



Isolation and identification of monkeypox virus MPXV-ROK-P1-2022 from the first case in the Republic of Korea

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ABSTRACT

Objectives: Monkeypox outbreaks in nonendemic countries have been reported since early May 2022. The first case of monkeypox in the Republic of Korea was confirmed in a patient who traveled to Europe in June 2022, and an attempt was made to isolate and identify the monkeypox virus (MPXV) from the patient's specimens.

Methods: Clinical specimens from the patient were inoculated in Vero E6 cells. The isolated virus was identified as MPXV by the observation of cytopathic effects on Vero E6 cells, transmission electron microscopy, conventional polymerase chain reaction (PCR), and sequencing of PCR products.

Results: Cytopathic effects were observed in Vero E6 cells that were inoculated with skin lesion swab eluates. After multiple passages from the primary culture, orthopoxvirus morphology was observed using transmission electron microscopy. In addition, both MPXV-specific (*F3L* and *ATI*) and orthopoxvirus-specific genes (*A39R*, *B2R*, and *HA*) were confirmed by conventional PCR and Sanger sequencing.

Conclusion: These results indicate the successful isolation and identification of MPXV from the first patient in the Republic of Korea. The isolated virus was named MPXV-ROK-P1-2022.

Keywords: Identification; Isolation; Monkeypox virus

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Introduction

Monkeypox virus (MPXV), a DNA virus, belongs to the family *Poxviridae*, genus *Orthopoxvirus* [1,2]. MPXV shows a characteristically large brick or mulberry shape with a dumbbell-shaped DNA core. The diameters of the viral particles have been reported to be about 150 to 200 nm. MPXV is a zoonosis that is transmitted from animals to humans, and several rodent species

and nonhuman primates are known to be susceptible hosts. Human-to-human transmission can also occur, mainly through close contact with the skin lesions or body fluids of an infected person [3]. In the past, MPXV was endemic in West and Central Africa, but it has recently spread in Europe and several countries, including the United States. As of August 11, 2022, more than 32,000 cases and 6 deaths across 74 countries have been reported since May 2022 [4]. On July 23, 2022, the MPXV outbreak was declared an international public health emergency by the World Health Organization [5]. In Korea, on June 22, 2022, the Korea Disease Control and Prevention Agency (KDCA) confirmed the first MPXV-infected case [6]. In this study, we report the isolation and identification of the first patient-derived MPXV. The virus was named MPXV-ROK-P1-2022.

Materials and Methods

Virus Isolation from Clinical Specimens

Clinical specimens, including skin lesion, nasopharyngeal, and oropharyngeal swabs, were collected at a medical center (where the patient was hospitalized) and sent to the KDCA. The specimens were diluted with antibiotics (penicillin/streptomycin and nystadine) at a 1:4 ratio, and then diluted with culture medium (2% fetal bovine serum [FBS]–Dulbecco's modified Eagle's Medium [DMEM]) at a 1:3 ratio and were incubated for 30 minutes at 4°C [7]. After centrifugation, the supernatant was inoculated into Vero E6 cells in 2% FBS-DMEM with antibiotics (penicillin-streptomycin) and was incubated at 37°C and 5% CO₂. For virus isolation, inoculated cells were incubated with 1.5% carboxymethyl cellulose overlay medium, and plaque isolation was performed. All virus isolation procedures were performed according to laboratory biosafety guidelines for the MPXV by the KDCA in a biosafety level 3 facility.

Conventional PCR for the Detection of MPXV

Viral DNA was extracted from 200 µL of virus culture supernatants using a QIAamp blood mini kit (Qiagen, Hilden, Germany) following the manufacturer's instructions. Conventional polymerase chain reaction (PCR) was performed with a selected primer set. The sequences of the primer sets for conventional PCR were as follows: F3L-forward primer, 5'-CATCTATTATAGCATCAGCATCAGA-3', F3L-reverse primer, 5'-GATACTCCTCCTCGTTGGTCTAC-3' for the F3L gene; A39R-forward primer, 5'-TGGGATAACG AATCCAATGTCA-3', A39R-reverse primer, 5'-GCGTGC TTCCAGCAACT-3' for the A39R gene [6]; 1995MPV-forward primer, 5'-CTGATAATGTAGAAGAC-3'; 1995MPV-reverse primer, 5'-TTGTATTTACGTGGGTG-3' for the B2R

gene [8]; 1997MPV-forward primer, 5'-AATACAAGG AGGATCT-3'; 1997MPV-reverse primer, 5'-CTTAACCTTTT CTTTCTC-3' for the ATI gene [9]; HAOUT-forward primer, 5'-CCATTGGAAAAAACACAGTAC-3'; HAOUT-reverse primer, 5'-CCAAATATATTTCCCATAGTC-3' for the HA gene [10]. The conventional PCR conditions were as follows: 94°C for 2 minutes, 35 cycles of 94°C for 30 seconds, 50°C for 30 seconds, and 72°C for 30 seconds, followed by 72°C for 5 minutes.

Sequencing Analysis of the Conventional PCR Products

The PCR products were purified using a gel extraction kit (Qiagen). Next, sequence reactions using the F3L, A39R, 1995MPV, 1997MPV, and HAOUT primers with the BigDye Terminator Reaction Mix (Applied Biosystems, Foster City, CA, USA) were performed. The reaction products were purified using a BigDye Purification Kit (Applied Biosystems), and sequence analysis was performed with a 3500xL Dx Genetic Analyzer (Applied Biosystems) [11]. A nucleotide blast was carried out with the sequencing results to confirm whether the sequences matched those of MPXV.

Transmission Electron Microscopy

In order to observe virus particles, inoculated cells were fixed with 0.1 M phosphate buffer (pH 7.4) containing 2% paraformaldehyde and 2.5% glutaraldehyde. After the fixation, a 3-mL drop of the fixed sample was adsorbed to a glow-discharged formvar-coated 200-mesh copper grid, washed with 3 drops of deionized water 3 times, and stained with 2 drops of freshly prepared 0.5% uranyl acetate, and then washed with deionized water and dry at room temperature. The staining grid was observed with a transmission electron microscope (Libra120; Carl Zeiss, Oberkochen, Germany) at an acceleration voltage of 120 kV. To perform transmission electron microscopy with sectioning, prefixed cells were fixed sequentially with 0.1 M phosphate buffer (pH 7.4) containing 2% paraformaldehyde, 2.5% glutaraldehyde, and 1% osmium tetroxide before negative staining. The fixed samples were sectioned at 80 nm, followed by staining with 4% uranyl acetate and 1% lead citrate. The prepared samples were observed with a transmission electron microscope.

Results and Discussion

Virus Isolation

Of the specimens from the first patient, 2 specimens—the skin lesion swab and the oropharyngeal plus nasopharyngeal swabs—showed cytopathic effects (CPEs) on monolayers of Vero E6 cells (Figure 1). In the inoculation of the skin lesion swab, CPEs were observed after 36 hours on the monolayers.

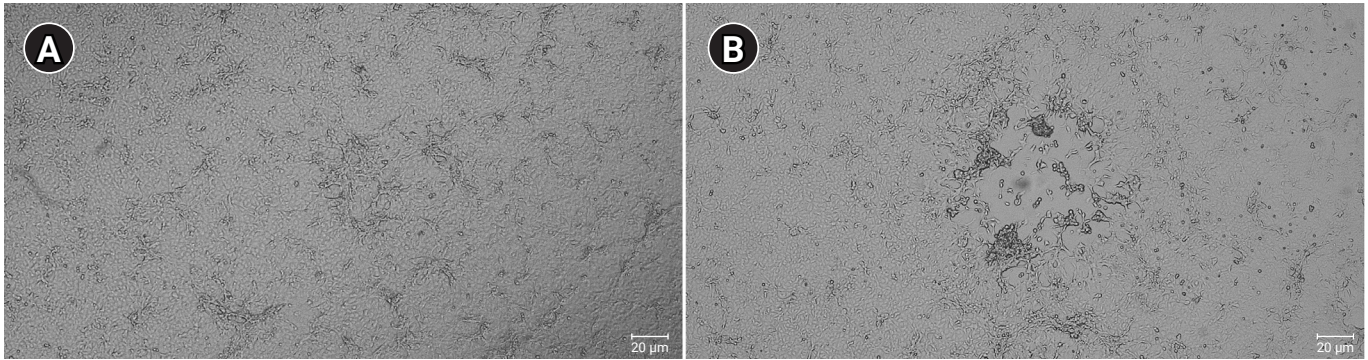


Figure 1. Cytopathic effects in Vero E6 cells after 36 to 48 hours. (A) Mock infected cells (B) Skin swab specimen-infected cells.

After 4 days, CPEs and detaching of cells were shown on the entire area of the monolayers. Through subsequent multiple passages and isolation of plaques, we obtained culture suspension and infected cells from a single plaque of the virus. The culture suspension and cells were used for conventional PCR and electron microscopy, as described in the following section. After confirmation of the virus as MPXV, we named the virus MPXV-ROK-P1-2022. Virus culture using the oropharyngeal plus nasopharyngeal swabs showed similar results (data not shown).

Confirmation of MPXV by PCR

The isolated virus was confirmed as MPXV by PCR assays. The conventional PCR assays with F3L primers for the F3L gene, A39R primers for the A39R gene, 1995MPV primers for the B2R gene, 1997MPV primers for the ATI gene, and HAOUT primers for the HA gene were performed, followed by Sanger sequencing. Conventional PCR revealed PCR products of the expected sizes: 79 bp for the F3L gene, 70 bp for the A39R gene, 405 bp for the B2R gene, 1,550 bp for the ATI gene, and 1,171 bp for the HA gene, as shown in Figure 2. The Sanger sequencing results showed that sequences of those genes were >99% identical to previously reported human MPXVs (OP022170.1, OP022171.1, OP018607.1) (data not shown). Among the conventional PCR procedures, PCR of the ATI gene with 1997MPV primers was designed to distinguish 2 clades of MPXV—clade I (Congo-Basin clade, 450 bp) and clade II (West African, 1,550 bp) [8,12]—with different PCR product sizes. As shown in Figure 2 the PCR product of MPXV-ROK-P1-2022 showed a 1,550-bp product, indicating that the virus belonged to the West African clade (i.e., clade II).

Transmission Electron Microscopy

The transmission electron microscopy experiment revealed MPXV-ROK-P1-2022 virus in Vero E6 cells. As shown in

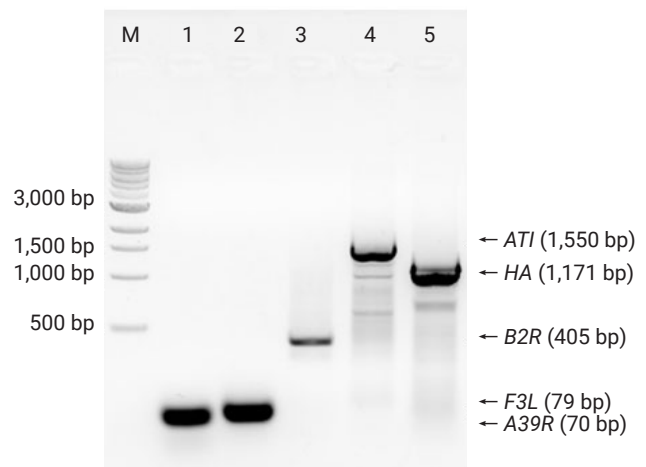


Figure 2. Conventional polymerase chain reaction products of MPXV-ROK-P1-2022. M, size maker; Lane 1, A39R; lane 2, F3L; lane 3, B2R; lane 4, ATI; lane 5, HA; lane.

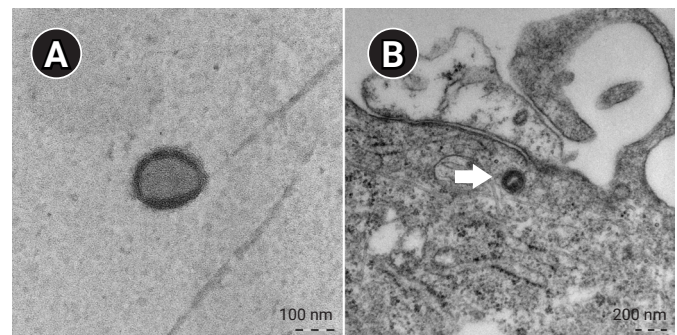


Figure 3. Electron microscopy of MPXV-ROK-P1-2022. (A) Mulberry-shaped virion from cell culture. (B) Virus particle (arrow) in an infected cell.

Figure 3, the virus itself or in the infected cells was visualized as a typical mulberry-shaped particle, which is characteristic of MPXV. In accordance with a previous report [13], the viral particle diameters were about 150 to 200 nm.

In summary, our results showed successful isolation and identification of MPXV-ROK-P1-2022 from the first monkeypox patient in Korea. The virus isolated using Vero E6 cells was identified as MPXV by electron microscopy, PCR, and sequencing. MPXV-ROK-P1-2022 will be used as a reference strain for diagnosis by the KDCA.

Notes

Ethics Approval

This study was approved by the Institutional Review Board of Korea Diseases Control and Prevention Agency (No: KDCA-IBC-2022-056).

Conflicts of Interest

The authors have no conflicts of interest to declare.

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Availability of Data

The datasets are not publicly available but are available from the corresponding author upon reasonable request.

Authors' Contributions

Conceptualization: GER, HY, JiWK, ML; Data curation: GER, HY, JiWK, ML, CHC; Formal analysis: HS, MMC; Funding acquisition: HY, GER; Investigation: HS, MMC, CHC; Methodology: HY, JiWK, ML, JeWK; Project administration: MMC, JiWK; Resources: JiWK, ML; Supervision: CKY, GER; Validation: HY, GER; Visualization: ML, JeWK; Writing—original draft: GER, HY, JiWK, ML; Writing—review & editing: all authors.

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