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High-temperature ethanol production using thermotolerant yeast newly isolated from Greater Mekong Subregion

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ABSTRACT

The application of high-potential thermotolerant yeasts is a key factor for successful ethanol production at high temperatures. Two hundred and thirty-four yeast isolates from Greater Mekong Subregion (GMS) countries, i.e., Thailand, The Lao People's Democratic Republic (Lao PDR) and Vietnam were obtained. Five thermotolerant yeasts, designated *Saccharomyces cerevisiae* KKU-VN8, KKU-VN20, and KKU-VN27, *Pichia kudriavzevii* KKU-TH33 and *P. kudriavzevii* KKU-TH43, demonstrated high temperature and ethanol tolerance levels up to 45 °C and 13% (v/v), respectively. All five strains produced higher ethanol concentrations and exhibited greater productivities and yields than the industrial strain *S. cerevisiae* TISTR5606 during high-temperature fermentation at 40 °C and 43 °C. *S. cerevisiae* KKU-VN8 demonstrated the best performance for ethanol production from glucose at 37 °C with an ethanol concentration of 72.69 g/L, a productivity of 1.59 g/L/h and a theoretical ethanol yield of 86.27%. The optimal conditions for ethanol production of *S. cerevisiae* KKU-VN8 from sweet sorghum juice (SSJ) at 40 °C were achieved using the Box–Behnken experimental design (BBD). The maximal ethanol concentration obtained during fermentation was 89.32 g/L, with a productivity of 2.48 g/L/h and a theoretical ethanol yield of 96.32%. Thus, the newly isolated thermotolerant *S. cerevisiae* KKU-VN8 exhibits a great potential for commercial-scale ethanol production in the future.

Introduction

At present, the world is facing an energy crisis caused by the continuous use of fossil oil. As a result, petroleum oil use has dropped sharply.[1] Bioethanol is an alternative energy source of particular interest whose production by microbial fermentation is increasing to replace gasoline.[2–4] Yeasts, particularly *Saccharomyces* spp., are the most common ethanol producers employed in industry.[5] Yeast has proven to be more effective for ethanol production than bacteria due to

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its good fermentative capacity and its ability to tolerate high concentrations of ethanol and the by-products formed during pretreatment and fermentation. *S. cerevisiae* is capable of fermenting different types of sugars, such as glucose, fructose and sucrose to ethanol via the glycolysis pathway under anaerobic conditions.[6,7] The optimal growth temperatures of yeasts range from 25 to 35 °C. However, heat stress as well as other stresses such as ethanol and osmotic pressure which are generated during fermentation process, greatly affect ethanol production and decrease the specific growth rate of yeast strains.[8,9]

Many types of raw materials can be used as potential substrates for ethanol production. They are classified into the following three major groups: (1) sugars such as sugarcane, sugar beet, sweet sorghum, whey and molasses[3,4,10,11]; (2) starches such as corn, wheat, cassava and potato[12–15]; and (3) lignocellulosic feedstock such as woody materials, agricultural wastes and crop residues.[16–18] In recent years, interest in the utilization of sweet sorghum juice (SSJ) for ethanol production has increased in the southern United States, India, China and other countries including Thailand. The advantageous attributes of SSJ include a shorter growing period for sweet sorghum, lower requirements for fertilizers and water, and lower cultivation costs compared to sugarcane.[10,11,19,20]

Ethanol production at high temperatures has garnered much interest due to several advantages, including reduced cooling costs and a reduced risk of contaminations.[21] To achieve high-temperature fermentation, the fermentation capability of yeasts and its ability to grow and produce ethanol under a variety of inhibitory conditions must be considered, particularly at elevated temperatures and the accumulation of high concentrations of ethanol. Many studies have examined various thermotolerant yeasts and raw materials for use in ethanol production under optimized conditions. *Pichia kudriavzevii* grows at 42 °C and produces ethanol concentrations in the range of 29–78.6 g/L from sugarcane and cassava starch hydrolysate at 40 °C.[4,15,17,22] Other yeasts, such as *S. cerevisiae* IR2 and IR2*, *S. cerevisiae* VS1, VS3 and *Kluyveromyces marxianus* DMKU 3-1042, grow at higher temperatures between 42 °C and 45 °C and achieve ethanol concentrations at 40 °C varying from 28 to 67.8 g/L when sugarcane and lignocellulosic biomass are used as substrates.[3,5,8] Although yeasts can grow at elevated temperatures above 40 °C, the optimal temperature for ethanol production is approximately 30 °C.[6,10,12,23]

SSJ contains an abundant carbon source and certain minerals essential for yeast growth and ethanol production[10] but lacks certain other inorganic constituents, vitamins and biogenic elements, potentially limiting ethanol fermentation under stresses such as high-temperature conditions.[24] Several researchers have reported the addition of exogenous nutrients, such as ammonium sulfate, magnesium sulfate, potassium dihydrogen phosphate, manganese sulfate or yeast extract to increase fermentation efficiency and ethanol yield.[3,15,24–28] Recently, statistically designed experiments have been widely employed to optimize ethanol production conditions.[24,26,28] These techniques provide several advantages, such as reductions in consumption time and operating costs due to the use of fewer experimental units and the evaluation of interactions between independent variables. Furthermore, optimal conditions are determined with a second-order polynomial equation.[29] Many factors, including incubation temperature, initial yeast cell concentration, initial pH and sugar concentration, affect the growth and ethanol production of yeast cells.[3,11,26,30]

Given the benefits of yeast thermotolerance, which can significantly reduce ethanol production costs, the search for thermotolerant yeasts is a major challenge in achieving high ethanol production levels. There is little information regarding thermotolerant yeast species and their distribution in the three countries of the Greater Mekong Subregion (GMS) (Thailand, Lao PDR and Vietnam) as well as their potential in ethanol production from SSJ at high temperatures. In addition, microbial populations from different geographic locations likely exhibit differential thermotolerance and fermentation capabilities. Therefore, the aims of this study were to isolate and identify thermotolerant and ethanol-tolerant yeast strains from specific regions of Thailand, Lao PDR and Vietnam. We identified five thermotolerant yeast strains, of which two were *P. kudriavzevii* and three were *S. cerevisiae*, exhibiting unique characteristics in ethanol production experiments employing YM medium and SSJ as substrates. The optimal conditions for ethanol production from SSJ by one of the newly isolated thermotolerant yeast strains were also investigated using the Box–Behnken experimental design (BBD). Finally, we described the ethanol production potential of the yeast strain using SSJ under high-temperature fermentation conditions.

Materials and methods

Sample collection

The samples used in this study were collected from fruits, flowers and other sources, such as banana, papaya, grape, orange, apple, mango, Vietnamese apple flowers, longan flowers, papaya flowers, alcoholic beverages, soil and sawdust, from Thailand, Lao PDR and Vietnam. One gram (or 1 mL if the sample was a liquid) of a sample was added to 100 mL of yeast extract-malt extract (YM) medium (3 g/L yeast extract, 3 g/L malt extract, 5 g/L peptone, 20 g/L glucose) and incubated at 35 °C for 24 h. One percent (v/v) inoculum was added into YM liquid medium containing 4% (v/v) ethanol and incubated at 35 °C, which were employed as selective pressures for obtaining thermotolerant yeasts, for 24 h.[3] Then, a spread plate technique was used to obtain isolated colonies on YM agar containing 4% (v/v) ethanol at 35 °C incubation temperature for 24 h. A single colony of each isolate was selected and streaked on YM agar containing an ethanol concentration of 4% (v/v) and incubated at 35 °C for 24 h. Samples were kept at −20 °C for further analysis.

Screening and isolation of thermotolerant and ethanol-tolerant yeasts

Thermotolerant yeast isolates were selected based on their growth performance at 37 °C, 40 °C and 45 °C on YM agar. Isolated yeasts capable of growing at 40 °C or higher were selected for further screening and identification. To screen for ethanol-tolerant yeasts, the thermotolerant yeast isolates were inoculated into YM medium at 35 °C for 24 h. An initial

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