanol in yeast is certainly the concerted heterologous expression of all types of cellulolytic enzymes to maximize their synergies (Baker et al. 1998; Fujita et al. 2004; Zhang and Lynd 2004).

Lignocellulosic plant biomass requires chemical pretreatment, exposing the polysaccharides (cellulose and hemicelluloses) to enzymatic hydrolysis and fermentation. Current pretreatment processes, which usually rely on high temperatures, acid hydrolysis and/or high pressure (Chandra et al. 2007; Galbe and Zacchi 2007; Lin and Tanaka 2006) form several degradation products with various inhibitory effects on yeast fermentation (Klinke et al. 2003, 2004). These substances fall into the following classes: carboxylic acids, furans, phenolic compounds and inorganic salts (Zaldivar et al. 2001). To overcome the various inhibitory substances, metabolic, genetic and evolutionary and gene disruption strategies were used.

33.6.3 Yeast Strains Tolerant of Ethanol Stress

Eukaryotic cells will encounter stresses during phases of their growth and reproductive cycles. As a result, signatures of stress response mechanisms are widespread in the genomes, cytoplasms and plasma membranes of cells. For example, in yeast, during the process of biomass propagation, yeast cells are dynamically exposed to a mixed and interrelated group of known stresses such as osmotic, oxidative, thermic,

ethanol tolerance and/or starvation. These stress conditions can dramatically affect the population dynamics and industrial fermentation, including ethanol production (Estruch 2000; Hohmann 2002). One of the most common stresses that yeast cells encounter during fermentation is the increased ethanol concentration.

Many researchers have attempted to analyze the mechanism of ethanol stress tolerance and to improve the ethanol stress tolerance of yeast. It was reported that yeast cells adapt to high concentration of ethanol by changing the components in the cytoplasmic membrane to maintain the membrane fluidity (Alexandre et al. 1994; Chi and Arneborg 1999; Chi et al. 1999; You et al. 2003). Additionally, it was reported that increasing intracellular accumulation of trehalose by deletion or expression of antisense for acid trehalase gene *ATH1* improved ethanol stress tolerance (Jung and Park 2005; Kim et al. 1996). Also it was reported (Takagi et al. 2005) that enhancement of intracellular proline accumulation improved ethanol tolerance of yeast used for Japanese rice wine (sake) brewing. On the other hand, genome-wide analysis are also useful to identify the genes responsible for ethanol stress tolerance of yeast except for the genes related to the characteristics described above. To analyze the transcriptional response upon ethanol stress and identify the genes responsible for ethanol tolerance, DNA microarray analysis have been performed (Alexandre et al. 2001; Ogawa et al. 2000). Moreover, genome-wide screening of ethanol-sensitive mutants has been carried out. The genes responsible for ethanol stress tolerance were identified by transposon mutagenesis (Takahashi et al. 2001). The knockout mutant library of *Saccharomyces cerevisiae*, which is now commercially available, has also been used for genome-wide screening of the genes responsible for ethanol stress tolerance (Fujita et al. 2006; Kubota et al. 2004; van Voorst et al. 2006). In these approaches, mutants showing ethanol sensitivity are screened, and the deleted genes are determined as the genes required for growth under high ethanol concentration conditions.

33.7 Conclusions and Prospective

Although date palm sap is the rich source of microorganisms, it could also serve as fermentation substrate. Recently lignocellulosic biomass represents the most prospective feedstock for ethanol production. The availability and low cost of a wide range of lignocellulosic materials offer many possibilities for the development of bioindustries that could support the growth of the international biofuel market and contribute to the reduction of greenhouse gas emissions worldwide. Fermentative microflora from date palm thus could be utilized for fermentation utilizing ethanol fermenting major feedstocks. These microorganisms could be modified utilizing genetic and metabolic engineering techniques for higher ethanol yield. The current research tendencies toward improving fuel ethanol production are thus linked to the nature of employed raw materials, processing steps and related process engineering issues.

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