RESEARCH ARTICLE



Enhancement of sustainable bioethanol production from microorganisms isolated from molasses and venasses

Alaa Fathy Mostafa¹*, Bahaa El-Din El-Sayed Abdel-Fattah², Mahmoud Abo El-Saud El-Rawy², Ameer Effat Elfarash²

Received: 15 February 2024\ Revised: 17 March 2024\ Accepted: 18 March 2024\ Published online: 22 March 2024

Abstract

Ethanol is one of the most important biofuels that can be produced from different renewable sources. Molasses and Venasses are used as cheap raw materials in the isolation of microorganisms and used molasses as renewable materials for ethanol production. Molasses and Venasses are considered important by-products in the sugar industry. This study aims to isolate and identify yeasts and bacteria present in both molasses and venasses to use them in the production of bioethanol. Molasses and venasses samples were collected from ten different sugar factories (Guirga, Savola, Deshna, Komombo, Abokorkaus, Delta, Dakahlia, Qus, Nag-hamdy, Armant) and were used to isolate different microorganisms that were screened for their bioethanol productivity. The results showed that the molasses samples contained more microbes than venasses. Twelve isolates were molecularly identified as S. cerevisiae by PCRspecific primers, while 64 isolates were bacterial isolates. All the yeast and bacterial isolates were screened for bioethanol productivity. Isolate M3 showed the highest bioethanol productivity (74%) and was identified by 16S rRNA gene sequencing as Klebsiella pneumoniae. Several factors affected the production of bioethanol, including sugar concentration, urea, and ammonium sulfate. When molasses was used as the carbon source, Klebsiella pneumoniae produced 1% (v/v) bioethanol by utilizing 20% molasses (sugar concentration), 0.4% urea, and 0.4% ammonium sulfate. When UV- mutagenesis was used to improve the bioethanol productivity, all the obtained mutants showed lower productivity compared to the wild-type (M3 isolate).

Keywords: Molasses; Venasses; Bioethanol; *Klebsiella pneumoniae*; Mutagenesis.

²Department of Genetics, Faculty of Agriculture, Assiut University, Assiut, Egypt.

*Correspondence: alaafathy5462@aun.edu.eg

Introduction

Bioethanol is a promising type of biofuel produced by sugar fermentation and used as a partial gasoline replacement in several parts of the world (Bhatia et al. 2012; Lee and Shah 2012; Sadik and Halema 2014). Bioethanol has many advantages over gasoline such as broader flammability limits, higher flame speeds, higher octane number(108) and increased heat of evaporation (Balat and Balat 2009). In contrast to petroleum fuel, bioethanol is less toxic, readily biodegradable and produces fewer airborne pollutants (John et al. 2011).

It is also used for cosmetics, medicines, industrial materials and its production is increasing every year (Cardona and Sánchez 2007). The sugarcane and beet molasses are by-products of sugar industries in Egypt and are cheap raw materials, readily available, and ready for conversion with fewer pretreatments as compared with starchy or cellulosic materials for bioethanol fermentation on an industrial scale. The majority of the sugars in molasses are available for fermentation (Razmovski and Vučurović 2011). Sugarcane molasses is a dark viscous fluid, and rich in nutrients necessary for the growth of microorganisms such as nitrogen, carbon, sodium, potassium, phosphorus and non-nitrogenous compounds. Beet molasses also is used as feedstock to produce bioethanol (Dodić et al. 2009).

Molasses composition is typically influenced by cane and beet variety and maturity, climate, soil, and factory processing conditions (El-Gendy et al. 2013). The primary components of molasses generally consist of sucrose (30–35%), fructose and glucose (10–25%), non-sugar compounds (2–3%), mineral and moisture content and about 45–55% of total fermentable sugars (Solomon 2011). Sugarcane and beet molasses are the final byproduct of sugar factories that is easily available and economically low in price for using it as a raw material for ethanol production (Khoja et al. 2015).



¹Industrial Biotechnology Department, Faculty of Sugar and Integrated Industries Technology, Assiut University, Assiut, Egypt.

Molasses have a significant quantity of sugar, around 40% to 50% (w/v), and ash content of around 5-15%, making it a suitable substrate for rum and bioethanol production for many years (Doelle and Doelle 1990).

A large variety of microorganisms can produce ethanol from polysaccharides. But an ideal microorganism used for ethanol production must have rapid fermentative potential, improved flocculating ability, appreciable osmo-tolerance, enhanced ethanol tolerance and good thermotolerance (Brooks 2008). The yeast (Saccharomyces cerevisiae) has been used widely on a commercial scale, but bacteria have not been practiced commercially vet due to some constraints. such as industrial robustness, substrate utilization, productivity and yield. Bacteria have some advantages over yeasts (Hahn-Hägerdal et al. 2006; Khoja et al. 2014; Sadik and Halema 2014). Industrially, bacteria are preferred over fungal strains as bacterial strains show higher yield, higher tolerance, shorter generation time, lower biomass generation, more effective substrate utilization and simpler downstream processing steps than fungal strains (Yang et al. 2016). There have been numerous reports of bacterial strains with the potential to generate ethanol, which Zymomonas mobilis is the oldest known. Apart from these, Lactobacillus plantarum M24, L. sakei, Weissella viridescens and Pediococcus acidilactici are also reported for efficient bioethanol production (Sharma et al. 2007). With the of molecular engineering, various progress recombinant strains with higher ethanol production capabilities have been created, including Escherichia coli and Klebsiella oxytoca (Soleimani et al. 2017). Certain yeast strains such as Pichia stipitis (NRRL-Y-7124), S. cerevisiae (RL-11) and Kluyveromyces fagilis (Kf1) were reported as good ethanol producers from different types of sugars (Mussatto et al. 2012). Most yeasts can convert a range of hexose sugars to ethanol via glycolysis. However, Saccharomyces cerevisiae is the most used yeast organism for alcoholic fermentation due to its robustness and tolerances. S. cerevisiae has several advantages over other yeasts as it is a facultative anaerobe capable of growing under both aerobic and anaerobic conditions in the presence of glucose (Krantz et al. 2004). Yeast is the most widely utilized microorganism for ethanol fermentation. S. cerevisiae is one of the well-known ethanol producer (Izmirlioglu and Demirci 2012). Yeast plays an essential role in bioethanol production by fermenting a wide range of sugars to ethanol. S. cerevisiae is used in industrial plants due to valuable properties in ethanol yield (> 90.0% theoretical yield), ethanol tolerance (> 40.0 g/L), ethanol productivity (> 1.0 g/L/h), growth in simple, inexpensive media and undiluted fermentation broth with resistance to inhibitors and retard contaminants from growth condition (Dien et al. 2003). There are many factors which affect the production of bioethanol including temperature, sugar concentration, pH, fermentation time, agitation rate, and inoculum size

(Zabed et al. 2014). The growth rate of the microorganisms is directly affected by the temperature (Charoenchai et al. 1998). Inoculum concentration affects the consumption rate of sugar and ethanol productivity but does not give significant effects on the final ethanol concentration (Laopaiboon et al. 2007). One important element in the production of ethanol has also been identified as the initial sugar content. Using a greater initial sugar concentration during batch fermentation will result in increased ethanol production and yield. But it requires more time for fermentation and higher recovery cost (Zabed et al. 2014). The aim of this study was to isolate yeasts and bacterial isolates and characterize their ethanol production and production enhance the by mutagenesis and investigating the factors that affect the production from molasses.

Materials and Methods

Samples collection

Several types (20 samples) of molasses and vinasse samples were collected from different Sugar factories (Guirga, Savol, Deshna, Komombo, Abokorkaus, Delta, Dakahlia, Qus, Nag hamdy, Armant) in Egypt which were used to isolate several bacterial and yeast isolates.

Isolation of microorganisms from molasses and vinasses

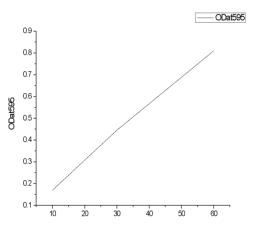
One ml of the molasses sample was suspended in 9 ml sterile distilled water and mixed well. A 0.1 ml of the suspension was plated on Luria broth medium which contains the following composition (g/L dist. H2O): yeast extract, 5 g; peptone,10 g and NaCl, 10 g (Nasiri et al. 2017). The plates were incubated at 37°C overnight, single colonies were re-streaked onto fresh plates. Colonies were picked up individually repeatedly sub- cultured for purification and maintained on sterile LB slants, coded and stored at 4 °C with regular transfer at monthly intervals and glycerol stock was used for culture preservation. These cultures were then screened for their ability to produce bioethanol.

Screening for bioethanol production

Screening for bioethanol productivity was conducted on the following media which contains (g/L dist. H2O): glucose 10 g; yeast extract,5 g; peptone,10 g and NaCl,10 g. Isolates (76 isolates) were cultured and incubated at 37°C for 24 h. After 24 h, the culture samples were centrifuged for 3 minutes at 10000 rpm and 750 μ l of the culture supernatant were mixed with 750 μ l of Tri-n-butyl phosphate (TBP) and then vortexed vigorously for 10 min.



After phase separation,750 μ l of solvent phase (upper) was transferred to a new tube and 750 μ l of dichromate reagent was added, and vortexed vigorously for 10 min. After phase separation, the lower phase was transferred and the optical density were measured using spectrophotometer at OD595 (Seo et al. 2009). All the obtained OD were compared to the standard curve (Figure 1) to determine the ethanol productivity. All the isolates were tested in 3 replicates.



Ethanol concentration (% V/V)

Figure 1. Standard curve used for bioethanol concentration determination.

Molecular identification of isolates

The isolates were identified molecularly by 2 different methods. Firstly, the yeast isolates were identified by species-specific PCR primers (the 5' specific primer (SC1) was designed from the ITS-1 region (between position 161 and 161 from the 3'-SSU end forward), the 3' specific primer (SC2) was located in the LSU gene (between positions 562 and 582 from the 5' end of this gene, backward) (Josepa et al. 2000), while the bacterial isolates were identified by 16S rRNA gene Sequencing.

DNA Isolation

Isolation of DNA was performed according to (Saghai-Maroof et al. 1984) with some modifications. A 1 ml of overnight liquid culture (Bacteria and Yeasts) was placed in a 1.5 ml disposable centrifuge tube. The cells were collected through centrifugation at 7,500 rpm for 10 minutes. The supernatant was discarded, and the pellets were resuspended in 0.2 ml of phosphate buffer. A 10 μ l of lysozyme was added to bacterial isolates and incubated at 37°C for 60 minutes. A 0.4 ml of Cetyltrimethyl Ammonium Bromide (CTAB) extraction buffer was added followed by 40 μ l of β -mercabtoethanol and mixed gently. The tube was placed in 60 °C water bath for 60 min. After cooling an

equal volume of Chloroform: Isoamyl alcohol (24:1) was added and mixed. This mixture was centrifuged for 5-10 min at full speed, and the aqueous supernatant was transferred to a new tube. An equal volume of cold ethanol 100% was added then cooled at -4° C for 30 min. Then centrifuge for 5 min. at 1300 rpm to pellet the DNA. Washing was done with ethanol 70% followed by centrifugation for 5 min. Finally, the pellets were kept for drying for 1hr at room temperature and then dissolved in warm dist. The quality and the quantity of the genomic DNA were checked spectrophotometry using nanodrop at wavelength of 260/280 nm.

Molecular identification of yeasts using PCR

PCR was used to identify yeast of S. cerevisiae. The SC1/SC2 (table1) primers were designed by (Josepa et al. 2000) to distinguish between yeast and bacteria.

 Table 1. Primer used for Molecular identification of yeast isolates.

| Primer | Primer sequence | Size | Reference | |
|--------|-----------------------------|--------|----------------|--|
| SC1-F | 5'-AACGGTGAGAGATTTCTGTGC-3' | 1170bp | (Josepa et al. | |
| SC2-R | 5'-AGCTGGCAGTATTCCCACAG-3' | | 2000) | |
| 5C2-K | 5 110010001101111000110105 | | | |

PCR Master Mix reactions (GeneDirex) were conducted in a 20µL total volume, containing 1x PCR master mix, 1 µL of primer (100 ng / µL) and 2 µL of DNA template, and 7 µL dH2O. The PCR program was as follows: Initial denaturation at 94°C for 5 min, followed by 35 cycles of denaturation at 94°C for 30 s, 30 s of annealing at 50°C, 30 s of extension at 72°C, and a final extension for 5 min at 72°C. PCR products were electrophoresed onto a submerged 1% agarose gel and the results were compared with a 100 bp ladder marker. The gel documentation system was used to visualize the banding patterns.

Molecular identification of bacterial isolates by 16S rRNA Sequencing

The16S rRNA gene sequencing was performed to identify the highest ethanol producing bacterial isolates. The sequencing was carried out as follows: the extracted DNA was used as a template in a PCR reaction to partially amplify the 16S rRNA gene with two universal bacterial primer sets, PS-1 (AGT CGA ACG GCA GCG GGG G) and Ps-2 (GGG GAT TTC ACA TCG GTC TTG CA) (Pastrik and Maiss 2000). Sequencing was done at SolGent Company, Daejeon, South Korea. The obtained sequence was compared with sequences available in the GenBank database (NCBI) using a BLAST search, and phylogenetic tree was constructed using а mega software (version 11).

56



Enhancing bioethanol productivity by mutagenesis

The Bacterial isolate producing the highest amount of bioethanol was selected to increase its bioethanol production by UV mutagenesis following the method used by (Al Makishah and Elfarash 2022). A 1 ml an overnight bacterial culture was spread on the Petri dish and irradiated with a UV lamp for different time periods (10 s, 20 s, 40 s, 1 min, 2 min, and 3 min). Plates were covered and incubated overnight to generate mutants. Different mutants were screened for ethanol production as described before.

Optimization for bioethanol production using molasses as a carbon source.

Several factors affect ethanol production, including sugar molasses (150, 180, 200 and 240 g/L), urea and ammonium sulphate concentration (1, 2, 3 and 4 g/L). Therefore, these factors were studied to increase ethanol production. Twenty-four hours old bacteria inoculum was used to inoculate the molasses at the rate of 15%,18%,20% and 24%. Production of ethanol was conducted in 100ml glass bottles that included 47ml of pretreated molasses and 3ml of 24h old culture. The bottles were incubated on a rotary shaker (150rpm) at 37oC and pH (7). An empty media without bacterial inoculum was used as Negative control.

These different concentrations were used to prepare several media inoculated that were incubated with the highest ethanol producing isolate and was incubated overnight statically at 37°C. After fermentation, the ethanol concentration in the samples were estimated based on volatility by an Ebulliometer used in distillation factories (Iland et al. 2000).

Results and Discussion

Isolation of different microbial isolates from molasses and vinasse

In the present investigation, seventy-six microbial isolates were isolated from the collected molasses and venasses (Figure 2). The microscopic investigation showed that the number of bacterial isolates was 64 while the yeast isolates were 12 (Figure 2).

As shown in Figure 2, the highest number of bacterial isolates obtained from Armant Sugar Factory, while the highest number of yeast isolates recovered from Nag Hamdy Sugar Factory.

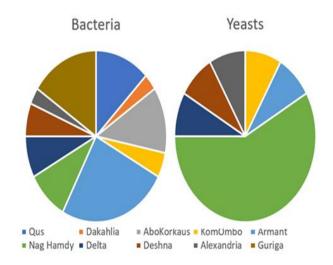


Figure 2. Number of bacterial and yeast isolates collected from different sugar factories.

Several investigation used molasses and venasses to isolate yeast and bacterial isolates. (Kechkar et al. 2019) used molasses, dates and figs to isolate three yeasts isolates. (Faiz Rasul et al. 2015) isolated 26 bacteria isolates from soil and molasses. (Farjana Islam and Narayan Roy 2018) isolated from molasses some bacterial isolates that were identified as Paenibacillus sp., Bacillus sp and Aeromonas sp.

Molecular identification of S. cerevisiae isolates

Agarose –gel electrophoresis showed that only 12 isolates (E2, G11, D2, D3, D4, D8, D10, D11, D12, C6, Z2 and f1) were able to produce the PCR specific band (1170 bp), so they were identified as S. cerevisiae (Figure.3). While this band disappeared by other isolates (64 isolate).

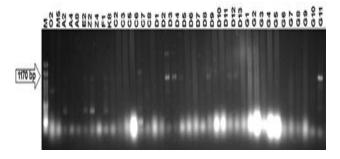


Figure 3. The agarose gel electrophoresis of the PCR product shows the presence of the 1170 bp band in some tested isolates.

These primers were also used by other researchers to identify the S. cerevisiae isolates from several isolates (Guimarães et al. 2006; Josepa et al. 2000).



Screening for bioethanol production

microorganisms

Both bacterial and yeast isolates (Seventy-six isolates) were screened for their bioethanol productivity after 24 hours, at pH 7 and 37°C. When the bacterial isolates were screened for bioethanol productivity (Figure 4), isolates M3 showed the highest ethanol While the lowest bioethanol productivity (74%). productivity was 1% in C2 isolate. Figure (6) showed that the productivity divided the bacterial isolates into 3 different groups (High, Moderate, and Low). The high isolates (8 isolates) produced ethanol with a percentage range from 40% to 74%, while the moderate isolates (15 isolates) ranged from 27%-36%, and 41 low productivity isolates which were only able to produce less than 27% of bioethanol.

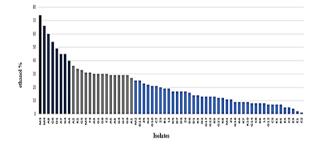


Figure 4. Bioethanol production by bacterial isolates

Yeast isolates showed lower ethanol productivity range (Figure 5) compared to the bacterial isolates (74% to 1%), since the range of yeast productivity was only from 29% to 2%. The highest yeast isolate (F1 isolate) in the productivity was 29%, while the lowest bioethanol productivity was 2% (G11 isolate). Since most yeast isolates were able to produce less than 21% of bioethanol, they were all considered as low ethanol producers.

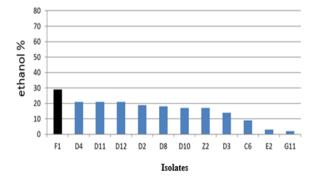


Figure 5. Ethanol production by yeast isolates.

Moreover, the results in Figure 6 showed a significant difference between the highest yeast isolate (G11 isolate) and the highest bacterial (isolates M3) in ethanol productivity when tested under the same condition (incubation time 24 hours and pH 7 and at temperature 37 °C.)

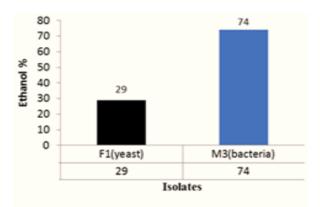


Figure 6. Comparison between the highest yeast isolate and the highest bacterial isolate in ethanol production.

Dien et al. (2003) reported that ethanol-producing bacteria have attracted much attention in recent years because their growth rate is substantially higher than that of the Saccharomyces. Among these ethanolproducing bacteria, Z. mobilis is a well-known species that has historically been employed in tropical regions to produce alcohol from plant sap (Skotnicki et al. 1983).

Bacterial isolates Identification by 16S rRNA

sequencing

The best bacterial isolate that produces bioethanol (M3 isolate) was selected for molecular identification by 16S rRNA gene sequencing. The sequencing was done by the Gene Analysis Unit (Macrogene Inc., Seuol, Korea) using universe primers; PS-1 (AGT CGA ACG GCA GCG GGG G) and Ps-2 (GGG GAT TTC ACA TCG GTC TTG CA).

The obtained partial sequences of the 16S rRNA were first analyzed using the advanced BLAST search program at the NCBI website (http://www.ncbi.nlm.nih.gov/BLAST/) to molecularly identify the isolate. The sequencing results indicated that M3 isolate can be identified as K. pneumonia (Figure 7). (da Silva et al. 2020; Oh et al. 2011) also found that K. pneumonia demonstrated a high potential for ethanol production.



Klebsiella pneumoniae subsp. rhinoscleromatis ATCC 13884 16S ribosomal RNA, partial sequence Sequence ID: NR_114507.1 Length: 1436 Number of Matches: 1

| 1504 bits(814) 0.0 822/828(99%) 0/828(0%) Plus/Minus uery 1 ACTECCATEGETGFACG665GEGTGFACAGAGECCCG666AGGTATTCACCGFAGACTTC 60 bjt 1483 ACTECCATEGETGFACG665GEGTGFACAGAGECCG666AGGTATTCACCGFAGACTTC 124 uery 1 GATCTACGATTCATGGGTGACG66CGGTGFGACAGAGCCCG666AGGTATTCACCGFAGACTTCCAATCGGAGATTCACGAGATCCGAGATCGCAGAGTCCAATCGGAGATCCCAATCGGAGATCCCAATCGGAGATCCCAATCGGAGATCCCAATCGGAGATCCCAATCGGAGATCCCAATCGGAGATCCCAATCGGAGATCCCAATCGGAGATCCCAATCGGAGATCCCAATCGGAGATCCCAATCGGAGATCCCAATCGGAGATCCCAATCGGAGATCCCAATCGGAGATCCCAATCGGAGATCCCAATCGGAGATCGCCAGATCGGAGATCGCCTGGTGAGCCCTGGTGGAGCCCTGGTGGAGCCCTGGTGGAGCCCTGGTGGAGCCCTGGTGGAGCCCTGGTGGAGCCCTGGTGGAGCCATGATGGACGACGAGATGGATG | - | to 1403 GenBank | | | Vext Match |
|--|------------------------|-----------------|-------------------|-------------------|-----------------|
| bjct 1449. ACTCCCATGGTGGGGGGGGGGGGGGGGGGGGGGGGGGGG | Score 1504 bits(81- | | | | |
| bjet 1483 ACTECCATGGTGGACGGGCGGTGGGAAGGGGCGGGGGGCGGGGACGGCGGGGACGTATTCACCGGTAGCATTC 134 uery 61 TGATCTACGGTTAGTAGGGCGGGGGTGGGCGGGGGGGGGG | uery 1 | | | | |
| bjct 1343 TGATCHACGATTACTAGCGATTCCGACTCALGAGTGAGATCCAGAGTCCAGAGTCCGATCCGA | bjct 1403 | | | | |
| bjet 1343 frårthåckatthacitaktoreskattickaktiveskattickaktickaktekski bjet 1343 frårthåckatthacitaktoreskattickaktiveskattickaktekski 128 cirkeskataktiveskattaktoreskattekski 128 cirkeskataktiveskattekskattekskattekski 128 attersakeksingen og konstruktiveskattekski 129 sin attersakeksingen og konstruktiveskattekski 120 sin attersakeksingen og konstruktiveskattekski 121 sin attersakeskatteksingen og konstruktiveskattekski 123 sin attersakeskatteksingen og konstruktiveskattekski 124 sin attersakeskatteksingen og konstruktiveskattekski 125 sin attersakeskatteksingen og konstruktiveskattekski 128 sin attersakeskatteksingen og konstruktiveskattekski 129 sin attersakeskatteksingen og konstruktiveskattekski 129 sin attersakeskatteksingen og konstruktiveskattekski 120 sin attersakeskatteksingen og konstruktiveskatteksisteksingen og konstruktiveskatteksisteks | uery 61 | TGATCTACGATTAC | | | TCCAATCCGGA 120 |
| bjet 1283 CHACGKATHACTTTATGAGGTCGCTTGCTCTCGGGAGGTGGCTTCTTTTGATATGCC 122- uery 181 ATTGTAGCAGTGGTGAGCCCTGGTGGAGGCATGATGGCTGGC | bjct 1343 | TGATCTACGATTAC | | | TCCAATCCGGA 128 |
| bjct 1283 CHAGAKAHACTTATAGAGATCGCTUGTCTCGGAGAGTCTCTCTTTGTATATCGC 122 attGTAGCAGTGGTGAGCCCTGGTGGAGCCCTGGTGGAGGGCCATGATGCCTGCC | uery 121 | | | | |
| bjct 1223 ATTGTAGCAGGTGGTGAGCCCTGGTGGTAGGGCCATGATGGCCTGGCGAACCAGC 116- very 241 TTCCTCCAGTTTATCACTGGCAGTCTCCTTGAGGTCCCCGGCCGAACCAGCTGGCAACAAA 300 bjct 1163 TTCCTCCAGTTTATCACTGGCAGTCTCCTTGAGGTCCCGGCCGAACCAGCTGGCAACAAA 300 very 301 GGATAGGGTTGGCCTGTTGGGGGACTAACCAGCACAACATTCGCAACGGCGGACCAACAACATGGCGCGGACCAACATTCGCCAGGTCGCGGAGTGGCGCTGGTGGCGGGACTAACCCAGACTTCTGCAAGGTCGCGGAGCAACAACATGCGACGAGCTGCGGCGGACCAAGGCGCGAGCCAAGGCTGCGGAGCAAGACCAGGCCGAGCCGAAGCAGGCGGAGCCAAGGCGCGAGCCGAGGCGGAGCCAAGGCGCGAGCCGAGGCGGAGCCAAGGCGGAGCCGAGGCGGAGCCAGGCGTAGCGCCGAGTCGCCGAGGGGGGGCGGAGGCGGAGGCGGAGGCGGAGGCGGAGCCGGGGGG | bjct 1283 | | | | |
| bjct 1223 ATGTAGCACGIGTAGCCCTGGTCGTAAGGGCATGATGAGCTCTGAACGCACCCCCCCC | uery 181 | | | | |
| bjet 1163 TTECTECAGTTAGEGETGECEGETGEGAGGAGCEGGEGEGGGGGGEGGGGGGGGGG | bjct 1223 | | | | |
| bjet 1163 TTCICCAGHTMATCATGGCAGTTGCGGGACTCHTGGCAGCGAACCGCGGCACCGAGCGGGCACCGAG Herry 301 GGATAGGGTTGCGCTGGTGCGGGGATTAACCCAGCATTGCAGACGGGACCGGGGCGCAGCG 104 Herry 31 GGATGGGGCGCCGTGGCGGGGATTGCGGGGGCTGCGGGGCGCGCGGCGGCGGCGGCGGCGGC | uery 241 | | | | |
| bjct 1183 GRATAAGGGTTGCCCCGTTGCGGSGACTTAACCCAACATTCACAACACGAGCTGACGAC 184 very 361 AGCCATGCAGCACCTGTTGCGGSGACTTAACCCAAGCATTCACAACACGAGCTGACGAC 184 very 361 AGCCATGCAGCACCTGTCTCACAGTTCCCGGAAGGCACCAAGCATCCTGCTACAGTCCTCGCA 420 very 421 TGGATGCAGGACCCTGTCTGACAGTTCCTGCGGTTGACGGATTAACCCAAGTGCTCCCCC 480 very 421 TGGATGCAGGACCCCGTCATTGATTGATGGTAAGGTAAG | bjct 1163 | | | | |
| bjct 1183 GATAGGSTTGGCCTGTTGCGSAGTTAACCAACATTCAACACAGGTGGAGCT184GC 144 very 161 ABCCATGCAGCACCTGTCTCACAGTTCCCGAAGGCACCAAGCTTCTTGCTAAGTTCCT 220 bjct 194 ABCCATGCAGCACCTGTCTCACAGTTCCCGGAAGGCACCAAGCATTCTTGCTAAGTTCTC 240 bjct 194 ABCCATGCAGCACCTGTCTCACAGTTCCCGGAAGGCACCAAGCATTCTTGCTAAGTTCTC 241 bjct 194 ABCCATGCAGCACCTGTCTCACAGTTCTCGCGCGTGACTCCAAGCAGTCTCCCCC 480 bjct 194 GGTGTGCGGGGCCCCGGTCAATTCATTGAGTTTTAACCTGCGGCGTGACTCCCCAAGCGCGGCGGGGGGGG | uery 301 | | | | |
| bjet 1943 ABECATEGLAGGACTIGTCTCLAAGSTCCCGAAGGACTCAAGGATTCTCTGCTAAGGATCCCAAGGATTCTCCAAGAGTAGTAAGGTAGGT | bjct 1103 | GGATAAGGGTTGCG | CTCGTTGCGGGACTTAA | CCAACATTTCACAACAC | SAGCTGACGAC 104 |
| bjct 1483 AddZATREGAGZCETBICTCALABITCCCCGAAABGZACZAAAGZCHCTCHCTALAGTTCC 488 very 100stTGTCAABAGTABGTABGTAAGGTCCCCGGTTGCATCGAATCHAACZACATGCTCCACC 488 983 TGGATGTCAABAGTABGTABGTAAGGTCATGCGATTCGATCGAATCHAACZACATGCTCCACC 488 very 481 GTGATGCGAGGCCCGTCAATCGCGTTGCGGTTGCATCGAATCHAACZACATGCTCCACCG 244 very 481 GTTGGCGGGGCCCGTCAATTCATTGGTTGGTTGATCGAATTGCATGCCCCAGGC 446 very 481 GTTGGCGGGGCCCGTCAATTCATTGGTTGGTTGATGCGGATGCCCCCAGGCCGTACTCCCCAGGC 464 very 481 GGTCTACTTATGGGTGGGCGCGCGGGAGCCCCGGCGCGCGC | uery 361 | | | | |
| bjct 983 TGSATGTCARGSGTAGSTAGSTAGSTGCCCCCGCGATTGATGCATGSATTGAACCACATGCTCCACC 924 very 481 GCTTGTGCGGGGCCCCGTCAATTGATGTGATGSATTGAACCAGCCTGCCAGGCGTACCCCCAGGC 923 GCTGGTGCGGGGCGCGCCGGCGATTGCATTGATGGGGGGGCGGCGGCGGCGGCGGCGGCGGCGGGGGGGG | bjct 1043 | | | | |
| bjct 983 TögAlförZABAGTAGGTAAGGTAAGGTAAGGTAAGGTATGATCTCCGGTTGGATCGAATGATCATCCCAAGG 924 981 GCTTGTGCGGGGCCCCGTCAATTCATTTGAGTTTGAGGTTTAACCTGGGGCCGTACTCCCCAGGG 540 987 SGTGGACTAAGGCCGGTAATTCATTGAGTTTAACCTGGGGCCGTACTCCCCAGGG 540 987 SGTGGACTAAGGCCGGTAGGCCGGACCGGGCGAGGCCGAGGCCGAAGCCGCCAATGGC 640 987 SGTGGACTAAGGGGTAGGCAGGGCCGGGGAGGCCGAGGGCCGAAGGCCCCCAAATGGC 640 986 SGTGGACTTAAGGCGTGGGACTACCAGGGCAAGCCCCCAAAGGGCCAAGCCCCCAAATGGC 640 99 641 TGGTTAGGCGGGGACTACCAGGGCCAGGCCCTGAGGGGCAAGCCCCCAAGGCCTTCGAAGGCGCGCCGGGGGCCGGGCGCTGGCCGCGGGGGGCCTTGGCCACGGGTATTCCTCCAAGTCTCTGAC 640 99 641 TGGTTAGGSCGTGGGACTACCAGGGGCCTGCGGCACTGCCGGCGGCCTTGCCACGGCGTTTGGCCC 640 90 91 613 TGGATGCGGGGACTACCAGGGGCCTTGCCACCGGGCTTTGCCCCCAGGTTTGCTCCAAGTCTCTA 780 92 CGGCATTGCCGGCAGCCCTGGGATTCGCCCCCGGGATTTCCCCCAGGTTGCCACGCGGCT 780 GGCATTGCCGGTAGCCCTGGAATTCTGGCCGCCCTGGAATTCCTCCAAGTCCCCAGGT 780 92 CGGCATTGCCGGTAGCCTTGGAATTCGGGCGGGCCCTGGGAATTCGCCCCGGGAATTCGCCAGCT 780 GGCCATTGCCGGTAGCCCTGGGAATTCGCCCCCCGGGAATTCGCACGGCGGCCGCGCGCCCCGGGAATTCGCACGGCGGGCCGGCGGGGGGCCCGCCGGGGAATTCGCAGGCCGGGGGGGCGCGCGC | uery 421 | | | | |
| bjct 923 GCTTGTCGGGGGCCCGGTGAATTGATTGAGTGGAAGGCCCGGAGCCGGGCGTACTCCCCABGGGGGGGGGG | bjct 983 | | | | |
| bjct 923 GCTTGTGCGGGCCCGGTUATTCATTGAGTTBAGTTBAGTTGAGCGGCGATACCCCAAGC 864 very 541 GGTCTACTTACGGGTBAGCTCCGGAAGCCAGCCTGAAGGGCAAACCTCCAAATGGAC 600 bjct 863 GGTCACTTACGGGTBAGCTCCGGAAGCCAGCCAGCCTCAAGGGCAAACCTCCAAATGGAC 600 very 641 ATGGTTACGGGTGGACTACCAGGGTTATATCCTGTGTGGCCCCCCAGGCTTTCGCCAC 600 bjct 863 ATGGTTTAGGGGGGGACTACCAGGGTTATATCCTGTGTGTG | uery 481 | | | | |
| bjct 883 GGTCGALTHANGGCGTGGALTACCAGGGCAGGCCTGAAGGGCAGALCTCCCAATCGAC 804 very 601 ATCGTTTACGGCGTGGALTACCAGGGCATCTAATCGTGTTGTCCCCCAGGCTTCGGCAC 600 bjct 803 ATCGTTTAGGGCGTGGALTACCAGGGTATCTAATCCTGTTGTCCCCCAGGCTTCGGCAC 600 bjct 803 ATCGTTTAGGGCGTGGALTACCAGGGTATCTAATCCTGTTGTGCCCCCGGGTATTCCCCAGGCTTCGGCAC 744 very 601 CTGAATGCGTGAGCTAGCCTGGGAATTCCAGGGGGCCTCGCCACCGGGTATTCCTCCAGGTCTCCCAGGTTCCCCCAGGT 780 bjct 733 CTGAATGCGGTGAGGCTGGGGCCGGGGGAATTCACCCCCCTGTGAAGGCTGCAGCCGGCAGT 780 bjct 683 GGCATTCACCGCTGGAATTCTGGGAGTCGGGGGATTCACCCCCCTGTGAAGGCCTGCCAGGT 624 very 781 TGGAATGCAGTTCCCAGGTGGGAGTTGGGCCGGGGGAGTTGCACCCGGGATTTGCACCGCGCAGT 826 | bjct 923 | | | | |
| bjet 883 GötGALTHAGGSGTHAGTECGAAGGSCHAGTECGAAGGSCHAGTECGAAGGSC wery 601 ATCGTTAGGSGGTGGALTACCAGGGTGATCTAATGCTGTTGETCCCCAGGTTTGGCAG 606 bjet 803 ATCGTTAGGSGGTGGALTACCAGGGGTATCTAATGCTGTTGETCCCCAGGTTTGGCAG 728 bjet 743 CTGAGGTGAGTCTTTGTCCCAGGSGSGCCTTGGCGACGGGTATTCCTCCAGATCTTA 728 617 cgaGGTGAGTCTTTGTCCCAGGSGSGCCTGCCCCCGGGTATTCCTCCAGATCTTA 728 618 cgaTTGCGGTGAGTCTTGTCCCAGGGSGSCCTGCCCCCGGGTATTCCTCCAGATCTTA 728 619 cgaTTGCGGTGAGTCTTGCCCGCGGGGATTCGCGCCGGGTATTCGCCGCGGTTGCGCGCGGTTGGCGCCGGTTGGCGCCGGGTTGTGGCGCCGGGTTGGCGCCGGGTTGGCGCCGGGTTGGCGCCGGGTTGGCGCCGGGTTGGCGCCGGGTTGGCGCCGGTTGGCGCCGGGTTGGCGCGGGTTGGCGCCGGGTTGGCGCCGGTTGGCGCCGGTTGGCGCCGGTTGGCGCCGGTTGGCGCGCGGTTGGCGCGCGGTTGGCGCGCGGTTGGCGCGCGGTTGGCGCGGGTTGGCGCGGGTTGGGCGCGGTTGGCGCGGGTTGGGCGCGGTTGGGCGCGGTTGGGCCGGGGTTGGGCCGGGGTTGGGCGCGGTTGGGCGCGGTTGGGCGGGGGTTGGGCGGGGGTTGGGCGGGGGTTGGGCGGGGGTTGGGCGGGGGTTGGGCGGGGGTTGGGCGGGGGTTGGGCGGGGGTTGGGCGGGGGTTGGGCGGGGGTTGGGCGGGGGG | uery 541 | | | | |
| bjct 883 ATCGTTTACGSCGTGGAFTACLAGSGTATCTAATCCTGTTTGCTCCCAGGCTTTCGCAC 744 very 661 CTGAGCGTCAGTCTTTGTCCGAGGGGCCGCTTCGCCACCGGTATTCCTCCAGGTTCTTCA 728 bjct 743 CTGAGCGTCAGTCTTTGTCCGAGGGGCCGCCTTCGCCACCGGTATTCCTCCAGGTTCTCA 684 very 721 CGCATTCACCGCTGCACTCGGAATTCTGAGACCCCCTGAGGCCTCTCCAGGCTCCCAGGT 788 bjct 683 CGCATTCACCGCTGGAATTCTGGGACTCGGGAATTCGAGCCGCCGGGGATTCGCAGCCGCGGGGATTCGAGCCGCGGGGATTCGAGCCGCCGGGGATTCGAGCCGCCGGGGATTCGAGCCGCGGGGATTCGAGCCGCGGGGATTCGAGCCGCGGGGATTCGAGCCGGCGGGGATTCGAGCCGGGGATTCGAGCCGGGGATTCGAGCCGGGGATTCGAGCCGGGGATTCGAGCGGGGGATTCGAGCGGGGGATTCGAGCGGGGGATTCGAGCCGGGGGATTCGAGCCGGGGGATTCGAGCCGGGGGATTCGAGCCGGGGGATTCGAGCGGGGGATTCGAGCGGGGGATTCGAGCGGGGGATTCGAGCGGGGGATTCGAGCGGGGGATTCGAGCGGGGGGGG | bjct 863 | | | | |
| bjet 883 ATGSTTTAGGSGBGGSACTACGAGSGTATCTAATCCTGTTTGCTCCCCAGGTTTCGGAC 744 very 661 CT636GSTGAGTCTTTGTCCAGAGSGCCGCCTTGCCACGGSTATTCCTCCAGATCTCTA 728 CT636GGTCAGTCTTTGTCCAGAGSGCCGCCTCGCCAGGSTATTCCTCCAGATCTCTA 743 CT636GGTCAGTCTTGTCCAGGGSGCCGCCCTCGCAGGSTATTCCTCCAGATCCTAG 744 very 721 CG6ATTCCACCGTGSAATTCTACCCCCTTGGAATCCTAGAGCCTGCCAGT 788 CGCATTCCACCGTGCACGTGSGATTCTACCCCCCTTGAAAGCCTCTAGCCTGCCAGT 780 TCGAATGCAGTTCCCAGGTTGAGCCCGGSGATTCCACCGACT 782 CGCATGCAGTCCCCAGGTGGGCCCGGSGATTCCACCGACT 784 Very 781 TCGAATGCAGTCCCAGGTGGGCCCGGSGATTCACCCCGACT 785 VGCCCAGGTCCCAGGTGGGCCCGGGGATTCACCCCGGTATTCACGCCGCCGCGGCGCGCCGGGCGCCCGGGGCCTCGGCGCGGGCGCGCGGGCCGGGGCCGGCGG | uery 601 | | | | |
| bjet 743 CTGARGETAGTCHTTGTCCARGSGEGCECTTG2CCACGGTATTCCTCCARATCHTA 684 wery 721 CGCATTC2ACGCTG2ACTCGAATTCTACCCCCCTCTACAACGACTCTAGCCTGCCAGT 788 bjet 683 CGCATTCACCGCTGAACTCGGAATTCTACCCCCCTCTACAAGGACTCTAGCCTGCCAGT 624 wery 781 TCGATGCCAGTTGC4CGCCGGGGATTCGACTCCGACT 826 | bjct 803 | | | | |
| bjet 743 čTGAGCGTCAGTCTTTGTCCAGGGGGCCGCCTTCGCCACCGGTATTCCTCCAGATCTCTA very 721 GGCTTTCACCGCTGGAATTCTACCCCTGGAATTCTACCGCCGCCAGT 780 bjet 683 GGCATTCACCGCTGGAATTCTACCCCCGTCTACAAGACTCTAGCCTGCCAGT 624 very 781 TCGAATGCAGTTCCCAGGTTGAGCCCGGGSATTCTACCGCCGT 826 | uery 661 | | | | |
| bjet 683 CGCATTTCACCGCTACACCTGGAATTCTACCCCCCTCTACAAGACTCTAGCCTGCCAGTT 624 uery 781 TCGAATGCAGTTCCCAGGTTGAGCCCCGGGGATTCACATCCGACTT 826 | bjct 743 | | | | |
| bjet 683 CGCATTTCACCCGCTACACCTGGWATTCTACCCCCCTCTACAAGACTCTAGCCTGCCAGT 624 uery 781 TCGMATGCAGTTCCCAGGTTGAGCCCCGGGGATTTCACATCCGACTT 826 TCGMATGCAGTTCCCAGGTTGAGCCCGGGGATTTCACATCCGACTT 826 | uery 721 | | | | |
| | bjct 683 | | | | |
| | uery 781 | | | | 26 |
| | bjct 623 | | | | 78 |

Figure 7. Sequence alignment of the M3 isolate (Query) against the partial 16S rRNA gene sequence data of K. pneumonia in GenBank showing 4 base substitutions.

Several sequences were selected from GenBank database for the construction of a phylogenetic tree to compare the M3 isolate with other closely related species. These strains were: K. pneumonia (NR 114507.1), K. quasipneumonia (NR 134063.1), Kluyvera ascorbate (NR 114589.1), K. intermedia (NR 028802.1), K. aerogenes (NR 113614.1).

The phylogenetic tree (Figure 8) of 16S rDNA gene sequences indicated that the M3 isolate and the K. pneumonia shared one clear clade with 99 % similarity.

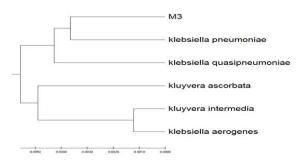


Figure 8. Phylogenetic tree relationships between the M3 isolate and other 16S rDNA gene sequences selected from GenBank database. In the phylogenetic tree, M3 and K. pneumonia were clustered together in one group.

Mutagenesis of the best ethanol producing

isolate by UV irradiation.

Mutagenesis is a technique commonly used to improve the performance of enzymes as well as for high productivity in several organisms such as bacteria (Joshi et al. 2013) and Fungi (Hasan et al. 2019). Mutagenesis has been used to enhance bioethanol production levels in the best ethanol producing wild type isolate (M3). Mutagenesis was performed for different periods of exposure to UV irradiation. Mutants were selected from surviving bacteria after exposure. A total of 20 different mutants obtained from the mutagenesis of M3 isolate were screened for bioethanol productivity. Results in Figure 9 showed that all the selected mutants produced lower amounts of bioethanol compared to the wild-type (M3 isolate),

while (Oh et al. 2011) reported that a mutant strain of K. pneumoniae showed increment in production of ethanol. This results agreed with Strub et al. (2004) who found that mutagenesis did not always improve productivity.

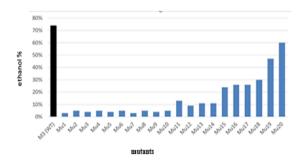


Figure 9. Bioethanol production of the 20 selected mutants produced from M3 isolate mutagenesis.

Table 2. Bioethanol production at different conditions.

| Molasses sugar concentration (%) | Urea | Ammonium sulphate | 3 days |
|-------------------------------------|------|-------------------|--------|
| 15% | 1 | 1 | 0 |
| 15% | 2 | 2 | 0 |
| 15% | 3 | 3 | 0.2 |
| 15% | 4 | 4 | 0.8 |
| 18% | 1 | 1 | 0 |
| 18% | 2 | 2 | 0.5 |
| 18% | 3 | 3 | 0 |
| 18% | 4 | 4 | 0 |
| 20% | 1 | 1 | 0 |
| 20% | 2 | 2 | 0.2 |
| 20% | 3 | 3 | 0 |
| 20% | 4 | 4 | 1 |
| 24% | 1 | 1 | 0 |
| 24% | 2 | 2 | 0 |
| 24% | 3 | 3 | 0.8 |
| 24% | 4 | 4 | 0.1 |



The obtained results are nearly similar to those recorded by (Al-Talibi et al. 1975; Rasmey et al. 2018), who observed that the alcohol produced by S. cerevisiae grown increased with increasing sugar concentration in the juice from 10-25% and then decreased.

Conclusions

Seventy -six different isolates were collected from contaminated Molasses and Vinasse. Yeast isolates were identified by specific primer, while one of the bacterial isolates were identified by 16S rDNA sequencing. When bioethanol productivity was screened, yeast isolates showed less production of bioethanol than bacterial isolates. The effect of different parameters in bioethanol production were studied for k. pneumoniae. The highest achieved production was 1%(v|v) of bioethanol production when the media were supplied with 20% of molasses, 0.4% of urea and 0.4% of ammonium sulphate after 3 days at 37 °C and pH=7. UV- mutagenesis could not improve the bioethanol productivity in all the selected mutants.

References

- Al-Talibi A, Benjamin N, Abboud A (1975) Relationship Al-Talibi A, Benjamin N, Abboud A (1975) Relationship between Ethyl Alcohol Production by Saccharomyces cerevisiae from Iraqi Dates, Pure Sugar Solution, and the Amount of Inoculum. Food/Nahrung19:335-340 Doi.10.1002/food.19750190405
- Al Makishah NH, Elfarash AE (2022) Molecular characterization of cellulase genes in Pseudomonas stutzeri. Electronic Journal of Biotechnology 59:55-61Doi.10.1016/j.ejbt.2022.07.004
- Balat M, Balat H (2009) Recent trends in global production and utilization of bio-ethanol fuel. Applied energy 86:2273-2282 Doi.10.1016/j.apenergy.2009.03.015
- Bhatia L, Johri S, Ahmad R (2012) An economic and ecological perspective of ethanol production from renewable agro waste: a review. Amb Express 2:1-19 Doi.10.1186/2191-0855-2-65
- Borzani W, Gerab A, De La Higuera G, Pires M, Piplovic R (1993) Batch ethanol fermentation of molasses: a correlation between the time necessary to complete the fermentation and the initial concentrations of sugar and yeast cells. World journal of Microbiology and biotechnology 9:265-268 Doi.10.1007/bf00327852
- Brooks A (2008) Ethanol production potential of local yeast strains isolated from ripe banana peels. African journal of Biotechnology 7.

- Cardona CA, Sánchez ÓJ (2007) Fuel ethanol production: process design trends and integration opportunities. Bioresource technology 98:2415-2457 Doi.10.1016/j.biortech.2007.01.002
- Charoenchai C, Fleet GH, Henschke PA (1998) Effects of temperature, pH, and sugar concentration on the growth rates and cell biomass of wine yeasts. American Journal of Enology and Viticulture 49:283-288 Doi.10.5344/ajev.1998.49.3.283_
- da Silva VZ, Ourique LJ, de David C, Ayub MAZ (2020) Construction of recombinant Klebsiella pneumoniae to increase ethanol production on residual glycerol fed-batch cultivations. Applied Biochemistry and Biotechnology 192:1147-1162 Doi.10.1007/s12010-020-03397-5
- Dien B, Cotta M, Jeffries T (2003) Bacteria engineered for fuel ethanol production: current status. Applied microbiology and biotechnology 63:258-266 Doi.org/10.1007/s00253-003-1444-y
- Dodić S, Popov S, Dodić J, Ranković J, Zavargo Z, Mučibabić RJ (2009) Bioethanol production from thick juice as intermediate of sugar beet processing. Biomass and Bioenergy 33:822-827 Doi.10.1016/j.biombioe.2009.01.002
- Doelle MB, Doelle HW (1990) Sugar-cane molasses fermentation by Zymomonas mobilis. Applied microbiology and biotechnology 33:31-35 Doi/10.1007/bf00170565
- Dragone G, Silva DP, e Silva JBdA (2004) Factors influencing ethanol production rates at high-gravity brewing. LWT-Food Science and Technology 37:797-802 Doi.10.1016/j.lwt.2004.03.006
- El-Gendy NS, Madian HR, Amr SSA (2013) Design and optimization of a process for sugarcane molasses fermentation by Saccharomyces cerevisiae using response surface methodology. International Journal of Microbiology 2013 Doi. 10.1155/2013/815631
- Faiz Rasul FR, Amber Afroz AA, Umer Rashid UR, Sajid Mehmood SM, Kalsoom Sughra KS, Nadia Zeeshan NZ (2015) Screening and characterization of cellulase producing bacteria from soil and waste (molasses) of sugar industry Doi.10.12692/ijb/6.3.230-238
- Farjana Islam FI, Narayan Roy NR (2018) Screening, purification and characterization of cellulase from cellulase producing bacteria in molasses Doi.10.1186/s13104-018-3558-4
- Guimarães TM, Moriel DG, Machado IP, Picheth CM, Bonfim T (2006) Isolation and characterization of Saccharomyces cerevisiae strains of winery interest. Revista Brasileira de Ciências Farmacêuticas 42:119-126 Doi.10.1590/s1516-93322006000100013



- Hahn-Hägerdal B, Galbe M, Gorwa-Grauslund M-F, Lidén G, Zacchi G (2006) Bio-ethanol-the fuel of tomorrow from the residues of today. Trends in biotechnology 24:549-556 Doi.10.1016/j.tibtech.2006.10.004_
- Hasan HA, Elfarash AE, Abdrabo KA (2019) An Inverse Correlation Between the Production of Itaconic and Mevinolinic Acids in Aspergillus terreus Mutants. Proceedings of the National Academy of Sciences, India Section B: Biological Sciences 89:1231-1237 Doi.10.1007/s40011-018-1041-6
- Iland P, Ewart A, Sitters J, Markides A, Bruer N (2000) Techniques for accurate chemical analysis and quality monitoring during winemaking. Wine Promotions, Campbell Town, Australia
- Izmirlioglu G, Demirci A (2012) Ethanol production from waste potato mash by using Saccharomyces cerevisiae. Applied Sciences 2:738-753 Doi.10.3390/app2040738
- John RP, Anisha G, Nampoothiri KM, Pandey A (2011) Micro and macroalgal biomass: a renewable source for bioethanol. Bioresource technology 102:186-193 Doi.10.1016/j.biortech.2010.06.139
- Josepa S, Guillamon JM, Cano J (2000) PCR differentiation of Saccharomyces cerevisiae from Saccharomyces bayanus/Saccharomyces pastorianus using specific primers. FEMS microbiology letters 193:255-259 Doi.10.1016/s0378-1097(00)00458-4
- Joshi SM, Inamdar SA, Jadhav JP, Govindwar SP (2013) Random UV mutagenesis approach for enhanced biodegradation of sulfonated azo dye, green HE4B. Applied biochemistry and biotechnology 169:1467-1481 Doi.10.1007/s12010-012-0062-5
- Kechkar M, Sayed W, Cabrol A, Aziza M, Ahmed Zaid T, Amrane A, Djelal H (2019) Isolation and identification of yeast strains from sugarcane molasses, dates and figs for ethanol production under conditions simulating algal hydrolysate. Brazilian Journal of Chemical Engineering 36:157-169 Doi.10.1590/0104-6632.20190361s20180114
- Khoja A, Ali E, Nawar A, Ansari A, Qayyum M, Ehsan H (2014) Bioethanol Production from sugarcane Molasses by using Zymomonas mobilis
- Khoja AH, Ali E, Zafar K, Ansari AA, Nawar A, Qayyum M (2015) Comparative study of bioethanol production from sugarcane molasses by using Zymomonas mobilis and Saccharomyces cerevisiae. African Journal of Biotechnology 14:2455-2462 Doi.10.5897/ajb2015.14569
- Krantz M, Nordlander B, Valadi H, Johansson M, Gustafsson L, Hohmann S (2004) Anaerobicity prepares Saccharomyces cerevisiae cells for faster adaptation to osmotic shock. Eukaryotic cell 3:1381-1390 Doi.10.1128/ec.3.6.1381-1390.2004

- Laopaiboon L, Thanonkeo P, Jaisil P, Laopaiboon P (2007) Ethanol production from sweet sorghum juice in batch and fed-batch fermentations by Saccharomyces cerevisiae. World Journal of Microbiology and Biotechnology 23:1497-1501 Doi.10.1007/s11274-007-9383-x
- Lee S, Shah YT (2012) Biofuels and bioenergy: processes and technologies. CRC Press Doi.10.1201/b12510
- Mussatto SI, Machado EM, Carneiro LM, Teixeira JA (2012) Sugars metabolism and ethanol production by different yeast strains from coffee industry wastes hydrolysates. Applied Energy 92:763-768 Doi.10.1016/j.apenergy.2011.08.020
- Nasiri V, Dalimi A, Ghaffarifar F (2017) LB brothlyophilized Rabbit serum (LLR) as a new and suitable culture medium for cultivation of promastigotes of Leishmania major. Journal of Parasitic Diseases 41:247-251 Doi.10.1007/s12639-016-0786-1
- Oh B-R, Seo J-W, Heo S-Y, Hong W-K, Luo LH, Joe Mh, Park D-H, Kim CH (2011) Efficient production of ethanol from crude glycerol by a Klebsiella pneumoniae mutant strain. Bioresource technology 102:3918-3922 Doi.10.1016/j.biortech.2010.12.007
- Pastrik KH, Maiss E (2000) Detection of Ralstonia solanacearum in potato tubers by polymerase chain reaction. Journal of Phytopathology 148:619-626 Doi.10.1111/j.1439-0434.2000.00567.x
- Rasmey A-HM, Hassan HH, Abdulwahid OA, Aboseidah AA (2018) Enhancing bioethanol production from sugarcane molasses by Saccharomyces cerevisiae Y17. Egyptian Journal of Botany 58:547-561 Doi.10.21608/ejbo.2018.1820.1126
- Razmovski R, Vučurović V (2011) Ethanol production from sugar beet molasses by S. cerevisiae entrapped in an alginate-maize stem ground tissue matrix. Enzyme and microbial technology 48:378-385 Doi.10.1016/j.enzmictec.2010.12.015
- Sadik MW, Halema AA (2014) Production of ethanol from molasses and whey permeate using yeasts and bacterial strains. Int J Curr Microbiol App Sci 3:804-818
- Saghai-Maroof MA, Soliman KM, Jorgensen RA, Allard R (1984) Ribosomal DNA spacer-length polymorphisms in barley: mendelian inheritance, chromosomal location, and population dynamics. Proceedings of the National Academy of Sciences 81:8014-8018 Doi.10.1073/pnas.81.24.8014
- Seo H-B, Kim H-J, Lee O-K, Ha J-H, Lee H-Y, Jung K-H (2009) Measurement of ethanol concentration using solvent extraction and dichromate oxidation and its application to bioethanol production process. Journal of industrial Microbiology and Biotechnology 36:285-292 Doi.10.1007/s10295-008-0497-4



61

- Sharma N, Kalra K, Oberoi HS, Bansal S (2007) Optimization of fermentation parameters for production of ethanol from kinnow waste and banana peels by simultaneous saccharification and fermentation. Indian Journal of Microbiology 47:310-316 Doi.10.1007/s12088-007-0057.
- Skotnicki M, Warr R, Goodman A, Lee K, Rogers P (1983) High-productivity alcohol fermentations using Zymomonas mobilis. Biochemical Society Symposium, pp 53-86 Doi.10.1007/bf00129544.
- Soleimani SS, Adiguzel A, Nadaroglu H (2017) Production of bioethanol by facultative anaerobic bacteria. Journal of the Institute of Brewing 123:402-406 Doi.10.1002/jib.437
- Solomon S (2011) Sugarcane by-products based industries in India. Sugar Tech 13:408-416 Doi.10.1007/s12355-011-0114-0
- Strub C, Alies C, Lougarre A, Ladurantie C, Czaplicki J, Fournier D (2004) Mutation of exposed hydrophobic amino acids to arginine to increase protein stability. BMC biochemistry 5:1-6 Doi.10.1186/1471-2091-5-9

- Thatipamala R, Rohani S, Hill G (1992) Effects of high product and substrate inhibitions on the kinetics and biomass and product yields during ethanol batch fermentation. Biotechnology and bioengineering 40:289-297 Doi.10.1002/bit.260400213
- Yang S, Fei Q, Zhang Y, Contreras LM, Utturkar SM, Brown SD, Himmel ME, Zhang M (2016) Zymomonas mobilis as a model system for production of biofuels and biochemicals. Microbial biotechnology 9:699-717 Doi.10.1111/1751-7915.12408
- Yue G, Yu J, Zhang X, Tan T (2012) The influence of nitrogen sources on ethanol production by yeast from concentrated sweet sorghum juice. Biomass and bioenergy 39:48-52 Doi.10.1016/j.biombioe.2010.08.041
- Zabed H, Faruq G, Sahu JN, Azirun MS, Hashim R, Nasrulhaq Boyce A (2014) Bioethanol production from fermentable sugar juice. The scientific world journal 2014 Doi.10.1155/2014/957102

