Data in Brief

Data on the isolation and identification of thermotolerant microorganisms from cow manure promising for organic waste processing

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ABSTRACT

Thermotolerant microorganisms play an essential role in the composting process of organic waste; they are responsible for the degradation process of organic compounds owing to their enzymatic activity in the high-temperature phase of composting. This article presents data on the isolation and identification of thermotolerant microorganisms from cattle manure. In total, three bacterial strains with the ability to grow at 50°C were isolated on different media. The cultural and morphological characteristics of the strains are presented. By analysis of the 16S rRNA gene sequence, the isolates were assigned to the species Bacillus coagulans and Bacillus licheniformis. Isolated strains were characterized for their enzymatic potential (lipolytic, amylolytic, and proteolytic). These results can be promising for further studies in order to design biological additive for accelerated composting of organic waste.

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Value of the data

- The market of Kazakhstan is missing domestic microbial additives based on effective microbial strains for accelerated composting of organic wastes. The value of this data is on the isolation and identification of the beneficial thermotolerant bacteria from cow manure composting.
- The data will be valuable for further studies on designing the microbial additive for accelerated organic waste composting.
- The data may contribute to the emergence of demand for domestic microbial additives for accelerated waste processing in Kazakhstan.
- The data could be useful for researchers who are engaged in isolation, identification of thermotolerant bacteria, and studying their enzymatic activity.

1. Data

Inadequate organic waste management leads to a plethora of problems such as environmental pollution, eutrophication, esthetic damage to urban landscape, greenhouse gases emission and effects on human health. Unwise and non-scientific disposal of wastes not only poses a grave threat to environmental quality but also results in loss of economic value of wastes. Inadequate organic waste management leads to a plethora of problems such as environmental pollution, eutrophication, esthetic damage to urban landscape, greenhouse gases emission and effects on human health. Unwise and non-scientific disposal of wastes not only poses a grave threat to environmental quality but also results in loss of economic value of wastes. Composting of organic waste is the process of conversion of solid waste materials into a stable product, free of pathogens and plant seeds that can be beneficially applied to land [3]. Thermotolerant microorganisms with the ability for the production of enzymes responsible for the degradation of organic compounds, play an essential role in accelerating the composting rate of organic waste. The dataset of this article provides information on the characterization of thermotolerant bacteria isolated from cow manure promising for composting organic waste. Three pure cultures of thermotolerant bacteria were obtained (Table 1). The cultural and morpholog-
Table 1
Isolates from cow manure, capable to grow at 50°C.

| Culturing temperature, OC | Growth media | Nutrient agar | Saburo | MRS-1 |
|--------------------------|--------------|---------------|--------|-------|
| 50                       | P1-6         | no single colonies | P1-5  |       |
|                          | P1-7         |               | P1-6  |       |

Table 2
Characteristics of thermotolerant isolates.

| Characteristics | P1-5         | P1-6         | P1-7         |
|-----------------|--------------|--------------|--------------|
| Size, mm        | 2-2.5        | 2-5          | 2-7          |
| Shape           | circular     | irregular    | irregular    |
| Surface         | smooth, shiny| rough, dull  | rough, shiny |
| Elevation       | convex       | flat         | umbonate     |
| Edge (Margin):  | entire       | undulate     | undulate     |
| Opacity         | opaque       | opaque       | opaque       |
| Colour of colony| creamy       | creamy       | creamy       |
| Consistency     | friable      | friable      | mucoid       |

Fig. 1. Photographs of thermotolerant bacteria isolated from cow manure: 1A. Bacillus coagulans P1-5 colony on MRS agar; 1B. Gram stain of Bacillus coagulans P1-5; 2A. Bacillus licheniformis P1-6 colony on Nutrient agar; 2B. Gram stain of Bacillus licheniformis P1-6; 3A. Bacillus licheniformis P1-7 colony on Nutrient agar; 3B. Gram stain of Bacillus licheniformis P1-7.

Fig. 1. Photographs of thermotolerant bacteria isolated from cow manure: 1A. Bacillus coagulans P1-5 colony on MRS agar; 1B. Gram stain of Bacillus coagulans P1-5; 2A. Bacillus licheniformis P1-6 colony on Nutrient agar; 2B. Gram stain of Bacillus licheniformis P1-6; 3A. Bacillus licheniformis P1-7 colony on Nutrient agar; 3B. Gram stain of Bacillus licheniformis P1-7.

By molecular identification based on the analysis of 16S rRNA gene nucleotide sequences, the isolates were assigned to the genus Bacillus. Table 3 presents the nucleotide sequences of isolate’s 16S rRNA gene and their similarity to available nucleotide sequences deposited in the Gene Bank databases. The data of the enzymatic activity (lipase, amylase, and protease) of isolated strains are presented in Table 4.
Table 3
Genetic identification of thermotolerant strains.

| Isolate | Accession number in GenBank | Accession number to an identical sequence in GenBank | Species affiliation | Percent identity (%) |
|---------|----------------------------|-----------------------------------------------------|---------------------|----------------------|
| P1-5    | MT378217.1 [https://www.ncbi.nlm.nih.gov/nuccore/MT378217](https://www.ncbi.nlm.nih.gov/nuccore/MT378217) | LC140744.1 | Bacillus coagulans | 99                   |
| P1-6    | MT378218.1 [https://www.ncbi.nlm.nih.gov/nuccore/MT378218](https://www.ncbi.nlm.nih.gov/nuccore/MT378218) | KY623056.1 | Bacillus licheniformis | 99          |
| P1-7    | MT378219.1 [https://www.ncbi.nlm.nih.gov/nuccore/MT378219](https://www.ncbi.nlm.nih.gov/nuccore/MT378219) | MG650748.1 | Bacillus licheniformis | 99          |

Table 4
Enzymatic activity of thermotolerant bacteria, isolated from cow manure.

| Strains                     | Proteolytic activity | Amylolytic activity | Lipolytic activity |
|-----------------------------|----------------------|---------------------|--------------------|
| Bacillus coagulans P1-5     | -                    | +                   | -                  |
| Bacillus licheniformis P1-6 | +                    | +                   | -                  |
| Bacillus licheniformis P1-7 | +                    | +                   | -                  |

2. Experimental design, materials, and methods

2.1. Culture media

Following culture media were used for data collection: for isolation and studying cultural properties of isolates: (1) Nutrient broth (HiMedia Lab Pvt. (India)), (2) MRS Broth (HiMedia Lab Pvt., India), (3) Sabouraud Dextrose broth (Titan Biotech, LTD, India), (4) Nutrient agar (HiMedia Lab Pvt. (India)), (5) Sabouraud Dextrose Agar - (HiMedia Lab Pvt. (India)), (6) MRS Agar (HiMedia Lab Pvt. (India)). The enzymatic activity was tested on: (7) starch containing media - peptone (Titan Biotech, LTD, India)-10g/L, KH2PO4-5g/L, Starch- (JSC "Proxima", Russia), - 2g/L Agar (Titan Biotech, LTD, India)-15 g/L, pH6.8-7; (8) fat containing media - Tween 80 (Titan Biotech, LTD, India)-10 g/L, peptone (Titan Biotech, LTD, India) - 10 g/L, NaCl -5 g/L, CaCl2-0.1 g/L, Agar 20 g/L, pH7.4; (9) protein containing media: skimmed milk powder (Polotsk Dairy Plant, Belarus) - 87g/L, Nutrient agar– 30g/L.

2.2. Cattle manure

The cattle manure sample used to produce the data was collected from a private compound located in the North Kazakhstan region. For the isolation purpose of thermotolerant bacteria, the samples of fresh cow manure were taken immediately after emptying the cow by sterile spatula and transferred to the glass tubes containing 7 ml of sterilized media (Nutrient broth, MRS Broth, Sabouraud Dextrose broth). The seeded glass tubes were delivered to the laboratory.

2.3. Isolation of thermotolerant microorganisms

After 24 hours of inoculating at room temperature and shaking an orbital shaker incubator (150 rpm), 1 ml of culture broth from seeded tubes was transferred to the new tube, with 7 ml of respective liquid media, and incubated at 50°C. After 24 hours from liquid media were made seedings on solid plate agar (MRS agar, Nutrient Broth, and Sabouraud Dextrose Agar) and incubated at 50°C. (Table 1).
2.5. Cultural and morphological properties of isolates

Cultural characters of the isolates were studied by inoculating the strain into respective sterile solid media. A series of culture tubes containing 9 ml of sterile water was prepared. From the stock culture, 1 ml suspension was transferred aseptically to the 1st tube (10⁻¹) and mixed well. Further serial dilutions were made to produce 10⁻⁵ suspensions were made. Suspension (0.1 ml) from each culture tube was spread on sterile solid media aseptically in a laminar-air flow cabinet [4]. The plates were incubated at 50 ± 2°C for 24 h and colony characteristics were observed [5].

2.5.1. Microscopical characterization

Gram staining method. A smear of the selected strains was prepared on a clean glass slide. The smear was allowed to air-dry and then fixed by heating. The fixed smear was flooded with crystal violet and was washed with water and flooded with mordant Gram’s iodine. The smear was decolorized with 95 % ethanol, washed with water, and then counterstained with safranin for 45 s. The glass slide was washed with water and dried with tissue paper and examined under oil immersion (100x) [4].

2.6. Molecular identification of bacterial isolates

Genomic DNA, of isolates extracted from an overnight pure bacterial culture grown in Nutrient broth at 50°C was isolated using the standard protocol of K. Wilson [6]. DNA concentration was determined spectrophotometrically using a NanoDrop1000 spectrophotometer. The PCR reaction was performed with universal primers: 8f (5′-agagtttgatccttgctcag-3’) and 806R (5′-ggactacgggtataaat-3’) in a total volume of 20 μl. All PCR amplicons were confirmed by electrophoresis, purified, and sequenced. Purification of PCR products was carried out by the enzymatic method using Exonuclease I (Fermentas) and alkaline phosphatase (Shrimp Alkaline Phosphatase, Fermentas). The sequencing reaction was carried out using the BigDye® Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems) according to the manufacturer’s instructions, followed by separation of fragments on a 3730xl DNA Analyzer (Applied Biosystems) automated genetic analyzer. The nucleotide sequences obtained using direct and reverse primers were analyzed and combined into a common sequence using SeqScape 2.6.0 software (Applied Biosystems). The obtained nucleotide sequences of the 16S rRNA gene were identified relative to the available nucleotide sequences deposited in the GenBank databases (http://blast.ncbi.nlm.nih.gov) using the BLAST algorithm [7].

2.7. Enzymatic activity of isolates

The isolated strains were tested for enzymatic activity by inoculating into starch-, fat-, protein- containing media for 24 hours at 50°C. Iodine was added to starch medium to determine amylolytic activity of strains. The presence of "clearance zones" surrounding the colonies was taken to indicate enzymatic activity of strains, responsible for the hydrolysis of casein, starch or fat particles.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships, which have, or could be perceived to have, influenced the work reported in this article.
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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.dib.2020.105761.

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