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Abstract : The second generation of bioethanol made from lignocellulosic biomass which is considered a clean energy source and has high potential of alternative and renewable energy sources. Yeast play an essential role in bioethanol production as fermentation agent a wide range of sugars to ethanol. The great yeast biodiveristy isolated from plants, fruit, or its part could be an potential source of strain. Indigenous yeasts screened and isolated from tuak, an traditional beverages, durian fruit, coconut water fermented some monosacharides of lignocellulose. Yeast isolates migh have 'superior yeast' which has characteristics such as tolerant to high temperature, acids, inhibitors, high ethanol level. These isolates are important for the development of efficient ethanol production, therefore very attractive for the fuel elcohol industry.

1. Introduction

A worldwide energy crisis has encouraged studies on bioethanol as an energy source from renewable resources. Bioethanol has become one of the most promising alternative energy source. It can be produced from various renewable biomass. In the conventional method at the first generation, it was made from kernel and starchy crops biomass. In the 2nd generation raw material using lignocellulosic biomass etc, while the third generation bioethanol produced from algae biomass, and at the forth generation it can be produced from industrial was CO_2.

Lignocellulosic biomass consists of carbohydrate polymers (40-50% cellulose and 25-35% hemicellulose), 15-20% lignin and small remaining fraction of extractive acid, salts, and mineral [1-2]. Conversion from lignocellulosic into bioethanol in the three main steps: pretreatment or delignification process, hydrolysis, and fermentation. Hydrolysis and Fermentation can be carried out either simultaneously namely simultaneous saccharification and fermentation (SSF) or separately namely separate hydrolysis fermentation (SHF) [3].

Some industries beganned commercial scale lignocellulosic as raw material for bioethanol plants, most of which utilize yeast for the fermentation step. Lignocelluse consists of cellulose, hemicellulose Several countries such as USA, Canada, Brazil, China, Italy, Norway have operated commercial scale plants for second generation bioethanol production from corn stover, corn cobs, corn fiber, waste stream, biomass crops, sorted municipal solid waste, woody biomass, crops residue, bagasse, sugarcane bagasse, rice straw, wheat straw, wood pulping residue, etc [4-5].

Saccharomyces cerevisiae is the most commonly used in conventional method for producing bioethanol . Unfortunately, wild type *S.cerevisiae* is able to metabolize mono and disasaccharides such as glucose, fructose, maltose, and sucrose, but not pentoses such as xylose and arabinose-composing hemicellulose, and it is can't assimilate directly of cellulose and hemicellulose [6]. However, in commercial scale the conversion process second generation production from lignocellulosic biomass into ethanol are mostly by engineered strains of *S.cerevisiae*. Engineered strain of *S.cerevisiae* based on heterologous expression for utilzitation of both xylose and arabinose.

The metabolic engineering strategy for constructing l- arabinose-fermenting *S. cerevisiae* is based on heterologous [7]. During fermentation, some stresses such as high concentration of ethanol, high osmolarity, high temperature, acids, inhibitors, and oxidative stress can impact on cell viability, growth rate, and ethanol yield [6]. From natural sources we are enable to screen potential yeast with better characteristics for bioethanol production. The great yeast biodiveristy isolated from plants, fruit, or its part could be an potential source of strain. Indigenous yeast especially superior yeast, that are tolerant to high temperatures, acids, inhibitors, high ethanol levels and sugar concentrations are therefore very attractive for the fuel alcohol industry.

2. Yeast as fermentation agent for bioethanol production

Fermentation to produce bioethanol mostly uses yeast strains. Not only wild type *S.cerevisiae* but also both hybrid and recombinant yeast have applied in this fermentation process. This is due to its ability to catabolize hexose such as glucose which produces two carbon components such as ethanol [8]. *S.cerevisiae* has some advantages such as 1) genetic accesibiliy to create recombinants, 2) Engineered *S.cerevisiae* strains migh have high tolerance to fermentatite stress, high temperature, high ethanol and ethanol contents, low pH; 3) and ideal physiology feature [6].

In fermentation, not only monosaccharide hexose such as glucose, fructose, mannose, and monoscahharide pentose such as arabinose and xylose, but also disaccharide or tri saccharide need to be fermented into ethanol. Recombinant *S.cerevisiae* strain which contains expression cellobiose transporter and b-glucosidase has fermented disaccharide cellobiose succesfully [9-10].

3. Indigenous yeast have identified from natural resources

Several indigenous yeast were isolated from variety of part of plants such as fruit, root, barks of trees, water of fruit, juice, etc. Yeast isolates are characterised morphologically and physiologically. Identification of yeast isolates can be carried out molecularly and biochemically. Based upon analysis of D1/D2 variable domain sequence of LSU (large subunit) rRNA genes by using two universisal primer pairs ITS1 (5′-TCCGTAGGTGAACCTGCGG-3′) and ITS4 (5′-TCCTCCGCTTATTGATATGC-3′) ; NL-1 (5′-GCATAT- CAATAAGCGGAGGA AAAG-3′) and NL-4 (5′-GGTCCGT- GTTTCAAGACGG- 3′) [11]. DNA sequencing data then aligned with all GeneBank database sequences of related species using Basic Local Alignment Search Tool (BLAST) at NCBI (http://blast.ncbi.nlm.nih.gov/ Blast.cgi) and ClustalW2 (http://www.ebi.ac.uk/Tools/msa/ clustalw2/). Identification by biochemical test using API 20C Biomerieux yeast strains, based on assimilation of 19 sugars usage is carried out as reccomendated by manufacture. It incubates at 30^{o}C for 48-72 h. Observation of sugar assimilation pattern conducted after incubating it at 25^{o}C for 72h. By this method, interpretation result is sometimes subjective [12].

Alok also has isolated novel indigenous yeast strains *Pichia farinose*, *Arxula adeninivorans*, *Rhodotorula colostri*, and *Stephanoascus cifemi* from curd and juice samples [13]. Yeast isolates from orange juice, cashew juice and coconut water showed *Aureobasidiu pullulans*, *C. akabanensis*, *C.boldinii*, *C. cylindracea*, *C. intermedia*, *C. krusei*, *C. mesenterica*, *C. oleophila*, *C. parapsilosis*, *C. pseudolambica*, *C. sake*, *C. santamariae*, *C. tropicalis*, *C. vartiovaarae*, *Clavispora lusitaniae*, *Cryptococcus diffluens*, *Crypt. flavescens*, *Crypt. heveanensis*, *Crypt. laurentii*, *Crypt. liquefaciens*, *Crypt. magnus*, *Crypt. sp*., *Hanseniaspora uvarum*, *Kloeckera apis*, *Lachancea fermentati*, *Lodderomyces elongisporus*, *Meyerozyma guilliermondii*, *Pichia fermentans*, *P. membranifaciens*, *P. occidentalis*, *Rhodosporidium diobovatum*, *R. mucilaginosa*, *R. graminis*, *S.cerevisiae*, *S. uvarum*,

Saccharomydes aff. Ludwigii, *Saccharomycopsis aff. selenospora*, *Sporidiobolus runineniae*, *Trichosporon ovoides*, *Zygoascus hellenicus*, *Eickerhamomyces anomalus* [14]. Some yeast strains were further assayed their potency for ethanol production as alternative fermentation agent. Rao identified *Pichia galeiformis* and *Kluyveromyces marxianus* isolated from pepaya; *Candida parapsilosis* and *Issatchenkia orientalis* from wine grape; *I. orientalis*, *C. tropicalis*, *C. viswanathii* and *P. guilliermondii* from sapota; *P.guilliermondii* and *P. membranaefaciens* from mangosteen; *C. tropicalis* from strawbery; *C. albicans* from guava; *P. barkeri* from fig ; *C. maltosa*, *C. parapsilosis*, and *P. mexicana* from Mango; *Clavispora lusitaniae*, *Debaryomyces hansenii*, *P. veronae*, *P. mexicana*, *Metschnikowia chrysoperlae*, *Rhodotoru laminuta*, *R. mucilaginosa*, *R.pallida*, *Cryptococcus saitoi*, *Crypt. albidosimilis*, and *Crypt. Albidus* from tree bark [15]. Ruriani also isolated 12 yeast from rootten fruit apple, papaya, melon and watermelon, and isolates obtained from apple and watter melon confirmed as *Pichia kudriavzeevii* [16]. *Candida tropicalis* was successfully isolated from tuak produced from spontaneous fermented sap water of sugar palm [11]. Our recently result showed that *Kodamae ohmeri*, *C. fumata*, *C. guilliermondii* , *C. laurentii*, *C. humicola*, *Rhodotorula glutinis*, *C. sphaerica*, *C. parapsilosis* were identified from durian fruit.

4. Fermentation Indigenous yeast to produce ethanol

In the first generation, raw materials composed of glucose can be converted to ethanol through sequetial pathways. Glycolysis, a metabolic consists of 10 reactions utilizes Embden Meyerhoff Parnas (EMP) pathway whbich corvents glucose (6 carbons) to pyruvate (2 carbons), 2 molecules of ATP molecules, and 2 molecules of NADH. Then under anaerobic condition, pyruvate can be fermented to ethanol (2 carbons) by sequential reactions of pyruvate decarboxylase (PDC) and alcohol dehydrogenase (ADH) and release one carbo as CO_2 [17]. At least 6 transporters Hxt1, Hxt2, Hxt3, Hxt4, Hxt 6, and Hxt7) facilitate glucose to enters the cell [6].

In the second generation, Lignocellulosic biomass, made of lignin and a rich fermentable material cellulose composed of monosaccharide hexose glucose and hemicellulose composed of hexose glucose and pentose arabinose and or xylose. Lignin content of lignocellulosic biomass should be removed or delignification by pretreatment process. Several indigenous yeast strains are capable to ferment glucose, and/ or xylose, arabinose to produce ethanol which produce different amount of ethanol. Fermentation contained 10% Glucose and Glucose-Xylose (10%-10%) substrates by *Pitch kudriavzeevii* resulted 1.09%, 1.68% and 1.32%, 1.14% of ethanol, respectively [16]. While *candida tropicalis* fermented 10% glucose at 30^{o}C and 42oC resulted 6.55 and 4.58% of ethanol , respectively. Two yeasts isolates *Pichia fairness* and *Stephanoascus ciferrii* were applied at 3 L Fermenter which contains household and agricultural bagasse as the carbon source resulted 31 g/L and 28.73 g/L ethanol [13].

Yeast isolates from fruits and tree bark fermented D-Xylose and incubated at 28^{o}C for 120 h, the result showed that ethanol produced per gram of Xylose as follows : 0.12% of ethanol by *Pichia galeiformis* ; 0.18% by *Kluyveromyces marxians* ; 0.34% by *Candida parapsilosis*, 0.16% by *Issatchenkia orientalist, 0*.14% by *I. orientalist*, 0.36% by *C. tropicalis*, 0.3% *C. viswanathii*, 0.38% by *P. guilliermondii* ; 0.16% by *P.guilliermondii, 0.16% P. membranaefaciens*; 0.32% by *C. tropical*; 0.16% by *C. albicans*; 0.24% by *P. barkeri* ; 0.34% by *C. maltosa*, 0.32% by *C. parapsilosis*, 0.26% by *P. mexicana*; 0.22% by *Clavispora lusitaniae*, 0.18% by *Debaryomyces hansenii*, 0.36% by *P. verona*, 0.32% by *P. mexicana*, 0.22% by *Metschnikowia chrysoperlae*, 0.12% by *Rhodotoru laminuta*, 0.16% by *R. mucilaginosa*, 0.14% by *R.pallida*, 0.22% by *Cryptococcus sati*, 0.18% by *Crypt. albidosimilis*, and 0.24% by *Crypt. Albidus; w*hile medium supplemented with 0.5% P.guilliermondii resulted 1.54% ethanol [15]. Lignocellulose i.e bagasse is applied , Pichia farinose and Stephanoascus ciferrii fermented baggase produced 1.31 g/L and 28.73% of ethanol, respectively [13]. Fermentation lignocellulose, xylose, or arabinose to produce ethanol involves reduction/oxidation pathway for pentose metabolism, and the improved pathways from these strains are often used to create a new recombinant *S.cerevisiae* (Bettiga et al, 2009).

5. Stress-tolerant Yeast

During fermentation to produce ethanol, yeast may undergo various stresses such as stress to high temperature, acid, high ethanol content, hyperosmolarity, etc. *Candida glabrata* NFRI 3164 has superior lactate acid and high temperature tolerances, *Schizosaccharomyces pombe* NFRI 3807 is an acetate -tolerant yeast, *Scheffersomyces shehatae* is high temperature tolerant strain, etc. [19]. By using yeasts which have various stresss tolerance will decrease some cost of production. Further, usage of stress-tolerant yeasts in industrial level is expected improving ethanol production efficiency.

6. Conclussion

Indigenous yeast instead of construction of engineered S.cerevisiae is alternative fermentation agent for bioethanol production. Indigenous yeast strains can be isolated from various natural sources such as part of plants including fruits, water coconut, tree bark etc. Superior yeast has various stress - tolerant yeast feature improvement, is expected to increase ethanol production.

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