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Effect of the Thermophilic Bacterial Biculture Consortium from Mudiak Sapan Hot Springs on Biofuel Production

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Abstract: Biofuels are fuels derived from biomass and consist of biogas, biodiesel and bioethanol. Bioethanol is a biofuel whose main ingredients are from plants and generally use microorganisms in the fermentation process. One way is to use thermophilic bacteria with the advantage of low contamination levels and better product quality. Bacteria that exist in nature are not only in a single form but also exist in the form of a consortium in which there are beneficial or detrimental bacterial interactions. Favorable bacterial interactions indicate that the bacteria are compatible. Using a consortium with compatible bacteria gives better results than using a single bacteria. This study aims to determine the compatibility and effect of a consortium of thermophilic bacteria from Mudiak Sapan hot springs on biofuel yields. This research is a descriptive research. To test the cooperation between isolates, a compatibility test was carried out using the disk diffusion method. Then the biculture consortium isolates of thermophilic bacteria were fermented in liquid TMM medium (Thermophilic Minimum Media) and the bioethanol content was measured using a distillation apparatus. The results of this study showed that the six pairs of compatible consortium and MS 9-12 consortium produced the highest bioethanol, namely 1.0003%. Then the biculture consortium isolates of thermophilic bacteria were fermented in liquid TMM medium (Thermophilic Minimum Media) and the bioethanol content was measured using a distillation apparatus. The results of this study showed that the six pairs of compatible consortium and MS 9-12 consortium produced the highest bioethanol, namely 1.0003%. Then the biculture consortium isolates of thermophilic bacteria were fermented in liquid TMM medium (Thermophilic Minimum Media) and the bioethanol content was measured using a distillation apparatus. The results of this study showed that the six pairs of compatible consortium and MS 9-12 consortium produced the highest bioethanol, namely 1.0003%.

Keywords: Bacteria consortium; Bioethanol; Biofuel; Thermophilic bacteria

Introduction

The production of fuel oil that cannot be met by the state is a problem that must be resolved. In addition, fuel oil (BBM) is a non-renewable natural resource and leaves gas emissions which cause air pollution (Mayzuhroh, 2015). Alternative energy solutions to meet national energy needs are being developed as a substitute for fuels other than petroleum (Winaya et al., 2002). Alternative energy can be developed through the processing of renewable natural resources such as biofuels. Biofuel is any solid, liquid or gaseous fuel produced from organic materials. Biofuels can be produced directly from plants or indirectly from industrial, commercial, domestic or agricultural waste and waters (Kasim et al., 2013).

Bioethanol is ethanol whose main ingredients are from plants and generally uses a fermentation process with the help of microorganisms with characteristics such as being volatile, flammable, soluble in water, biodegradable, low toxicity and does not cause major air pollution when leaked (Novia et al., 2014). The

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microorganisms used in the manufacture of bioethanol are *Saccharomyces cerevisiae* and *Zymomonas mobilis*. Both of these microorganisms belong to the mesophilic group, and have a weakness, namely the growth temperature which ranges from 30-37^0 C. Another weakness is that the separation in ethanol requires energy supply because the distillation is carried out at a temperature of 70-80^0 C, the use of a limited substrate in producing ethanol is glucose (Lynd L, 1989).

To overcome this problem, alternative microbes are needed in the production of bioethanol, one of which is thermophilic microbes. Thermophilic microorganisms are types of microbes that are stable to high temperatures (heat) or thermostable. Usually thermophilic bacteria can be found in nature in various places such as areas of volcanic activity, the seabed which has hot springs and hot springs (Fifendy et al., 2015).

Other advantages of this thermophilic bacteria have a low level of contamination, the resulting product is higher, the energy supply for product separation is lower. According to research conducted by Scully et al. (2015) thermophilic bacteria are more beneficial in bioethanol production because thermophilic bacteria are able to degrade various kinds of substrates, are able to work in a high temperature range,

Ridha (2022) used 12 monoculture isolates of thermophilic bacteria (MS 4, MSS 5, MSS 8, MS 9, MSS 10, MSS 11, MS 11, MS 12, MSS 15, MS 16, MS 17 and MS 18) which were fermented and then the result is distillation and the bioethanol content is measured. The results showed that the monoculture isolate that had the potential to produce bioethanol with the highest yield was MS 9 isolate with an average bioethanol content of 1.0001%.

In nature bacteria are not only in the singular form but also exist in mixed forms called consortia. Using a consortium of bacteria the results are better than using a single isolate. Microbes in a consortium have a great opportunity to obtain energy and survive, because they can mutually utilize coenzymes excreted by other microbes. Notodarmojo (2005) said that several advantages of using a microbial consortium are (1) it can carry out degradation sequentially, (2) the consortium can produce the enzymes or substances needed, (3) it can increase the overall rate of substrate degradation. Within the consortium, of course, there are interactions between microorganisms that are beneficial and detrimental (Millati, 2018).

Compatibility is a collection of two or more bacteria that work together to form a community that has cooperative, commensal and mutualistic relationships. Community members who have relationships will associate, so they are more successful in degrading chemical compounds than single isolates (Jovanita et al., 2022). The use of compatible bacteria gives better results than single isolates because the enzyme work of each type of bacteria can complement each other, so that they can survive using the same source of nutrients in culture media (Siahaan et al., 2013).

Vinotha's research, (2019) used a new microbial consortium to produce bioethanol consisting of *Pseudomonas aeroginosa*, *Bacillus clausii*, and *Enterobactor cloacais* with orange peel as a substrate. The results of this bacterial consortium increased bioethanol production effectively with a bioethanol titer yield of 10.91 ± 0.49(g/l) or 1.32%-1.44% compared to the results of monoculture of *Pseudomonas aeroginosa* bacteria (1.02%-1, 21%), *Bacillus clausii* (1.13%-1.15%), and *Enterobactor cloacais* (1.15%-1.26%).

Research by Scully et al. (2015) used a co-culture of *Clostridium thermocellum* bacteria with a bioethanol yield of 4.19 g/l or 2.4%. Meganandi (2016) stated that from the results of his research the microorganism that produced the highest levels of bioethanol was the *Saccharomyces cerevisiae* – *Pichia stipitis* consortium where the highest bioethanol yield was 2.1% with a consortium concentration of 10% and during 24 hour fermentation.

This study aims to look at the collaboration between consortium isolates and the ability of the consortium to produce biofuels. Based on the background that has been described, the researchers decided to conduct research on "The Effect of the Thermophilic Bacteria Biculture Consortium from Mudiak Sapan Hot Springs on Biofuel Production".

Method

This research is a descriptive research. The compatibility test was used to test isolates of thermophilic bacteria producing bioethanol using the scatter method. Then the consortium isolate was fermented in liquid TMM medium and the bioethanol content was measured using a distillation apparatus.

Preparation of Liquid TMM Medium

The medium for growing bioethanol-producing thermophilic bacteria uses Thermophilic Minimum Media (TMM) medium consisting of 0.01% MgSO4.7H2O, 0.1% K2HPO4, 0.1% NaCl, 0.35% (NH4)2SO4, 0.05% mold extract, 0.05% peptone, 6% glucose (Zilda et al., 2008). Dissolved with distilled water up to 1000 ml. Then heated until homogeneous and sterilized by autoclave at 1210 C with a pressure of 15 psi for 15 minutes.

Compatibility Test Thermophilic Bacteria

Compatibility Test using the Spread Plate Method. The compatibility test for thermophilic bacterial isolates MS 9 and MS 12 was carried out by taking 5 doses of MS 12 then putting them in a tube containing 5 ml of sterile distilled water and adjusting the turbidity with Mc Farland scale 1 solution. Then pouring it into a petri dish containing NA medium and spread with a spreader. Next, 4 pieces of sterile paper discs were taken which had previously been dripped with 0.1 ml of the MS 9 bacterial suspension. The four paper discs were placed in a medium that had been inoculated with the MS 12 bacterial suspension and incubated for 24 hours in an incubator at a temperature of 50^0 C (Jovanita et al., 2022).

Activation and Fermentation of Thermophilic Bacteria Biculture Consortium Isolates

MS 9, MS 12, MS 18 and MS 17 isolates 5 ose were taken each from the slanted agar and put into a test tube containing 5 ml of physiological salt (0.85% NaCl) which would be equivalent to 0.5 of Mc Farland's solution. Then 2.5 ml of the bacterial suspension was put into an Erlenmeyer containing 22.5 ml of liquid TMM medium, then incubated for 24 hours in an incubator at 60^0 C. After 24 hours 0.25 ml of activation medium was taken and put into a test tube contains 5 ml of physiological salt (0.85% NaCl) and will be compared with 0.5 Mc Farland solution. Bacterial suspension taken as much as 10 ml to make a biculture consortium (MS 9-MS 12; MS 9-MS 18; MS 9-MS 17; MS 18-MS 17; MS 18-MS 12; and MS 17-MS 12) with a ratio of 1:1 then put into 40 ml of liquid TMM medium and then incubated in an incubator with a temperature of 60^0 C, pH 8. After completion, distillation was carried out with a pycnometer to measure the level of bioethanol (Safari et al., 2022; Vinotha., 2019).

Result and Discussion

Compatibility Test Thermophilic Bacteria

Of the six pairs of thermophilic bacterial isolates that were tested for compatibility, all isolates showed compatible results as indicated by the absence of a clear zone (Table 1).

Table 1. Compatibility Test Results for Thermophilic Bacteria from Mudiak Sapan Hot Springs

MS couple	Formation of Inhibition Zone	Results
MS 9 and MS 18	-	Compatible
MS 9 and MS 12	-	Compatible
MS 9 and MS 17	-	Compatible
MS 18 and MS 12	-	Compatible
MS 18 and MS 17	-	Compatible
MS 17 and MS 12	-	Compatible

Note: (-) no inhibition zone

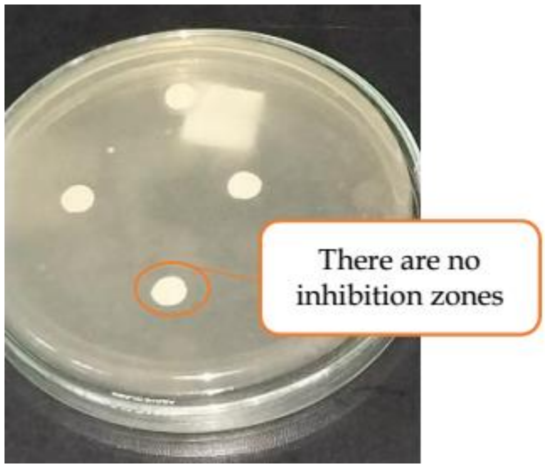

Figure 1. Bacterial consortium compatibility test

Based on the research that has been done, it can be seen that all isolates of thermophilic bacteria are compatible or able to work together physiologically well. The indicator is the absence of a clear zone after 2 x 24 hour incubation.

Bacterial compatibility is an association between two species or genera of bacteria that do not mutually interfere with one another, but the activities of each species or genus are mutually beneficial and share the same nutrient sources in the same living medium (Asri et al., 2016). In line with the opinion (Prayudyaningsih et al. (2015) that not all microorganisms have properties that are detrimental to other organisms, but there are also bacteria that can be beneficial. The use of compatible bacteria gives better results than single isolates because the enzyme work of each type of bacteria can complement each other, so that they can survive using the same source of nutrients in culture media (Siahaan et al., 2013).

Bacteria are said to be synergistic or compatible if the interactions between the genus or species of bacteria are mutually beneficial and share the same source of nutrients in the same living medium. Bacteria are said to be antagonistic or incompatible when in activities with other organisms they compete with each other for space, air, water, food (nutrients) (Rifai et al., 2020).

The thermophilic bacteria used are bacteria that come from water sourcesin South Solok Regency, to be precise, the Mudiak Sapan hot springs, Jorong Balun, Nagari Pakan Rabaa, Koto Parik Gadang District in Ateh. This hot spring has a temperature of 93^0 C with a pH of 8. The degree of acidity (pH) will also affect the variant of the isolate because the conditions around the hot spring are alkaline, so it is estimated that the isolates of thermophilic bacteria are more diverse (I. Irdawati et al., 2017).

According to Sari (2012) hot springs alkaline so that it has a high mineral content and allows thermophilic microorganisms to live well. This ability is due to a different protein structure than thermophilic bacteria so that they can survive extreme temperatures. Thermophilic bacteria include bacteria that are amylotic, namely bacteria that produce amylase enzymes that can degrade starch (F. Irdawati, 2011).

Activation and Fermentation of Thermophilic Bacteria Biculture Consortium Isolates

The bioethanol content of the biculture consortium isolates of thermophilic bacteria can be seen in (Table 2) which shows that 10 treatments of thermophilic bacterial isolates were able to produce bioethanol.

Table 2. Average Value of the Biculture Consortium of Thermophilic Bacteria from Mudiak Sapan Hot Springs

Bacterial Isolate	Average Content of Bioethanol (%)
MS 9	0.93
MS 12	0.43
MS 18	0.34
MS 17	0.31
MS 9-17	0.53
MS 18-17	0.60
MS 12-18	0.63
MS 12-17	0.93
MS 9-18	0.96
MS 9-12	1.00

From the research results obtained in Table 2, it is known that the MS 9 control produces a high bioethanol content of 0.9378%, followed by MS 12 with a yield of 0.4388% ethanol content. Then MS 9 and MS 12 were paired as a biculture consortium to produce twice the bioethanol content of the monoculture isolate, namely 1.0003% because the two paired isolates were the best bacterial isolates in producing bioethanol in monoculture. Then MS 9 in consortium with MS 18 produced the second highest bioethanol content of 0.9612%. This result was lower than the MS 9-12 consortium because MS 18 in monoculture produced the third lowest bioethanol content so that when paired with MS 9 which produced the highest bioethanol content MS 18 affected the results of the biculture consortium.

Isolate MS 12 which is a monoculture isolate with the second highest bioethanol content was consortium with MS 17 with the lowest bioethanol content in monoculture. This consortium produces the third highest bioethanol content of 0.9345%. This shows that the MS 12 and MS 17 consortium are compatible and able to cooperate physiologically, marked by a doubling of the bioethanol yield compared to the monoculture. Then MS 12 was consortium with MS 18 to produce a bioethanol content of 0.6365%. The results of the bioethanol content of the two isolates were double compared to the monoculture isolates. Likewise, with other biculture consortium isolate pairs, namely and MS 17-18.

Furthermore, MS 17, which yielded the lowest monoculture content of 0.3121%, was consortium with MS 9, whose monoculture produced high levels of bioethanol. The MS 9-17 consortium produced the lowest bioethanol content among the other consortium isolates with a yield of 0.5359%. Based on the results of compatibility, the two isolates were compatible, but in working together to produce bioethanol MS 17 gave unfavorable results to the biculture consortium with MS 9.

Bacteria in synergistic consortia have higher metabolic activity compared to pure cultures (Deng et al., 2016). In line with the opinion of (Prescott et al., 2002), bacterial consortia formed naturally and artificially have the advantage of having complementary metabolic functions in an ecosystem. Strengthened by the opinion of Siahaan et al. (2013) who said the use of microbial consortia tends to give better results than the use of a single isolate, because the work of enzymes from each type of microbe complements each other to be able to survive using the available nutrient sources in the media used.

Enzymes that work in bacterial consortia enable cooperation in using available nutrients to survive (Komarawidjaja et al., 2009). According to research by Widjaja et al. (2016) using a consortium between Pichia stipitis and Saccharomyces cerevisiae with a ratio of 1:1 (one ose each) proved that biculture consortia were better than monocultures. Based on the results of Irdawati's research (2019) showed that the thermophilic bacteria Mudiak Sapan are Bacillus bacteria. Ou et al. (2009) suggested that the genus Bacillus is more effective in fermenting sugar than yeast. Strengthening the opinion of Khasanah (2003) who argued that the use of yeast in producing bioethanol is imperfect because not all available glucose is converted to ethanol. This is because the available glucose will also be used for the formation of cell biomass and other by-products such as glycerol and succinic acid.

Conclusion

From the results of this study it can be concluded that biculture consortium isolates of thermophilic bacteria are compatible. Then the optimum biculture consortium isolate formulation in producing bioethanol is MS 9 with MS 12 with a yield of 1.0003%.

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References

Agusri, R. (2022). *Optimization of Thermophilic Bacteria Temperature in Producing Bioethanol as Biofuel*. Padang State University. Retrieved from http://repository.unp.ac.id/39806/

Asri, A. C., & Zulaika, E. (2016). Synergism Between Consortium Azotobacter Isolates. *Journal of Science and Arts ITS*, *5*(2), 2337–3520. https://doi.org/10.12962/j23373520.v5i2.20693

Deng, Y.-J., & Wang, S. Y. (2016). Synergistic growth in bacteria depends on substrate complexity. *Journal of Microbiology*, *54*(1), 23–30. https://doi.org/10.1007/s12275-016-5461-9

Fifendy, M., Irdawati, I., & Yenti, N. (2015). Screening of thermophilic bacteria that produce amylase enzymes from the Sapan Aro hot spring, South Solok Regency. *Scientific Periodical Exacts in Mathematics and Natural Sciences*, *1*, 73–81. Retrieved from http://ejournal.unp.ac.id/index.php/eksakta/article/view/5266

Irdawati. (2019). *Diversity of Thermoxilanolytic Bacteria from Habitat of South Solok Hot Springs and Their Potential as Pulp Additives*. Andalas University. http://scholar.unand.ac.id/42929/

Irdawati, F. (2011). *Isolation of Amylase Producing Thermophilic Bacteria from Rimbo Panti Pasaman Hot Springs*. Retrieved from http://repository.unp.ac.id/48/1/3. Penelitian 2011- 2.pdf

Irdawati, I., Syamsuardi, S., Agustien, A., & Alberida, H. (2017). Growth Profile of Alkaline Xylanase Producing Thermophilic Bacteria from Mudiak Sapan Hot Springs, South Solok. *Proceedings of the National Seminar on Biology Education*. Retrieved from http://repository.unp.ac.id/27774/

Jovanita, L., & Advinda, L. (2022). Compatibility Test of Fluorescent Pseudomonas Isolated from Plant Rhizosphere. *Journal of Porch Biology*, *7*(1), 65–69. Retrieved from https://serambibiologi.ppj.unp.ac.id/index.php/srmb/article/view/21

Kasim, S., & Usman, H. (2013). Utilization of ARS-CHAT Medium in Potential Marine Phytoplankton Biomass Production As Raw Material for Bioethanol-type Biofuels. *Proceedings of the MIPA National Seminar*. Retrieved from https://ejournal.undiksha.ac.id/index.php/semnasmipa/article/view/2729

Khasanah. (2003). *Formulation, Physical-Chemical Characterization and Organoleptic of Sweet Potato Breakfast Food Products* [Faculty of Agricultural Technology, IPB]. Retrieved from http://repository.ipb.ac.id/handle/123456789/25878

Komarawidjaja, W., & Lysiastuti, E. (2009). Status of local oil-degrading microbial consortia. *Journal of Environmental Technology*, *10*(3), 347–354. https://doi.org/10.29122/jtl.v10i3.1481

Lynd L, R. (1989). Production of ethanol from lignocellulosic materials using thermophilic bacteria : critical evaluation of potential and review. *Adv In Biochem. Eng. Biotechnol. Springer-Verlag*, *38*, 1–52. https://doi.org/10.1007/BFb0007858

Mayzuhroh, A. (2015). *Production of Bioethanol Using Commercial Starters With and Without Providing Agitation Aeration in Molasses Media*.

Meganandi, K. A. (2016). Production of Bioethanol from Sorghum Bicolor Stems. *Journal of Purification*, *16*(2). Retrieved from https://purifikasi.id/index.php/purifikasi/article/download/43/41/

Millati, G. Z. (2018). *Practical Work Report on the Use of Compatibility Test of 7 LOB Isolates with Pseudomonas fluorescens PF32 Using the Cross Streak and Modified Kirby-Bauer Methods*. Microbiology Study Program, Bandung Institute of Technology.

Notodarmojo, S. (2005). *Soil and Groundwater Pollution*. ITB Publisher.

Novia, A., & Rosmawati, W. (2014). Pembuatan Bioetanol dari Jerami Padi Dengan Metode Ozonolisis–Simultaneous Saccharification and Fermentation (SSF). *Journal of Chemical Engineering*, *3*(20). Retrieved from https://repository.unsri.ac.id/73119/2/180

Ou, M., Mohammed, N., Ingram, L. O., & Shanmugam, K. T. (2009). Thermophilic Bacillus coagulans Requires Less Cellulases for Simultaneous Saccharification and Fermentation of Cellulose to Products than Mesophilic Microbial Biocatalysts. *Journal of Applies Biochemistry and Biotechnology*, *155*, 76–82. https://doi.org/10.1007/s12010-008-8509-4

Prayudyaningsih, R., & Nursyamsi, R. S. (2015). Beneficial Soil Microorganisms in Rooting Rhizosphere Under Community Forest Stands in South Sulawesi. *Proceedings of the National Seminar on Community Biodiv Indonesia*, *1*, 954–959. https://doi.org/10.13057/psnmbi/m010453

Prescott, L. M., Harley, J. P., & Klein, D. A. (2002). *Microbiology* (5th ed.). McGraw-Hill.

Rifai, M. R., H, W., & Susanto, A. (2020). Synergism and Antagonism of Several Types of Consumed Bacterial Isolates. *BIOLOVA*, *1*(1), 21–26. https://doi.org/10.24127/biolova.v1i1.31

Safari, W. F., & Intercession, M. (2022). Effect of Pretreatment and Composition of Trichoderma Viride and Zymomonas Mobilis Consortium on Bioethanol Production from Leaf Litter. *Indonesian Journal of Biotechnology and Biodiversity*, *6*(2), 52–60. https://doi.org/10.47007/ijobb.v6i2.134

Sari, U. M. (2012). *Screening and Characterization of Thermophilic Cellulolytic Bacteria in Medang River Hot Springs, Kerinci, Jambi*. Andalas University.

Scully, S. M., & Orlygsson, J. (2015). Recent advances in second generation ethanol production by thermophilic bacteria. *Energies*, *8*(1), 1–30. https://doi.org/10.3390/en8010001

Siahaan, S., M, H., & Hasibuan, R. (2013). Determination of the Optimum Conditions of Temperature and Carbonation Time in Making Charcoal from Rice Husk. *Journal of Chemical Engineering*, *2*(1), 1–6. Retrieved from https://talenta.usu.ac.id/jtk/article/view/1423

Vinotha., U. (2019). Bioethanol Production Using Bacterial Consortia From Waste Cellulosic Waste. *Think India Journal*. Retrieved from https://thinkingdiaquarterly.org/index.php/think-india/article/view/11440

Widjaja, T., Altway, A., Nurkamidah, S., Endahwati, L., Lini, F. Z., & Oktafia, F. (2016). The Effect of Pretreatment and Variety of Microorganisms to the Production of Ethanol from Coffe Pulp. *ARPN. Journal of Engineering and Applied Science*, *11*(2), 1056–1060. Retrieved from https://www.arpnjournals.org/jeas/research_papers/rp_2016/jeas_0116_3453.pdf

Winaya, R., & Kristanto, P. (2002). Use of Vegetable Oils as Alternative Fuels in Direct Injection Diesel Engines. *Journal of Mechanical Engineering*, *2*, 99 – 103. https://doi.org/10.9744/jtm.4.2.pp.99-103

Zilda, D. S., Kusumarini, A., & Chasanah, E. (2008). Protease Screening and Characterization of P5-A Thermo-Acidophilic Bacteria from Tambarana Hot Springs. *Journal of Postharvest and Marine and Fishery Biotechnology*, *3*(2), 113–121. https://doi.org/10.15578/jpbkp.v3i2.17