# Isolation and identification of indigenous lactic acid bacteria on corn flour BISI-16 during spontaneous fermentation process

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# Isolation and identification of indigenous lactic acid bacteria on corn flour BISI-16 during spontaneous fermentation process

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**Abstract**. Lactic acid bacteria (LAB) is one of the dominant bacteria species that have an important role during spontaneous fermentation process of corn flour BISI-16. LAB are known to change the chemical structure of natural corn starch through the activity of enzyme and lactic acid produced during fermentation process. This change of structure causes the modification of physicochemical properties of corn flour. The objectives of this study were to isolate and identify indigenous LAB involved in spontaneous fermentation process of BISI-16 cornflakes. Isolation and identification of early LAB was done by isolation morphology characterization including cell shape, Gram staining, catalase test, and endospora staining. Furthermore, isolates LAB were identified by their genotypes using Polymerase Chain Reaction (PCR) method and 16S rRNA sequencing analysis. The results showed two isolates (ASN3 and ASN5) that had been isolated from corn flour during spontaneous fermentation and were grown on MRSA media having rounded colonic and creamy, rod-shaped cells, Gram (+), catalase (-), and endospores (- ). The initial identification stage shows both isolates as candidate LAB. The results of the 16S rRNA sequence analysis showed both isolates were genotypically similar with *Lactobacillus fabifermentans* with 97% similarity for ASN3 isolates and ASN5 isolates of 98%.

## 1. Introduction

The use of corn into food products is still very low. The condition of hard corn seeds with large seed shape causes the processing to take longer. Therefore, processing corn into corn flour products is needed. When compared with corn shaped, corn flour will be more easily applied to food products, although the application of corn flour is highly dependent on its physicochemical properties.

Physicochemical properties are one of the properties associated with viscosity and gelatinization of corn flour during the heating process, namely the peak viscosity and hot paste viscosity, where these parameters are describes the ability of a granule to experience maximum development during heating [1]. Breakdown viscosity or changes in heat paste are physicochemical properties that describe the resistance of a granule to the heating process and mechanical treatment during processing, while the cold paste viscosity and setback parameters are physicochemical properties that describe the ability of the granule to retrograde during cooling [2]

Corn flour as natural starch still has a non-uniform gel viscosity, is not resistant to high temperatures, cannot stand acid conditions, cannot withstand mechanical treatment, has limited

solubility, and is still susceptible to cirrhesis. This causes the application of corn flour to food products is still very limited, so we need an effort to modify corn flour which is expected to modify the physical properties of corn flour so that the potential of the application becomes greater.

Modification of the characteristics of corn flour can be done by fermentation. Modification of flour with fermentation has the potential to be developed because of low operating costs. The fermentation process is defined as the decomposition of starch by an enzyme produced by microorganisms on a substrate, so that the fermentation of corn starch is the decomposition of corn starch carried out by the amylase and amyloglucosidase enzymes produced by indigenous microbes. Starch degradation process depends on the composition of amylose and amylopectin corn flour which also affects the performance of the enzyme, the amylase enzyme will degrade more starch with high amylose content [3]. Corn fermentation process has been investigated by [4], who reported that the fermentation of corn flour can increase the protein content and amino acid lysine which has been the inhibiting factor of corn flour. [5] also found that corn fermentation can reduce total acid from corn so that the quality of corn flour becomes better. Likewise with the discovery of [6], which shows that the fermentation process can increase globulin and albumin from corn flour. In addition, [7] reported that the process of spontaneous fermentation of corn can improve food safety because the fermentation process can reduce M1 aflatoxin, citrine, and cyclopiazonic acid.

Corn flour which had been spontaneously had better nutritional content compared to unfermented corn flour [8]. [9] also reported that two modified types of corn flour (BISI-2 and pop corn) using spontaneous fermentation caused a decrease in enthalpy value, reduced crystalline characteristics of starch, and decreased value of peak viscosity, reverse viscosity, initial temperature and corn starch peak temperature

Modification of corn flour using the method of spontaneous fermentation is considered to have weaknesses, namely the type of living microbes that can vary and are very dependent on conditions and environment so that it is difficult to control. Several studies have reported that the dominant bacteria involved during the spontaneous fermentation process of corn flour is from the group of lactic acid bacteria (LAB). Lactic acid bacteria are beneficial bacteria in the fermentation process and are always involved in spontaneous fermentation because they are indigenous. Lactic acid bacteria are able to inhibit the growth of pathogenic and decay bacteria due to this bacteria capable of producing several anti-bacterial compounds such as bacteriocin, hydrogen peroxide, fatty acids, reuterin, diasetil and lactic acid.

Research studies on the involvement of LAB during the process of spontaneous fermentation of corn flour have been reported by several researchers. However, studies on BISI-16 corn which are hybrid corn have not been reported. Corn BISI-16 is a corn that has been given genetic engineering technology. The process of genetic engineering allows indigenous microbes in BISI-16 corn to differ from other types of corn. Therefore, this study focused on the study of LAB involved during the spontaneous fermentation process of BISI-16 corn. The purpose of this study was to isolate and identify LAB involved in the spontaneous fermentation process of BISI-16 corn flour

## 2. Materials and Methods

### *2.1. Materials and Tools*

The main ingredient is BISI-16 hybrid corn obtained from Jeneponto Regency, South Sulawesi. The microbial growth media used were PCA media, NA media, and MRSA media. The materials used for analysis were water and distilled water, NaCl, 95% ethanol, immersion oil, crystal violet solution, 70% alcohol, methylated spirits, cotton, aluminum foil and safranin solution.

The equipment used for analysis includes blenders, test tubes, centrifuge tube racks, vortices, hot plates, glass beakers, volumetric flasks, clasps, analytical scales, measuring cups, volumetric pipettes, drop pipettes, PCR (polymerase chain reaction), pH meter, Water bath, Erlenmeyer, Petri dish, incubator, autoclave, burette, 9FZ-23 type disc mill and PPK N 70 type corn seed disposal machine.

### *2.2. Research Methods*

*2.2.1. Isolation and Identification Indigenous Bacteria*

Corn seeds that have been cleaned from dirt and defective seeds are soaked for 1 hour at room temperature with a ratio of corn and the amount of water 1: 2 (b / v). Furthermore, the seeding of corn kernels was carried out using PPK N 70 type buffering machine. The corn grain which was silo was added with water that had been cooked in a ratio of 1: 2 (b / v) fermented spontaneously for 48 hours using the microaerophilic method. During the fermentation process, the fermentation liquid is inoculated into PCA, NA, and MRSA growth media. Inoculation and analysis are carried out at fermentation time 24 and 48 hours.

Indigenous bacteria on PCA, NA, and MRSA media were isolated using quadrant scratching techniques. After obtaining isolates with separate colonies a simple identification was made consisting of typical colonies, morphological forms (cocci or bacil), Gram staining test (+/-), catalase test , and Endospora staining test. After that, the pure isolates obtained with LAB characteristics were tested for their genotypic properties (16sRNA coding DNA sequence analysis, 16sRNA encoding DNA amplification, 16sRNA coding DNA sequencing).

*2.2.2. Genotive Identification Using PCR and Analysis of 16S rRNA Coding DNA Sequences*

Genotification of bacterial culture was carried out by extracting 16S rRNA encoding DNA which was then amplified and sequenced. Isolates to be analyzed are classified as Gram positive (+) bacteria. Therefore, DNA extraction uses the GenAid method to extract DNA from Gram positive (+) bacteria with the *Geneaid Presto ™ Mini gDNA Bacteria Kit Protocol* . Culture cells as much as $10^9$ cells/ml were centrifuged at a speed of 14-16,000 g for 1 minute. Theformed supernatant is removed. Furthermore, pellets were added 200 µl buffer GT and lysozyme (4 mg/ml), occasionally distorted, and incubated at 37°C for 30 minutes. Supernatant was added with 20 µl proteinase K, then reexorted until mixed, and incubated at 60ºC for 10 minutes. After incubation, the supernatant was added 200 µl of buffer GB, vortexed occasionally, and incubated at 70ºC for 10 minutes. After that, 200 µl of absolute ethanol was added, distorted for 10 seconds, put into GD column, and centrifuged at 14-16000 g for 2 minutes. The GD column holder is replaced with a new one. The next process, W1 buffer of 400 µl was added, centrifuged at a speed of 14-16,000 g for 30 seconds, the liquid contained in the container GD column was removed. Next, 600 µl of W1 buffer was added, centrifuged at 14-16,000 g for 30 seconds, the GD column was transferred to sterile eppendorf, the liquid contained in the GD column container was discarded. Centrifugation was carried out again for 3 minutes to dry the GD column. The next step, EB 100µl solution was added, it was transferred from the GD column to the new eppendorf, then centrifuged again for 30 seconds with a speed of 14-16,000 g. DNA samples are produced.

*2.2.3. DNA amplification of 16S rRNA encoders with PCR*

The amplification reaction of DNA samples was carried out in a 0.2 ml PCR tube. Each tube of PCR reaction was added with RBC *Taq* (5 units / ml) as much as 0.25 µL, 10 x buffer Taq (containing $Mg^{2+}$ ) as much as 5 µl, dNTP 2.5 mM as much as 4 µl. The primers used were universal primers, namely 63F (5'-CAGGCCTAACACATGCAAGTC-3') and 1387R (5'-GGGCGGWGTGTACAAGGC-3'), respectively 1.25 µl (20 pmol). The genome extract was 2.5 µl (100 ng) and added with $ddH_2O$ until the volume became 50 µl. PCR amplification was carried out at an initial denaturation temperature of 95ºC for 5 minutes, primer attachment at 94ºC for 30 seconds with 30 cycles, and extension at 50ºC for 1 minute, 72ºC for 2 minutes, and the final stage 72ºC for 2 minutes. PCR products are taken and stored at 4ºC. Furthermore, PCR products (DNA amplification as much as 5 µl) were put into wells of 1.5% agarose gel and TAE buffer solution submerged in the tank. Agarose gel 1.5% and TAE buffer solution consisted of 1.5 g agarose powder and 100 ml TAE buffer and 8 µl ethidium bromide.Electrophoresis is run for 1 hour with a constant voltage of 100 Volts. DNA tape (gel formed) is observed under UV light.

*2.2.4. Analysis of 16S rRNA encoding DNA sequences*

DNA sequencing of 16S rRNA coding was carried out in 1st *Singapore BASE sequencing* facilitated by PT. Genetics Science Indonesia. Analysis of sequencing results was carried out with the BLASTN

2.5.1+ program, which is by matching the nucleotide sequence from the 16S rRNA sequencing results with the data base available at the site www.ncbi.nlm.nih.gov

## 3. Results and Discussions

### *3.1. Isolation and identification of lactic acid bacteria*

The results showed that indigenous microbes involved during the spontaneous fermentation process of cornstarch-BISI 16 were mold, yeast, bacteria, and LAB. Theresults of isolation and identification of mold using slide culture techniques found 7 types of mold involved in the spontaneous fermentation process of BISI-18 corn flour, namely *Aspergillus fumigatus* , *A. flavus* , 3 classified species of *Aspergillus* sp, C *unninghamellaelegans* , and *Dendryphiopsisatra* [10].

The dominant indigenous microbes in the spontaneous fermentation of corn flour BISI-16 is LAB, so that during the fermentation there is a change in the pH value (the more acidic) the total acid content increases with the fermentation time interval. LAB produces lactic acid and other organic acids as the main metabolite products. Identification data on mold and yeast are not shown.

The results of the simple identification of 6 isolates of indigenous bacteria that had been isolated showed that there were four isolates of LAB bacteria, namely isolates ASN3, ASN4, ASN5, and ASN12. ASN9 and ASN11 bacterial isolates have been reported as *Enterobacter cloacaesubsp. cloacae* [11]. Both of these bacteria were found to be involved spontaneously during the fermentation process of BISI-16 corn flour. The results of simple identification of isolates are presented in Table 1.

**Table 1.** Simple identification of indigenous bacterial isolates in corn flour spontaneous fermentation

| No. | Bacterial Isolates | Characteristics | | | | |
|---|---|---|---|---|---|---|
| | | Gram | Catalase | Endospora | Cell Form | Typical Colonies |
| 1 | ASN3 | + | - | - | Short stem | Creamy round |
| 2 | ASN4 | + | - | - | Stem | Creamy round |
| 3 | ASN5 | + | - | - | Stem | Round, large beige colonies |
| 4 | ASN9 | - | + | - | Stem | Round white, the edges of the surface are slimy |
| 5 | ASN11 | - | + | - | Stem | White round |
| 6 | ASN12 | + | - | - | Short stem | Creamy round |

The isolates of LAB ASN3 and ASN5 were further analysed in the genotypic characterization of LAB so that the two species of isolate were known. Genotypic characterization analysis of LAB was only carried out on these two isolates because the isolates of LAB ASN4 and ASN12 had similar cell form and typical colonies of isolates LAB ASN3 and ASN5.

Isolates LAB ASN3 and ASN5 are included in Gram (+) bacteria which have catalase (-) properties and do not produce endopora. Both of these isolates belong to the LAB group, because LAB is included in the Gram (+), catalase (-), and endospore groups. (-). ASN3 isolates were obtained after spontaneous fermentation of BISI-16 corn flour for 24 hours, while ASN5 isolates were obtained at the end of fermentation time, which was 48 hours. Typically, the two isolates of this bacteria have similarities, namely cream-colored bacterial colonies. However, ASN5 isolates have the characteristics of a larger colony.

### *3.2. Genotypic characterization of indigenous lactic acid bacteria*

Characterization of indigenous LAB bacterial genotypes was carried out using 16S rRNA coding DNA. Primers used in the DNA amplification process were 63F (5'-CAGGCCTAACACATGCAAGTC-3') and 1387R (5'-GGGCGGWGTGTACAAGGC-3'). The primary specificities of 63F and 1387R have been systematically tested with various types of bacteria and environmental samples , this primer is better used for 16S rRNA gene amplification, both ecologically and systematic studies compared to PCR amplitude that is more commonly used

[12]. The results of the 16S rRNA encodingsequence *alignment* analysis of the ASN3 isolates are presented in Figure 1, while ASN5 isolates can be seen in Figure 2.

```
Query  10    TTGATTGGTGCTTGCATCATGATTTACATTTGAGTGAGTGGCGAACTGGTGAGTAACACG  69
ASN3   96    TTGATTGGTACTTGTATCATGATTTACATTTGAGTGAGTGGCGAACTGGTGAGTAACACG  155

Query  70    TGGGAAACCTGCCCAGAAGCGGGGGATAACACCTGGAAACAGATGCTAATACCGCATAAC  129
ASN3   156   TGGGAAACTTGCCCAGAAGCGGGGGATAACACCTGGAAACAGATGCTAATACCGCATAAC  215

Query  130   AACTTGGACCGCATGGTCCGAGTTTGAAAGATGGCTTCGGCTATCACTTTTGGATGGTCC  189
ASN3   216   AACTTGGACCGCATGGTCCGAGTTTGAAAGATGGTTTCGGCTATCACTTCTGGATAGTCC  275

Query  190   CGCGGCGTATTAGCTAGATGGTGGGGTAACGGCTCACCATGGCAATGATACGTAGCCGAC  249
ASN3   276   CGCGGCGCATTAGCTAGATGGTGAGGTAACGGCTCACCATGGCAATGATGCGTAGCCGAC  335

Query  250   CTGAGAGGGTAATCGGCCACATTGGGACTGAGACACGGCCCAAACTCCTACGGGAGGCAG  309
ASN3   336   CTGAGAGGGTAATCGGCCACATTGGGACTGAGACACGGCCCAAACTCCTACGGGAGGCAG  395

Query  310   CAGTAGGGAATCTTCCACAATGGACGAAAGTCTGATGGAGCAACGCCGCGTGAGTGAAGA  369
ASN3   396   CAGTAGGGAATCTTCCACAATGGACGAAAGTCTGATGGAGCAACGCCGCGTGAGTGAAGA  455

Query  370   AGGGTTTCGGCTCGTAAAACTCTGTTGTTAAAGAAGAACATATCTGAGAGTAACTGTTCA  429
ASN3   456   AGGGTTTCGGCTCGTAAAACTCTGTTGTTAAAGAAGAACATATCTGAGAGTAACTGTTCA  515

Query  430   GGTATTGACGGTATTTAACCAGAAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAAT  489
ASN3   516   GGTATTGACGGTATTTAACCAGAAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAAT  575

Query  490   ACGTAGGTGGCAAGCGTTGTCCGGATTTATTGGGCGTAAAGCGAGCGCAGGCGGTTTTTT  549
ASN3   576   ACGTAGGTGGCAAGCGTTGTCCGGATTTATTGGGCGTAAAGCGAGCGCAGGCGGTTTTTT  635

Query  550   AAGTCTGATGTGAAAGCCTTCGGCTCAACCGAAGAAGTGCATCGGAAACTGGGAAACTTG  609
ASN3   636   AAGTCTGATGTGAAAGCCTTCGGCTCAACCGAAGAAGTGCATCGGAAACTGGGAAACTTG  695

Query  610   AGTGCAGAAGAGGACAGTGGAACTCCATGTGTAGCGGTGAAATGCGTAGATATATGGAAG  669
ASN3   696   AGTGCAGAAGAGGACAGTGGAACTCCATGTGTAGCGGTGAAATGCGTAGATATATGGAAG  755

Query  670   AACACCAGTGGCGAAGGCGGCTGTCTGGTCTGTAACTGACGCTGAGGCTCGAAAGTATGG  729
ASN3   756   AACACCAGTGGCGAAGGCGGCTGTCTGGTCTGTAACTGACGCTGAGGCTCGAAAGTATGG  815

Query  730   GTAGCAAACAGGATTAGATACCCTGGTAGTCCATACCGTAAACGATGAATGCTAAGTGTT  789
ASN3   816   GTAGCAAACAGGATTAGATACCCTGGTAGTCCATACCGTAAACGATGAATGCTAAGTGTT  875

Query  790   GGAGGGTTTCCGCCCTTCAGTGCTGCAGCTAACGCATTAAGCATTCCGCCTGGGGAGTAC  849
ASN3   876   GGAGGGTTTCCGCCCTTCAGTGCTGCAGCTAACGCATTAAGCATTCCGCCTGGGGAGTAC  935

Query  850   GGCCGCAAGGCTGAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTG  909
ASN3   936   GACCGCAAGGTTGAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTG  995

Query  910   GTTTAATTCGAAGCTACGCGAAGAACCTTACCAGGTCTTGACATACTATGCAAATCTAAG  969
ASN3   996   GTTTAATTCGAAGCTACGCGAAGAACCTTACCAGGTCTTGACATACTATGCAAATCTAAG  1055

Query  970   AGATTAGACGTTCCCTTCGGGGACATGGATACAGGTGGTGCATGGTTGTCGTCAGCTCGT  1029
ASN3   1056  AGATTAGACGTTCCCTTCGGGGACATGGATACAGGTGGTGCATGGTTGTCGTCAGCTCGT  1115

Query  1030  GTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTATTATCAGTTGCCAGCA  1089
ASN3   1116  GTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTATTATCAGTTGCCAGCA  1175

Query  1090  TTAAGTTGGGCACTCTGGTTGAGACTGCCGGGTGACAAACCGGAAGGAAGGTGGGGGATG  1149
ASN3   1176  TTAAGTTGGGCACTCTGGT-GAGACTGCC-GGTGACAAACCGGA-GGAAGGT-GGGGATG  1231
Query  1150  ACGTCAAATCATCATGGCCCCTTATGACCTGGGGCTAC-CACCGTGGCTACAATGGAATG  1208
ASN3   1232  ACGTCAAATCATCAT-GCCCCTTATGACCTGGG-CTACACAC-GTG-CTACAATGG-ATG  1286

Query  1209  GTACAACGAGTTGCGAACTCCCCAGAGTAAGCTAATCTCTTAAAGCCATTCCCAGTTCGG  1268
ASN3   1287  GTACAACGAGTTGCGAACTCGCGAGAGTAAGCTAATCTCTTAAAGCCATTCTCAGTTCGG  1346

Query  1269  ATTGGAGGCTGGCACTCGCCCTAC-TGAAAATCGGAATCCCTTAGAAATCCCGGAATCAC  1327
ASN3   1347  ATTGTAGGCTGCAACTCGCC-TACATGAAG-TCGGAATCGC-TAGTAATCGCGGA-TCAG  1402

Query  1328  AATGCCCCGGGGGAAACC-TCCCGCCCCTTGT  1358
ASN3   1403  CATGCCGCGGTGAATACGTTCCCGGGCCTTGT  1434
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**Figure 1.** Alignment of DNA base sequences of the 16S rRNA code for ASN3 isolates

The results of sequencing analysis of ASN3 and ASN5 isolates showed that there was a type of suitability of DNA base sequence with *Lactobacillus fabifermentans*, the length of DNA strands of the two isolates were 1615. ASN3 isolates had similar DNA base strings with *L. fabifermentans* at a maximum of 2228 bits, while ASN5 isolates had a maximum of 2266 bits.

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Query 9     GGTTTTGATTGGTGCTTGCATCATGATTTACATTTGAGTGAGTGGCGAACTGGTGAGTAA  68
ASN5 92     GGTATTGATTGGTACTTGTATCATGATTTACATTTGAGTGAGTGGCGAACTGGTGAGTAA  151

Query 69    CACGTGGGAAACCTGCCCAGAAGCGGGGGATAACACCTGGAAACAGATGCTAATACCGCA  128
ASN5 152    CACGTGGGAAACTTGCCCAGAAGCGGGGGATAACACCTGGAAACAGATGCTAATACCGCA  211

Query 129   TAACAACTTGGACCGCATGGTCCGAGTTTGAAAGATGGCTTCGGCTATCACTTTTGGATG  188
ASN5 212    TAACAACTTGGACCGCATGGTCCGAGTTTGAAAGATGGTTTCGGCTATCACTTCTGGATA  271

Query 189   GTCCCGCGGCGTATTAGCTAGATGGTGGGGTAACGGCTCACCATGGCAATGATACGTAGC  248
ASN5 272    GTCCCGCGGCGCATTAGCTAGATGGTGAGGTAACGGCTCACCATGGCAATGATGCGTAGC  331

Query 249   CGACCTGAGAGGGTAATCGGCCACATTGGGACTGAGACACGGCCCAAACTCCTACGGGAG  308
ASN5 332    CGACCTGAGAGGGTAATCGGCCACATTGGGACTGAGACACGGCCCAAACTCCTACGGGAG  391

Query 309   GCAGCAGTAGGGAATCTTCCACAATGGACGAAAGTCTGATGGAGCAACGCCGCGTGAGTG  368
ASN5 392    GCAGCAGTAGGGAATCTTCCACAATGGACGAAAGTCTGATGGAGCAACGCCGCGTGAGTG  451

Query 369   AAGAAGGGTTTCGGCTCGTAAAACTCTGTTGTTAAAGAAGAACATATCTGAGAGTAACTG  428
ASN5 452    AAGAAGGGTTTCGGCTCGTAAAACTCTGTTGTTAAAGAAGAACATATCTGAGAGTAACTG  511

Query 429   TTCAGGTATTGACGGTATTTAACCAGAAAGCCACGGCTAACTACGTGCCAGCAGCCGCGG  488
ASN5 512    TTCAGGTATTGACGGTATTTAACCAGAAAGCCACGGCTAACTACGTGCCAGCAGCCGCGG  571

Query 489   TAATACGTAGGTGGCAAGCGTTGTCCGGATTTATTGGGCGTAAAGCGAGCGCAGGCGGTT  548
ASN5 572    TAATACGTAGGTGGCAAGCGTTGTCCGGATTTATTGGGCGTAAAGCGAGCGCAGGCGGTT  631

Query 549   TTTTAAGTCTGATGTGAAAGCCTTCGGCTCAACCGAAGAAGTGCATCGGAAACTGGGAAA  608
ASN5 632    TTTTAAGTCTGATGTGAAAGCCTTCGGCTCAACCGAAGAAGTGCATCGGAAACTGGGAAA  691

Query 609   CTTGAGTGCAGAAGAGGACAGTGGAACTCCATGTGTAGCGGTGAAATGCGTAGATATATG  668
ASN5 692    CTTGAGTGCAGAAGAGGACAGTGGAACTCCATGTGTAGCGGTGAAATGCGTAGATATATG  751

Query 669   GAAGAACACCAGTGGCGAAGGCGGCTGTCTGGTCTGTAACTGACGCTGAGGCTCGAAAGT  728
ASN5 752    GAAGAACACCAGTGGCGAAGGCGGCTGTCTGGTCTGTAACTGACGCTGAGGCTCGAAAGT  811

Query 729   ATGGGTAGCAAACAGGATTAGATACCCTGGTAGTCCATACCGTAAACGATGAATGCTAAG  788
ASN5 812    ATGGGTAGCAAACAGGATTAGATACCCTGGTAGTCCATACCGTAAACGATGAATGCTAAG  871

Query 789   TGTTGGAGGGTTTCCGCCCTTCAGTGCTGCAGCTAACGCATTAAGCATTCCGCCTGGGGA  848
ASN5 872    TGTTGGAGGGTTTCCGCCCTTCAGTGCTGCAGCTAACGCATTAAGCATTCCGCCTGGGGA  931

Query 849   GTACGGCCGCAAGGCTGAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGTGGAGCA  908
ASN5 932    GTACGACCGCAAGGTTGAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGTGGAGCA  991

Query 909   TGTGGTTTAATTCGAAGCTACGCGAAGAACCTTACCAGGTCTTGACATACTATGCAAATC  968
ASN5 992    TGTGGTTTAATTCGAAGCTACGCGAAGAACCTTACCAGGTCTTGACATACTATGCAAATC  1051

Query 969   TAAGAGATTAGACGTTCCCTTCGGGGACATGGATACAGGTGGTGCATGGTTGTCGTCAGC  1028
ASN5 1052   TAAGAGATTAGACGTTCCCTTCGGGGACATGGATACAGGTGGTGCATGGTTGTCGTCAGC  1111

Query 1029  TCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTATTATCAGTTGCC  1088
ASN5 1112   TCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTATTATCAGTTGCC  1171

Query 1089  AGCATTAAGTTGGGCACTCTGGTGAGACTGCCGGTGACAAACCGAAGGAAGGTGGGGATG  1148
ASN5 1172   AGCATTAAGTTGGGCACTCTGGTGAGACTGCCGGTGACAAACCGGAGGAAGGTGGGGATG  1231

Query 1149 ACGTCAAATCATCATGCCCCTTATGACCTGGGCTACCCACGTGCTACAATGGATGGTACA  1208
ASN5 1232   ACGTCAAATCATCATGCCCCTTATGACCTGGGCTACACACGTGCTACAATGGATGGTACA  1291

Query 1209  ACAAGTTGCGAATTCCCGAGAATAAGCTAATCCCTTAAAGCCATTCCCAGTTCGGATTGG  1268
ASN5 1292   ACGAGTTGCGAACTCGCGAGAGTAAGCTAATCTCTTAAAGCCATTCTCAGTTCGGATTGT  1351

Query 1269  AGGCTGCAACTCCCCTAACTGAAAGTCGGAATCCCTAGTAATCCCGGAT  1317
ASN5 1352   AGGCTGCAACTCGCCTACATG-AAGTCGGAATCGCTAGTAATCGCGGAT  1399
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**Figure 2** . Alignment of DNA base sequences of the 16S rRNA code for ASN5isolates

The results of identification of 16S rRNA DNA sequence analysis using the BLASTN 2.5.1 + program are presented in Table 2. Identification of DNA strand sequences showed ASN3 isolates had

similar levels to *L. fabifermentans* DSM 21115 NODE_181, whole genome shotgun sequence, which was 97%, while ASN5 isolates had similarity level of 98%.

**Table 2.** Results of 16S rRNA DNA sequence analysis using the BLASTN 2.5.1+ program

| Isolate | Description (Homolog) | Maximum score | Total Score | *Query Cover* | value E | Identification | Access Code |
|---|---|---|---|---|---|---|---|
| ASN3 | *L. fabifermentans*DSM 21115 NODE_181, whole genome shotgun sequence | 2228 | 2228 | 96% | 0.0 | 97% | NZ_AYGX02000121.1 |
| ASN5 | *L. fabifermentans*DSM 21115 NODE_181, whole genome shotgun sequence | 2266 | 2266 | 94% | 0.0 | 98% | NZ_AYGX02000121.1 |

*L. fabifermentans is* included in *Lactobacillus* . *Lactobacillus* is the most diverse genus among LAB, *Lactobacillus is a* group of Gram positive bacteria that produce lactic acid as the main end product of fermentation, this group of bacteria is often involved in food fermentation. Several studies have reported the involvement of LAB in spontaneous fermentation of corn. LAB involved during spontaneous fermentation of grits, including *L. plantarum* , *Pediococcuspentosaceus* , *L. brevis* , and *L. paracaseisspparacasei* [13]. Other types of LAB involved during spontaneous fermentation of corn have also been reported, including *L. cellobiosus, L. pentosus, Leuconostocmesenteroides* [14] and *L. fermentum* and *P.acidilactici* [15].

Unlike previous studies, LAB which was successfully isolated during BISI-16 corn flour fermentation was *L. fabifermentans. L. fabifermentans* is one of the bacteria that has been proposed as a new species. Initially, *L fabifermentans*was associated with spontaneous fermented cocoa beans. In addition, this bacterium was also isolated from Marc grapes collected after a prolonged storage period to allow spontaneous alcohol fermentation in northeastern Italy [16].

*L. fabifermentans*is included as a species of *L. plantarum* group, this group is very homogeneous in terms of metabolic features, this group is facultative heterofermentative, with GC content, which ranges between 44 and 47 mol% [17]. The *L. plantarum* group consists of 5 species, of which two species are *L. fabifermentans* and *L. xiangfangensis* .

*L. fabifermentans*is involved in spontaneous fermentation of corn because this type of LAB is capable of producing carbohydrate-breaking enzymes , especially glycoside-binding enzymes which are the main bonds in carbohydrates. *L. fabifermentans* is one of the LAB capable of producing glycoside hydrolase (GH) enzymes [18].

General characteristics of *L. fabifermentans*have been reported by [19]. *L. fabifermentans* is Gram positive, catalase negative, facultative and non motile anaerobe. *L. fabifermentans* cells are long rod shaped (1.0–3.0 mm wide and 10.0 mm long) that appear singly, in pairs or in short chains. The grayish colonies are white (creamy), opaque, smooth and round with convex elevation and the entire margin (diameter of about 1.0 mm). Cell growth can be observed at temperatures from 10 ° C (from 8th day of incubation) to 37 ° C (direct growth from day 1 of incubation). The ratio of production of D- and L-lactic acid isomers is 80: 20. *L. fabifermentans* does not produce gas and dehydrated arginine. Acids are produced from L-arabinose, ribose, D-xylose, galactose, glucose, fructose, mannose, mannitol, N-acetylglucosamine, amygdalin, arbutin, aesculin, salicin, selobiose, maltose, sucrose, trehalose and gentiobiose. Acid is not produced from glycerol, erythritol, D-arabinose, L-

xylose, adonitol, methyl βD-xylopyranoside, sorbose, rhamnose, dulcitol, inositol, sorbitol, methyl αD-mannopyranoside, methyl αD-glucopyranoside, lactose, melibiose, inulin, melezitose , raffinose, starch, glycogen, xylitol, turanose, D-lyxose, D-tagatose, D- or L-fucose, D- or L-arabitol, gluconate or 2- or 5-ketogluconate.

## 4. Conclusions

The spontaneous fermentation of BISI-16 corn flour involved LAB, namely ASN3 and ASN5 isolates, with characteristic creamy colonies, rod-shaped cells, Gram (+), catalase (-), and endospores (-). Genotypic properties based on sequencing analysis of 16S rRNA DNA strands showed that both isolates had homologous similarities with *Lactobacillus fabifermentans DSM 21115 NODE_181* ie 97% (ASN3) and 98% (ASN5).

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