



## Propagation of the Aujeszky's disease virus isolate in continuous cell lines

Svetlana A. Gryn\*, Evgenia V. Markova, Valentina I. Klyukina, Marina A. Frolova, Vera M. Popova, Larisa S. Lyulkova, Irina N. Matveeva, Yuriy N. Fedorov

All-Russian Scientific Research and Technological Institute of Biological Industry, Moscow region, 141142, Russia



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### ABSTRACT

The objective of this study was to investigate the cultural properties of Aujeszky's disease virus isolate and the optimization of the conditions of obtaining virus-containing material in the most prospective cell cultures. As a result, it was established that the study virus isolate is highly pathogenic for laboratory animals: rabbits, guinea pigs, and rats. The following continuous cell lines were sensitive to the virus isolate: MDBK, Taurus-1 and BHK-21. CPE (cytopathogenic effect of viruses) nature was similar in different cell cultures. The ability for propagation in cultures of continuous cell lines of the test virus isolates reached the maximum value to 4-6 passage, equaling to 6.85-7.65 lgTCD<sub>50</sub>/cm<sup>3</sup>. The obtained results show that the dynamics of the virus isolate dynamics had no significant differences, and following the adaptation, the virus isolate maintained the stable inherent propagation activity level throughout the study. As the study result, the most sensitive cell culture Taurus-1 was selected at passaging, with the highest virus accumulation observed, the infection activity was 7.55±0.12 lg TCD<sub>50</sub>/cm<sup>3</sup>. The presence of Aujeszky's disease virus was confirmed by the bioassay and the other laboratory methods, including PCR (polymerase chain reaction). The PCR method allowed defining the virus genome in the virus-containing cultural and organ and tissue material. PCR allowed amplifying the target gene fragment with the size of 194 base pairs (b.p.) in samples containing Aujeszky's disease virus DNA. No non-specific reactions were observed; PCR products did not exhibit the expected size.

### \*Corresponding Author

Name: Svetlana A. Gryn  
Phone: 89033146175  
Email: Smolentsev82@mail.ru

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### INTRODUCTION

Aujeszky's disease is an acute virus disease of domestic and some wild animals, including fur-

bearing animals, and rodents. It is characterized by fever, pneumonia, signs of lesion of cerebrospinal axis, which is exhibited by various nervous collapses (high fever, convulsions, palsies), strong itch and scratching in all animals, except for pigs, minks, and Russian sables (Brabander *et al.*, 2009; Ilyasovich *et al.*, 2016).

Aujeszky's disease is spread almost in all countries of the world and afflicts large economic damage to cattle breeding in developed countries (Viñas *et al.*, 2004). The infecting agent is a virus belonging to the Herpesviridae family, Alphaherpesvirinae subfamily. Pigs are the virus natural hosts, and remain its and remain its life-long carriers, and the latent agent reactivation is possible at any moment (Cristina *et al.*, 2012; Zai *et al.*, 2013; Singh *et al.*, 2015).

Continuous cell lines are promising for virus cultivation because they provide the obtaining of large volumes of virus-containing material that is used in studies of biological, molecular and genetic virus properties, and is also used as a laboratory model for investigating the virus evolution, developing means of diagnostics and specific prevention (Udalova *et al.*, 2015). The success of developing a vaccine and diagnostic drugs, studies of biological, molecular and genetic properties of the virus isolates highly depends on the successful selection of the cultivation system. Therefore, it is initially required to define the sensitivity of cell cultures to the isolated virus isolate (Singh *et al.*, 2015).

The objective of this study was to investigate the cultural properties of Aujeszky's disease virus isolate and the optimization of the conditions of obtaining virus-containing material in the most prospective cell cultures.

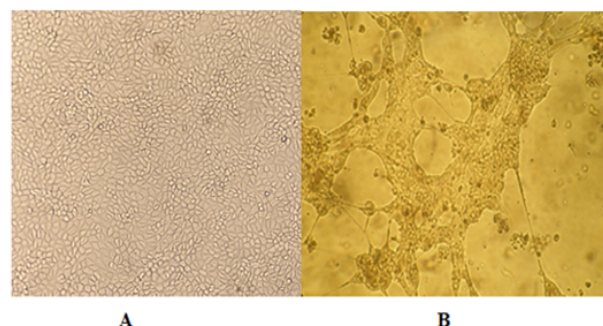
## MATERIALS AND METHODS

Aujeszky's disease virus isolate that was isolated in Moscow from the cat was used in the study. Continuous cell lines of the calf kidney were used in the study: MDBK and Taurus-1, and the continuous monolayer-suspension subline of the baby golden hamster kidney cell BHK-21. Eagle's MEM (Minimum Essential Medium) media of domestic and foreign production were used as cell growth and cell maintenance media. The 10% suspension in the Eagle's medium (MEM; Sigma, USA, HyClone, USA) with the addition of antibiotics (penicillin and streptomycin at the dose of 200-1000 IU/ml, nystatin 20 IU/ml) was prepared from organs of the cat that died from Aujeszky's disease (liver, lungs, lymph nodes, spleen).

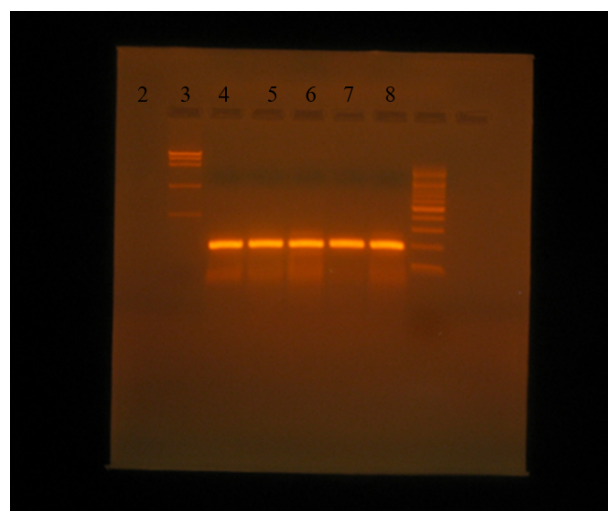
After the centrifugation clarification at 2000 rpm, the suspension was introduced into the culture flasks with the formed cell monolayer. 1 hour after the adsorption, the suspension was removed, and the cell maintenance medium, containing 2% bovine cattle serum, was introduced, and was incubated until the typical signs of the virus cytopathogenic effect (CPE) appeared (Carrasco-Pancorbo *et al.*, 2008; Mahmoudi *et al.*, 2014; Khristoforovich *et al.*, 2016). The status of the cell monolayer for determining the virus CPE was evaluated during the view of the culture flasks under the Olympus CKX31 inverted microscope (Olympus Co., Japan). The virus infection activity was defined by titering in 1-2-day cultures of the continuous cell lines incubated in 96-well microtitration plates. Virus titre was calculated according to Rid and Mench and was expressed in  $\lg \text{TCD}_{50}/\text{cm}^3$ . Nucleic acids were iso-

lated with the use of the RIBO-sorb kit (InterLab-Service LLC, Russia). The material for establishing PCR was represented by samples of cultural virus-containing fluid, organs of a rabbit, infected with the filed Aujeszky's disease virus isolate, of a lamb brain and a corpse of a cat that died from the strain of the pseudorabies virus, circulating at the territory of Moscow city. The specific primers were constructed with the use of Primer Select software (DNASTAR). The glycoprotein D gene coding sequence was selected as a genetic marker. The primary evaluation of the specificity of primers and amplicons was carried out with the use of BLAST (<http://blast.ncbi.nlm.nih.gov>). Primer synthesis was carried out in Litekh Research and Production Company. The PCR results were included by analyzing the amplification products through 1-2% agarose gel electrophoresis.

## RESULTS AND DISCUSSION



**Figure 1: Virus cytopathic action in the continuous cell line: A— control cell culture, B—cell culture on 3 day after contagion (X40 zoom, Olympus CKX31 microscope, Olympus Co., Japan)**



**Figure 2: Determining the Aujeszky's disease virus DNA by PCR lines**

**Table 1: Accumulation of the virus isolate in various continuous cell lines, (n=4)**

Cell culture	Passage	Cultivation duration, hours	Virus titre, lgTCD <sub>50</sub> /cm <sup>3</sup>
MDBK	4-6	48-72	6.81-7.06
		48	7.05-7.11
		48	6.90-7.10
Taurus-1	4-6	48-72	7.21-7.43
		48	7.55-7.62
		48	7.45-7.55
BHK-21	4-6	48-72	7.22-7.33
		48	7.14-7.25
		48	7.23-7.31

**Table 2: Accumulation of the Aujeszky's disease virus isolate in the MDBK cell line, depending on the infecting dose and cultivation terms, (n=3)**

Multiplicity of TCD <sub>50</sub> /ml infection	Biological activity in lg TCD <sub>50</sub> /cm <sup>3</sup> indifferent cultivation terms, hour					
	12	24	36	48	60	72
0.0001	2.30±0.06	3.30±0.21	4.4±0.07	4.7±0.19	4.1±0.08	3.56±0.12
0.001	3.20±0.21	3.6±0.12	4.00±0.09	4.83±0.14	4.5±0.08	4.2±0.15
0.01	4.10±0.07	5.7±0.09	6.75±0.19	7.00±0.13	6.83±0.03	6.55±0.20
0.1	5.20±0.14	5,76,20	6.5±0.18	6.84±0.02	7.05±0.09	6.84±0.15
1.0	5.6±0.03	6,30,15	7.00±0.12	6.75±0.14	6.5±0.12	6.20±0.14

**Table 3: Accumulation of the virus isolate in Taurus-1 cell culture with different native bovine cattle serum concentration (n=3)**

Bovine cattle serum concentration in the cell maintenance medium, %	Term of the virus CPE appearance in the cell culture, hours	Virus cultivation period, hours	Virus infection titre, lg TCD <sub>50</sub> /cm <sup>3</sup>
1.0	28	48-72	6.3±0.13
2.0	28	48-60	7.6±0.19
5.0	28	48-60	7.5±0.21

CPE nature was similar in different cell cultures (Figure 1). In the process of preliminary studies of virus material of the Aujeszky's disease virus isolate in monolayer continuous cell lines MDBK, BHK-21 or Taurus-1, it was established that the virus reached maximal titres by 4-6 passages. The virus infectivity accumulation value was evaluated under the 70-90% cytopathic effect occurrence. The propagation capability of the test virus isolate reached the maximal value by 4-6 passages and was 6.85-7.65 lgTCD<sub>50</sub>/cm<sup>3</sup>.

And further passaging did not exceed the initial activity. The obtained results show that the dynamics of the virus isolate dynamics had no significant differences, and following the adaptation, the virus isolate maintained the stable inherent propagation activity level throughout the study. As the result of the experiments conducted, the high infection activ-

ity of the virus isolate in the continuous cell lines BHK-21, MDBK, Taurus-1, was established, which is shown in (Table 1).

The highest level of propagation of the Aujeszky's disease virus was observed in the continuous cell culture Taurus-1 in titres that, in average, amounted to 7.55±0.12 lg TCD<sub>50</sub>/cm<sup>3</sup>.

Studies for defining the optimal infecting dose were carried out in triplicate, with the subsequent definition of biological activity of virus-containing suspensions. The results of the studies conducted are given in (Table 1).

According to the data of (Table 2), the level of virus accumulation in the cell culture, infected at doses of 0.0001 and 0.001 TCD<sub>50</sub>/ml, reached its maximal value in 48 and then decreased. The activity of virus material from samples infected at the dose of

1.0 TCD<sub>50</sub>/cm<sup>3</sup>, reached the maximum value in 36 h, with the subsequent trend to decrease observed. At cell infecting with the virus at the dose of 0.01-0.1 TCD<sub>50</sub>/cm<sup>3</sup>, the maximal virus accumulation level was detected in 48-60 h.

The efficiency of the serum concentration in the cell maintenance medium composition was evaluated by the virus accumulation results and the cell culture condition. The study results are given in (Table 3). According to the data given in (Table 3), the use of 2 and 5% bovine cattle serum in the cell maintenance medium has no significant effect on the activities of the obtained virus-containing strain materials. Virus titres were in the range of 7.00 lg TCD<sub>50</sub>/cm<sup>3</sup>. At 1% serum concentration, the virus titre was considerably lower and equalled 6.3lg TCD<sub>50</sub>/cm<sup>3</sup>.

Therefore, as the study result, the most sensitive cell culture Taurus-1 was selected at passaging, with the highest virus accumulation observed, the infection activity was 7.55±0.12 lg TCD<sub>50</sub>/cm<sup>3</sup>. The maximal virus accumulation in virus-containing material occurred at 48-60 hours. This cultivation period was optimal. At this, CPE comprised over 90% are of the infected monolayer.

The comparative evaluation of the virus virulence carried out on laboratory animals, demonstrated that the study virus isolate was highly pathogenic for laboratory animals: rabbits, guinea pigs, and rats.

Belonging of the virus accumulated in cell cultures to the specific species was confirmed by the genome definition by the PCR method (Figure 2).

Figure 2 shows that,

1, 7 - marker - DNA fragment of the known molecular weight;

2- lamb brain tissue,

3 - virus-containing cultural material

4 - cat brain tissue;

5.6 - brain tissue of a rabbit infected with the virus isolate (bioassay)

The PCR method demonstrated high specificity and sensitivity in the course of the study. PCR allowed defining the nucleic acid of the Aujeszky's disease virus in virus-containing cultural and organ and tissue material of fallen domestic animals in the minimal quantity of 22 pg.

According to the results given in 2, PCR allows amplifying the target gene fragment with the size of 194 base pairs (b.p.) in samples containing the Aujeszky's disease virus DNA.

## CONCLUSIONS

The virus isolate was isolated from samples of organs of a cat that died from Aujeszky's disease. The following continuous cell lines were sensitive to the virus isolate: MDBK, Taurus-1 and BHK-21. CPE nature was similar in different cell cultures. The ability for propagation in cultures of continuous cell lines of the test virus isolate reached the maximum value to 4-6 passage, equaling to 6.85-7.65 lgTCD<sub>50</sub>/cm<sup>3</sup>. The highest level of the virus isolate propagation was observed in the continuous cell line Taurus-1 - 7.55±0.12 lg TCD<sub>50</sub>/cm<sup>3</sup>. Belonging of the virus accumulated in cell cultures to the specific species was confirmed by genome definition by the PCR method and bioassay on rabbits.

The obtained experimental data demonstrated that only the samples containing the Aujeszky's disease virus DNA revealed the specific PCR reaction products with a size of 194 b.p. The virus-containing material of the Aujeszky's disease virus isolate, obtained in different cell cultures, is planned for use in further studies with the purpose of investigating its molecular and biological properties.

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