Synergism of cellulolytic bacteria in consortium as starter culture agent "MOIYL" for fiber feed

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Abstract. The study aimed to examine the synergism of cellulolytic cellulosic consortium degrading cellulose as a fibrous starter culture agent. The study used streak plate method on nutrient agar medium [NA]. The isolated bacterial isolates were isolates of potential cellulolytic bacteria isolated from oil palm plantation waste, corn, and coconut wastes, namely: Bsp1, BJ1, BJ2, BP3, BP6, BP11, BP13, and BP16. The isolates were scratched on the nutrient agar medium [NA] and incubated for 24 hours. Observations were made there was a clear zone or inhibit zone between two isolates that intersect. The results showed that from 8 isolates tested 5 isolates can synergize well against other isolates. Conclusion: naturally, the cellulolytic isolate from oil palm waste, corn waste, and coconut waste can be consortium as starter culture agent “MOIYL” for fibrous feed.

1. Introduction

The availability of cellulosic biomass derived from agricultural or plantation waste in nature is abundant, but its use as a source of energy for livestock is not yet optimal. This is because the structure of the components of cellulose is difficult to digest by livestock. Efforts that can be done in order to overhaul the structure of cellulose component composition are more optimal one of them through the utilization of microorganisms.

In life, microorganisms play a role in decomposing complete compounds (polysaccharide) to be simple. The final product of this compound decomposition may be simple sugars and organic acids. Given this process, agricultural or plantation wastes as cellulosic biomass will be utilized more optimally by livestock.

Microorganisms in nature can naturally live together to form communities that interact with each other. Interactions formed between microorganisms can be synergistic, successional, antagonistic and others. Bacteria as part of microorganisms can communicate with each other. Bacteria can synergize if the substrate is available enough and there is no competition between each other.

Bacteria that live together (consortium) can increase metabolism products that can be utilized for the benefit of humans [1]. The consortium is a mixture of microbial populations that have cooperative, commensal, and mutualistic relationships. Members of the associated microbial community will associate, thus more successfully degrading chemical compounds than single isolates. The relationship between the consortium bacteria in sufficient substrate state will not interfere with each other, but synergize in order to produce higher reform efficiency during processing [2].

In general, a consortium of microbes can be defined as a collection of various microbes that live together, forming a community of mutual synergy with each other. According to [3] the use of
microbial consortium tends to give better results than the use of single isolates, because it is expected that the enzyme work of each type of microbe can be complementary to survive using the available nutrient sources in the carrier medium.

The bacteria to be sponsored consists of isolated cellulolytic bacteria from plantation waste [4], which is an indigenous microorganism YL [MOIYL] that has a high ability to degrade fibers. These bacteria will be synergized with isolated bacteria from maize and bacterial waste from Piek-U. Synergism between bacteria provides an opportunity to be sponsored as a starter culture agent "MOIYL" for fibrous feed.

By adopting the nature of microorganisms in nature which form a community with cooperative, commensal, and mutualistic relationships, it is necessary to study a synergism of cellulolytic cellulose degradation consortium as a fibrous starter culture agent. The aim of this research is to know whether potential cellulolytic bacteria isolate from oil palm waste [Bsp]), corn crop [BJ1 and BJ2], and coconut waste [BP1, BP3, BP11, BP13, and BP16] can synergize so that it can be revitalized.

2. Material and Methods

2.1. Materials and Equipment
Isolates used were isolates of potential isolation from oil palm waste [Bsp1], corn waste [BJ1 and BJ2] and from coconut waste [BP3, BP6, BP11, BP13 and BP16]. The medium used is NA [Nutrient Agar], 70% alcohol, 96% alcohol, distilled, spirits, hot plate, autoclave, incubator, vacuum cabinets, wire loop, laminar air flow, analytical balance, oven, burners, and petri dish.

2.2. Methods
Synergism test between bacteria using the scratch method. Each isolate was scratched to each other so that the isolates would meet each other, then incubate. Observations are made after 24 hours of incubation. Synergistic isolates are shown by the formation of inhibitory zones [5], isolates are said to be compatible if there is no inhibition zone in the meeting area of the two isolates, and it is said to be incompatible if there is an inhibition zone in the area where the two isolates meet [6].

2.3. Procedure
- Making Medium. Preparation of medium by weighing nutrient agar medium (NA) then dissolved in water. The solution homogenized over hot plate then sterilized by autoclave. The sterile medium is then poured aseptically into a sterile Petri dish.
- Bacterial Rejuvenation. The bacteria to be tested were taken from the stock to be tilted then inoculated on a new agar medium.
- The result of the rejuvenated bacteria isolate is used for the synergism test. The test was performed by inoculating the bacterial isolate in the nutrient agar medium by using a scratch method between the tested isolates.
- Inoculation result was incubated at temperature ± 37 °C for 24 hours.
- Isolates that have been incubated for 24 hours were observed for either clear zones or inhibit zone between the two isolates intersecting.
- Isolates are said to be synergic if there is no inhibition zone in the second enclosure of isolates, and it is said to be non-synergistic if there is a zone of inhibition in the enclosure of the two isolates.
3. Results and Discussion
The result of synergy test between isolates cultured together on nutrient agar media showed that from 8 isolates tested, 5 isolates were able to synergize with each other and 3 isolates were not. From the 24-hour incubation results, the synergistic isolates did not form a clear zone or inhibition zone in their confusion Figure 2. The synergism between the test isolate can be seen in Table 1.

Figure 1. Stretch method [A, B and C are bacterial isolates]

Figure 2. The synergism test of between isolates bacteria
Figure 2 shows generally isolates can be well synergized except in isolate BJ1, BP3, and BP16. This isolate shows a drag zone in the intersection of both lines. The existence of the inhibitory zone shows that bacteria can not synergize well in the same substrate so that one isolate grows better than the other. This is thought to be due to the ability of individual bacteria to utilize nutrients or the ability to degrade organic material that is different so there is a fast-growing isolate while others are hampered.

Table 1. Synergism between cellulolytic bacterial isolates

| Isolate Bacteria | Bsp1 | BP3 | BP6 | BP11 | BP13 | BP16 | BJ1 | BJ2 |
|------------------|------|-----|-----|------|------|------|-----|-----|
| BSp1             | 0    | +   | +   | +    | +    | +    | +   | +   |
| BP3              | +    | 0   | +   | +    | +    | -    | +   | +   |
| BP6              | +    | +   | 0   | +    | +    | +    | +   | +   |
| BP11             | +    | +   | +   | 0    | +    | +    | +   | +   |
| BP13             | +    | +   | +   | 0    | +    | +    | +   | +   |
| BP16             | +    | +   | +   | +    | 0    | -    | +   | +   |
| BJ1              | +    | -   | +   | +    | +    | -    | 0   | +   |
| BJ2              | +    | +   | +   | +    | +    | +    | +   | 0   |

+: synergistic
-: antagonist

Table 1 shows the bacteria from palm waste [Bsp1] can synergize with bacteria from corn and coconut waste. This indicates that in the Bsp1 bacterial consortium co-operative against the bacteria from corn waste and coconut. Declare [7], the Bacterial consortium is a collection of bacteria that work together to form a community, to produce a significant product.

Bacterial isolates derived from coconut waste can synthesize each other [positive synergistic]. This indicates that isolates of the same species or genera can be cultured in utilizing nutrients from the same substrate so that they can support each other's growth between isolates and other isolates.

BJ1 bacteria from corn waste can not synergize with B3 BP3 and BP16 from coconut waste. However, BJ2 bacteria can synergize with other bacteria. This suggests that bacterial synergism can occur unspecified from which bacterial origin, but is determined by the ability of the individual genus or species of the bacteria. According to [8] Bacteria with the same genus or species can interact and synergize, and share the same source of nutrition. This shows the cooperative behavior between bacteria in a habitat in the form of a consortium. A consortium will produce products that can be harnessed together, so as to support each other's growth of isolates and others.

The existence of compatibility or synergism of two or more bacteria is a very important factor in order for the bacteria to cooperate well. The mechanism of synergism between isolates within the consortium is still not known with certainty, but some studies suspect it is caused by several factors including: 1) one member of the genus is able to provide one or more nutritional factors that can not be synthesized by members of other genera, 2) one member of the genus that is unable to degrade a certain organic material will depend on the member of the genus capable of providing the degradation of the organic material, 3) one member of the genus protects members of another genus sensitive to certain organic matter by lowering the concentration of the material organic toxic by producing specific and non specific protective factors.

4. Conclusions

Consortium cellulolytic bacteria can be synergized not only determined by the same species or genera but also determined by the individual ability of the species or genus. The syntactic bacterial isolates can be shown by the absence of clear zones or inhibit zone.
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