ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Isolation and Identification of Bacillus Species from Intestinal Tract of Livestock and Other Environmental Samples of Animal Farms of East Kazakhstan Region.

Abdrakhman Baygazanov, Artem Finogenov, Maral Nurkenova, Tatyana Bleim, Aleksandra Tleubayeva, Yesengeldy Omarbekov, Nurgul Ikimbayeva, Dinara Zeynettinova, Sandeep Sharma, and Zhanibek Yessimbekov*.

Shakarim State University of Semey, Glinki street, 20A, Semey 071412, Kazakhstan.

ABSTRACT

This study presents the results of the isolation and identification of Bacillus bacteria from the ampullaceous rectum of healthy animals and their excrement and environmental samples (soil, hay, haylage, and straw). Bacterial colonies were isolated and characterized by morphological (Gram's staining), cultural-morphological, staining, biochemical, and physical properties. By the results of bacterial identification, Bacillus was presented with *B. licheniformes, B. subtilis, and B. cereus.*

Keywords: bacteria, Bacillus, isolation, intestinal tract, soil, hay

Corresponding author

ISSN: 0975-8585

INTRODUCTION

Bacillus is one of the most diverse and widely distributed groups of bacteria and main components of the exogenous flora of human being and animal [1]. Moreover, these bacteria are the producer of enzymes, antibiotics, and insecticides. The family of *Bacillus* has been used for decades because of its high capability of producing many extracellular enzymes, such as xylanase, amylase, protease, pullulanase, chitinase, lipase, among others. These enzymes are produced commercially, and their production represents approximately 60% of the global market demand [2]. High adaptability and survivability against the different environmental conditions are a contributing cause of Bacillus dissemination in the soil, water, air, food, human and animal body, and et.al. Also, Bacillus strains are used in the production of alkaline proteases [3].

Bacillus activity is particularly articulated in relation to pathogenic and potentially pathogenic microorganisms [4]. Alongside with the familiar bacteria, mainly the representatives of the normal microflora of intestinal tract, *Bacillus* species are of great interest to researchers in the last decades. This type of bacteria is known as the bacteria of the transient microflora of intestinal tract [5].

B. subtilis and *B. licheniformis* are not part of the normal microflora of human's and animal's bacterial community, but play an important role in the function of natural microbiocenosis; metabolism improvement; delivering the organism with the biologically active and essential building materials; improving digestion naturally [6]. Bacteria lives in GI not more than one month and removes naturally. These bacteria are not killed in the stomach due to the high resistance to the stomach acid.

B. subtilis is Generally Regarded As Safe (GRAS) organism by the Food and Drug Administration. It is a non-pathogenic gram positive, rod-shaped, and endospore-forming aerobic bacterium, which is found in soil and rotting plant material [7, 8].

Bacillus species are a major component of the microbial flora, which live in close association with various types of agricultural crops [9]. The purpose of this study was to isolate and identify the bacterial colony from the ampullaceous rectum of healthy animals and their excrement and also from the soil, hay, haylage, and straw.

MATERIALS AND METHODS

Sampling

Bacillus species isolated from the ampullaceous rectum of healthy animals and their excrement and also from the hay, haylage, and straw, collected from four animal farms, such as "Lazarev," "Zaitenov," "Ornek," "Sakhnovskoe," located in East Kazakhstan region of the Republic of Kazakhstan.

Isolation method

Bacillus species isolated in meat-peptone agar (MPA). Further cultivation was performed in MPA, meat-peptone broth (MPB), Hottinger's agar, Hottinger's digest, and Bacilluscereus Agar Base (Holbrook и Anderson).

The capability of bacterial growth at +50 °C was analyzed by cultivation in MPB with the addition of 7% NaCl within 24h.

For determination of Bacillus cereus bacteria growth, the agar developed by Holbrook and Anderson was used. This agar allows growing the cells and spores, even with the presence of a vast quantity of other microbes, which contaminates the sample.

The flotation technique is used for studying the microorganism concentration. The sample was pounded with a pestle in physiological solution until creamy consistency. Then, 10 sm^3 of the sample was placed into the conical vessel (250 sm^3), and additionally, 10 sm^3 of 1% sodium hydroxide was poured and mixed for 10 min. After that, water (at the ratio mixture:water 1:9) and 1-2 sm^3 of dimethylbenzene were

added and mixed for 5-10 min. Then, the mixture was stored for 30 min at room temperature. Obtained flotation rings of bacteria seeded and strokes used for Gram's stain

For bacteria identification, the culture medium is incubated at different temperature modes: 30-32 ^{0}C, 35–37 ^{0}C, 42 ^{0}C, and 50 ^{0}C.

Further analyses were conducted with pure cultures isolated bacteria.

For identification of isolated *Bacillus,* the saccharolytic activity is determined by usingHesse agar withAndrade's Indicatormethod and MikroLaTest (PLIVA-LachemaDiagnostika) diagnostic set.

RESULTS AND DISCUSSION

The results of bacteria isolation from the animal's rumen, soil, and fodders were presented in Table 1.

Table 1: Bacteria isolation from the animal rumen, soil and fodders

Animal farm	Number of samples	Number of isolated culture	Including *Bacillus*
"Lazarev"	80	57	12 (21,0 %)
"Zaitenov"	100	61	18 (29,5 %)
"Ornek"	150	122	50 (40,9 %)
"Sakhnovskoe"	60	10	4 (40,0 %)

From the given table, the overall percentage of isolated *Bacillus* species is 40.9% in the samples from Ornek animal farm. Special mention can be made to the key role of *Bacillus* in the formation of soil's humus, and is intended to be used for normal microflora of the digestive tract of ruminant animals. About 60% of the total *Bacillus* species are isolated from the fodder (hay, straw, and haylage).

After the identification, 4 strains of *Bacillus* bacteria with strongly expressed activity were selected and numbered as strain 1, 2, 3 and 4.

Strain 1 gets muddy and forms a flocculent precipitate with a thin film in meat-peptone broth. Strain 1 forms gray-white, opaque, rough colony (2-3 mm) on meat-and-peptone agar (Fig. 1).

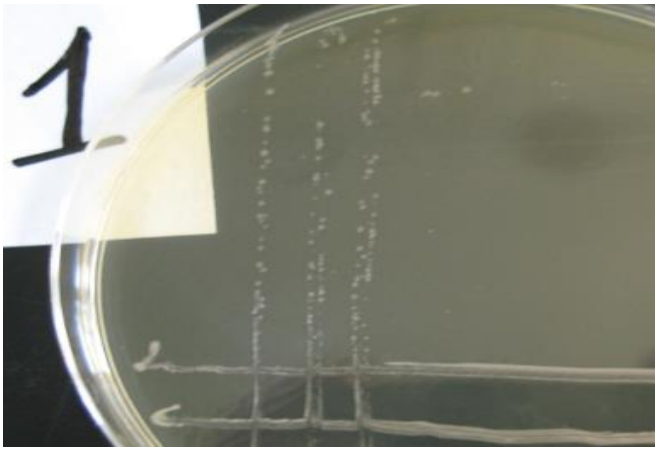

Fig. 1: Daily Bacillus species culture on MPA, strain 1.

Agar stroke microscopic evaluation (Gram's Staining Method) shows the thin Bacillus located in chains or single positions (fig. 2). The microscopic evaluation of Bacillus by Peshkov's Staining Method shows the oval spores, off-centered (Fig. 3).

ISSN: 0975-8585

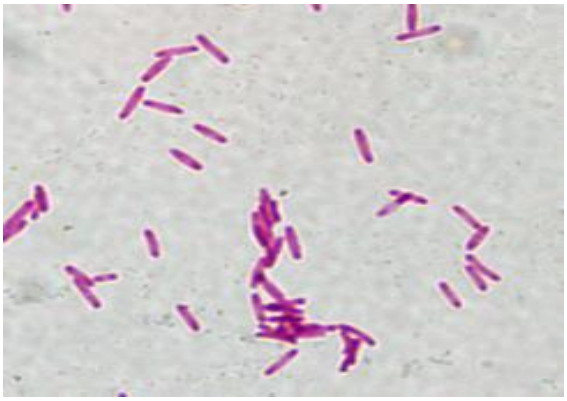

Fig. 2. Gram's staining of daily *Bacillus* species culture, strain 1 (10x100x1,5)

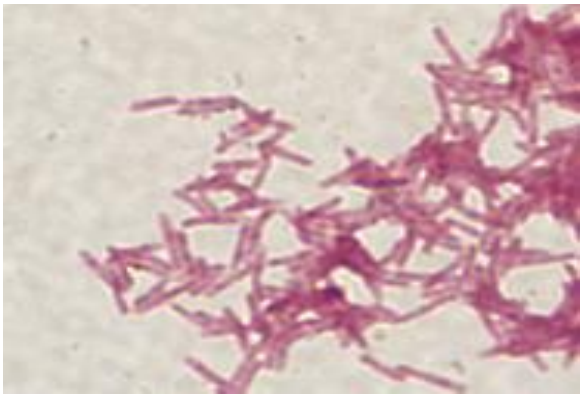

Fig. 3. Peshkov's staining of daily *Bacillus* bacteria species, strain 1 (10x100x1,5)

Strains 2 and 5 get muddy and form a flocculent precipitate with a thin film in meat-peptone broth. Strains form gray-white, opaque, rough colony with ridges of 2-4 and 8-10 mm on meat-and-peptone agar (Fig. 4, 5).

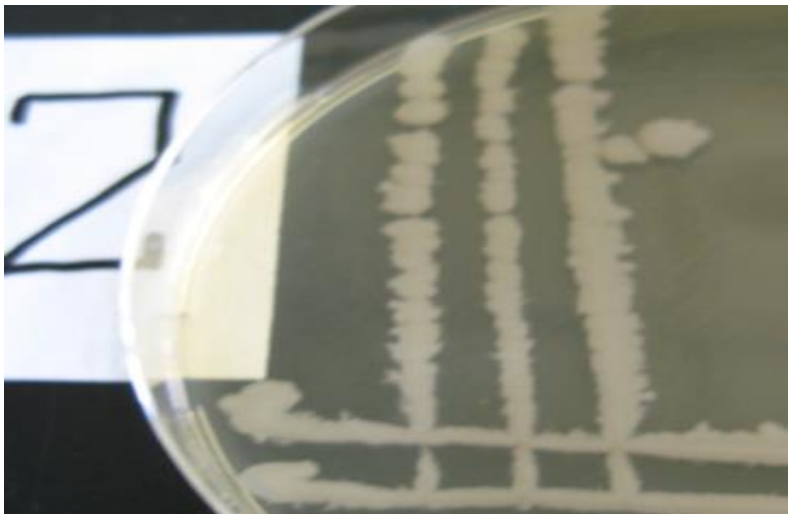

Fig. 4. Daily Bacillus species culture on MPA, strain 2.

Fig.5. Daily Bacillus species culture on MPA, strain 5.

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Agar stroke microscopic evaluation (Gram stain procedure) shows Gram-positive, large rods located in a series of chains (Fig. 6, 8). The microscopic evaluation of Bacillus bacteria by Peshkov Method showed the oval spores without thickening of the rods and located at or near the center of the cell (Fig. 7, 9).

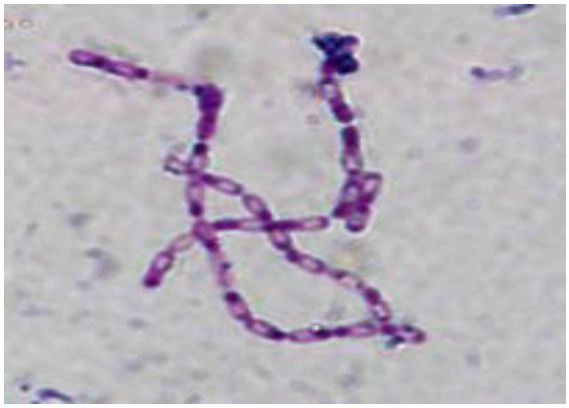

Fig. 6. Gram's staining of daily *Bacillus* species culture, strain 2 (10x100x1,5)

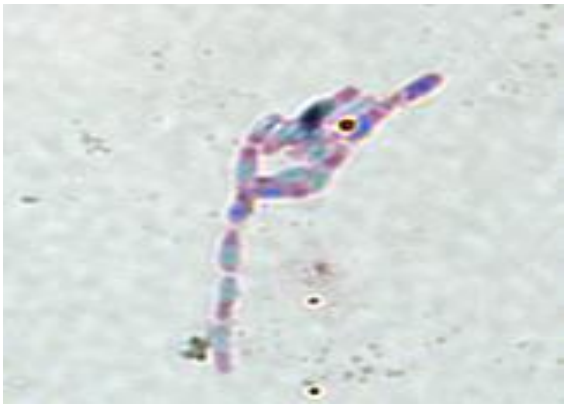

Fig. 7. Peshkov's staining of daily *Bacillus* species culture, strain 2 (10x100x1,5)

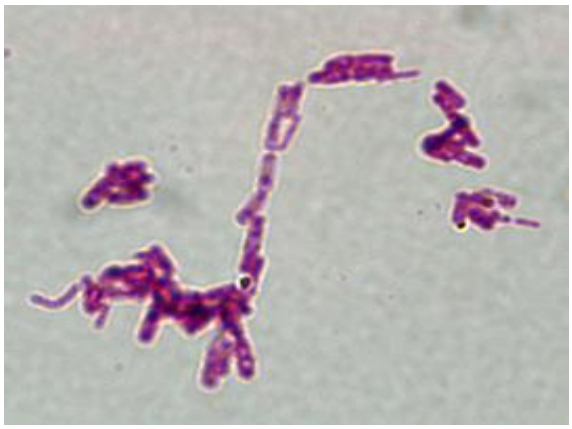

Fig. 8. Gram's staining of daily *Bacillus* species culture, strain 5 (10x100x1,5)

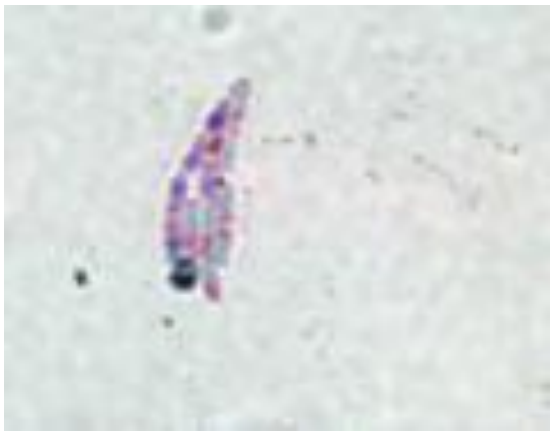

Fig. 9. Peshkov's staining of daily *Bacillus* species culture, strain 5 (10x100x1,5)

Strain 4 gets muddy and forms a flocculent precipitate with a thin film in meat-peptone broth. The addition of starch changes the color of the colony to yellow-brown.

Strains form gray-white, opaque, rough colony with ridges of 2-4 and 8-10 mm on meat-and-peptone agar (Fig. 10).

ISSN: 0975-8585

Fig. 10

Agar stroke microscopic evaluation (Gram stain procedure) shows Gram-positive, large rods located in a series of chains (Fig. 11).

The microscopic evaluation of *Bacillus* bacteria by Peshkov Method showed the oval spores without thickening of the rods and located at or near the center of the cell (Fig. 12).

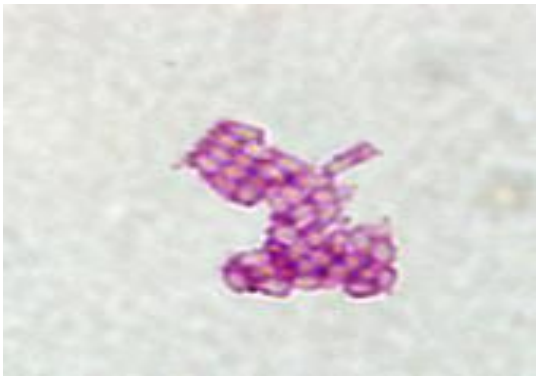

Fig. 11. Gram's staining of daily *Bacillus* species culture, strain 4 (10x100x1,5)

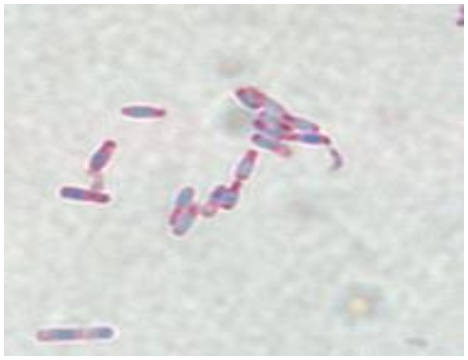

Fig. 12. Peshkov's staining of daily *Bacillus* species culture, strain 4 (10x100x1,5)

Biochemical properties of strain were presented in Table 2.

Table 2. Biochemical properties of strain 1, 2, 3, 4 of *Bacillus* species

Test	Strain			
	№1	№2	№4	№5
Indole	+	+	+	+
Hydrogen sulfide	-	-	+	-
Lysine	+	+	+	+
Ornithine	+	+	+	+
Urease	-	-	+	-
Arginine	+	-	-	-
Simmons Citrate	-	-	+	-
Malonate	-	-	+	-

Phenylalanine	-	-	-	-
Beta-galactosidase	+	+	+	+
Inositol	-	-	+	-
Adonitol	-	-	+	-
Cellobiose	-	-	+	-
Sucrose	-	+	+	+
Trehalose	+	+	+	+
Mannitol	+	+	+	+
Acetoin	-	-	-	-
Esculin	+	-	+	+
Sorbitol	+	+	+	+
Rhamnose	+	+	+	+
Melibiose	+	+	+	+
Raffinose	+	+	+	+
Dulcitol	-	-	+	-
Glucose	+	+	+	+

For determining the identity of bacterial strains, Bergey's manual of Systematic Bacteriology was used (Table 3).

Based on the conducted tests (cultural-morphological, staining, biochemical, and physical properties), the strain 1 identified as *B. licheniformes*, strains 2 and 5 - *B. subtilis*, strain 4 - *B. cereus*.

It is determined that strain 1 showed good growth on meat-peptone broth with the addition of 7% NaCl at the temperature of 50 °C; digests glucose, sorbit and starch; Voges-Proskauer reaction was negative; catalase reaction was positive.

Strains 2 and 5 grew at the temperature of 45 °C; digests glucose, sucrose, sorbit and starch; catalase and the indole reaction was positive; Voges-Proskauer reaction was negative.

Strain 4 did not grow on meat-peptone broth with the addition of 7% NaCl at the temperature of 50 °C; there was no positive reaction to starch hydrolysis; formed small quantity of yellow-brown pigments; digests glucose, sucrose, sorbit and inositol; formed hydrogen sulphide and indole; Voges-Proskauer reaction was negative; Simmons citrate reaction was positive.

Table 3. Strain 1, 2, 4, 5 identification

Test	**Strain**				***Bacillus* bacterial species determined by Bergey's Manual**		
	№1	№2	№4	№5	*B. cereus*	*B. subtilis*	*B. licheniformes*
Glucose	+	+	+	+	+	+	+
catalase	+	+	+	+	+	+	+
Simmons Citrate	-	-	+	-	+	-	-
Sucrose	-	+	+	+	+	+	-
Hydrolysis of starch	+	+	-	+	-	+	+
Chromogenesis on starch medium	-	-	+	-	+	-	-
Inositol	-	-	+	-	-	-	-
Sorbitol	+	+	+	+	+	-	-
Hydrogen sulfide	-	-	+	-	+	+	-
Indole	+	+	+	+	+	+	+
Growth on MPB 7 % NaCl	+	-	-	-		+	-
Growth at 50 ºC	+	-	+	-		-	+

Growth at 45 ºC	+	+	+	+		+	+
Voges-Proskauer reaction (Acetoin VPT)	-	-	-	-	+	-	-
Growth after heating for 30 min on water bath	+	+	+	+		+	+
Strain identification	***B. licheniformes***	***B. subtilis***	***B. cereus***	***B. subtilis***			

CONCLUSIONS

Based on the conducted studies, it is found that the frequency of bacteria in the animal body and in the environmental samples was 21-40%. The most quantity of *Bacillus* species is isolated from the environmental samples. By the results of bacterial identification, Bacillus was presented with *B. licheniformes, B. subtilis*, and *B. cereus*.

REFERENCES

[1] Blinkova L.P., Semenov S.A., Butova L.G. Journal of Microbiology of Epidemiology and Immunology. 1994, 5:71-72. (In Russian).
[2] Ho H.L., Chinonso A.M. British Microbiology Research Journal. 2016, 14(1).
[3] Bai Y., D'Aoust F., Smith D.L., Driscoll B.T. Canadian Journal of Microbiology. 2002, 48(3): 230-238.
[4] Schallmey M., Singh A., Ward O.P. Canadian journal of microbiology. 2014, 50(1): 1-17.
[5] Van der Waaij D. Molecular Nutrition & Food Research. 1987, 31(5-6): 507-517.
[6] Wyrick P.B., Rogers H.J. Journal of bacteriology. 1973, 116(1): 456-465.
[7] Sneath P.H.A., Mair N.S., Sharpe M.E., Holt J.G. Bergey's manual of systematic bacteriology. 1st edn., Williams and Wilkins, Baltimore, USA. 1986.
[8] Edkie G.N., Theertha Prasad D. Research Journal of Microbiology. 2014, 9: 115-128.
[9] Marei E.M., Elmaghraby I. Current Research in Bacteriology. 2016, 9: 1-8.