AMERICAN SOCIETY FOR MICROBIOLOGY | Microbiology Resource Announcements

GENOME SEQUENCES

Coding-Complete Genome Sequences of an Omicron Subvariant (BA.5.2.20) of SARS-CoV-2

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ABSTRACT Here, we present the complete coding sequences of two severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) strains that were recovered from a nasopharyngeal swab from a female patient and the second viral passage in cell culture. After testing, both strains were identified as BA.5.2.20, a subvariant of Omicron.

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), a member of the genus *Betacoronavirus* and family *Coronaviridae*, is the causative agent of coronavirus disease 2019 (COVID-19) (1). First reported in Morocco in March 2020, it has resulted in 1.27 million cases and 16,294 deaths (2). In November 2021, the WHO announced the emergence of a new variant of concern (VOC) named Omicron (B.1.1.529) (3). We report here the coding-complete genome sequence of 2 SARS-CoV-2 isolates exhibiting low threshold cycle (C_T) values. Sample 1 was a nasopharyngeal specimen collected from a 25-year-old female patient (RdRp gene C_T value, 14.66), and sample 2 was recovered from the second viral passage on 293TACE2.TMPRSS2 cells (ATCC

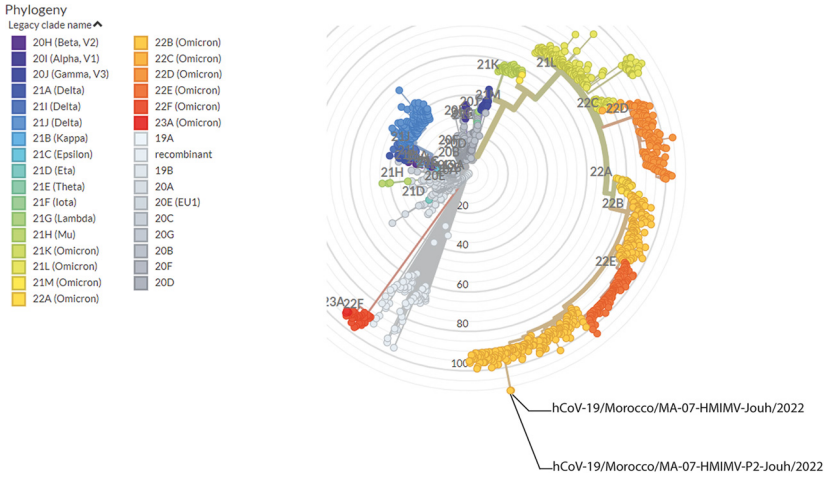

FIG 1 Phylogenetic tree displaying the sequenced SARS-CoV-2 strains (with reference to the global GISAID dataset), according to the clades assigned to the virus in the phylogenetic analysis using the Augur toolkit (with default settings) run by the Nextstrain server. The location of our strains, hCoV-19/Morocco/MA-07-HMIMV-Jouh/2022 (sample 1) and hCoV-19/Morocco/MA-07-HMIMV-P2-Jouh/2022 (sample 2), is marked by a large yellow circle with a red outline.

Editor John J. Dennehy, Queens College Department of Biology

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The authors declare no conflict of interest.

Received 2 February 2023
Accepted 9 May 2023
Published 24 May 2023

TABLE 1 Amino acid changes, all present in both hCoV-19/Morocco/MA-07-HMIMV-Jouh/2022 (sample 1) and hCoV-19/Morocco/MA-07-HMIMV-P2-Jouh/2022 (sample 2) compared to the reference strain[a]

Mutation location	Mutation type
S	T19I[b]
	A27S
	G339D
	S371F
	S373P
	K417N
	N440K
	L452R
	D614G
	H655Y
	N679K
	P681H
	N764K
	D796Y
	Q954H
	N969K
ORF1a	S135R
	T842I
	G1307S
	T1682I
	L3027F
	T3255I
	P3395H
ORF1b	R1315C
	T2163I
ORF9b	P10S
	D16G
E	T9I
M	D3N
	A63T
N	P13L
	R203K
	G204R
	S413R

[a] Reference strain:Wuhan-Hu-1 (GenBank accession number NC_045512.2).
[b] T19I, T-to-I change at position 19.

NR-55293_12-2021). These cells were modified to express TMPRSS2 and ACE2, hence increasing their permissivity to Omicron (4).

Both samples were sequenced. Viral RNAs were extracted using the NucleoSpin RNA virus isolation kit (Macherey-Nagel, Germany). Sequencing reagents were purchased from Thermo Fisher Scientific (USA). The RNA quantity and quality were evaluated using the Qubit RNA high-sensitivity (HS) assay kit; cDNA was then synthesized from a sufficient amount of RNA (1 to 10 ng) using the SuperScript VILO reverse transcriptase kit. Libraries were prepared using the Ion AmpliSeq SARS-CoV-2 research panel, according to the manufacturer's instructions, and then were loaded into the Ion Chef instrument for automated template preparation and chip loading. Sequencing was carried out on the Ion S5 sequencer. Finally, consensus sequences were generated by mapping the reads against the Wuhan-Hu-1 reference sequence (GenBank accession number NC_045512.2) and the reference sequence of Omicron variant BA.1 (Pango lineage B.1.1.529; OL672836.1) using Unipro UGENE version 45 and a *de novo* mapping tool (UGENE version 46.0). All tools were run with default parameters unless otherwise specified.

We obtained 495,971 and 654,008 reads (length, 29,903 bp; GC content, 37.92%). Phylogenetic analysis using the Pangolin web application (https://pangolin.cog-uk.io/) identified the strains as belonging to subvariant BA.5.2.20 of Omicron lineage B.1.1.529 (Fig. 1). This lineage was first reported in South Africa on 9 November 2021 (5).

The Nextclade Web application (https://clades.nextstrain.org/) was used to identify 34 identical amino acid substitutions in both samples against the Wuhan-Hu-1 reference sequence (Table 1). Both genomes had 16 amino acid changes in the spike protein, including

the S-to-F change at position 371 (S371F) and S373P in the receptor binding domain (RBD) region and L452R in the receptor binding motif (RBM) region. These mutations alter the site conformations of the S protein, resulting in reduced sensitivity to neutralizing antibodies (6, 7), a decrease in treatment efficacy (8, 9), and high transmissibility (10). Other protein mutations (N, E, M, and ORF1ab) were also detected with P681H in the S1/S2 furin cleavage site, which can prompt a rapid fusion between the virus and the cell membrane, resulting in increasing viral pathogenesis (11, 12).

Genomic surveillance and cell culture are two complementary approaches for understanding SARS-CoV-2 evolution, as cell culture helps us understand and study the cell entry of SARS-CoV-2 variants.

Data availability. The SARS-CoV-2 genome sequences were submitted to the GISAID database under the identifiers EPI_ISL_16683686 and EPI_ISL_16683687 and to NCBI GenBank under the accession numbers OQ341628 and OQ341629. The reads Binary Alignement Map (BAM) were also submitted to the SRA under BioProject accession numbers PRJNA945815 and PRJNA945874.

ACKNOWLEDGMENTS

This study was supported and financed by the Hassan II Academy of Science and Technology and Inspection du Service de Santé Militaire, Kingdom of Morocco. We declare no conflict of interest.

REFERENCES

1. Wu A, Peng Y, Huang B, Ding X, Wang X, Niu P, Meng J, Zhu Z, Zhang Z, Wang J, Sheng J, Quan L, Xia Z, Tan W, Cheng G, Jiang T. 2020. Genome composition and divergence of the novel coronavirus (2019-nCoV) originating in China. Cell Host Microbe 27:325–328. https://doi.org/10.1016/j.chom.2020.02.001.
2. Ministry of Health and Social Welfare of Morocco. 2023. The official coronavirus portal in Morocco. http://www.covidmaroc.ma. (In Arabic.) Accessed 1 February 2023.
3. Tian D, Sun Y, Xu H, Ye Q. 2022. The emergence and epidemic characteristics of the highly mutated SARS-CoV-2 Omicron variant. J Med Virol 94:2376–2383. https://doi.org/10.1002/jmv.27643.
4. Meng B, Abdullahi A, Ferreira IATM, Goonawardane N, Saito A, Kimura I, Yamasoba D, Gerber PP, Fatihi S, Rathore S, Zepeda SK, Papa G, Kemp SA, Ikeda T, Toyoda M, Tan TS, Kuramochi J, Mitsunaga S, Ueno T, Shirakawa K, Takaori-Kondo A, Brevini T, Mallery DL, Charles OJ, Bowen JE, Joshi A, Walls AC, Jackson L, Martin D, Smith KGC, Bradley J, Briggs JAG, Choi J, Madissoon E, Meyer KB, Mlcochova P, Ceron-Gutierrez L, Doffinger R, Teichmann SA, Fisher AJ, Pizzuto MS, de Marco A, Corti D, Hosmillo M, Lee JH, James LC, Thukral L, Veesler D, CITIID-NIHR BioResource COVID-19 Collaboration, Genotype to Phenotype Japan (G2P-Japan) Consortium, Ecuador-COVID19 Consortium, et al. 2022. Altered TMPRSS2 usage by SARS-CoV-2 Omicron impacts infectivity and fusogenicity. Nature 603:706–714. https://doi.org/10.1038/s41586-022-04474-x.
5. Ren S-Y, Wang W-B, Gao R-D, Zhou A-M. 2022. Omicron variant (B.1.1.529) of SARS-CoV-2: mutation, infectivity, transmission, and vaccine resistance. World J Clin Cases 10:1–11. https://doi.org/10.12998/wjcc.v10.i1.1.
6. Li Q, Wu J, Nie J, Zhang L, Hao H, Liu S, Zhao C, Zhang Q, Liu H, Nie L, Qin H, Wang M, Lu Q, Li X, Sun Q, Liu J, Zhang L, Li X, Huang W, Wang Y. 2020. The impact of mutations in SARS-CoV-2 spike on viral infectivity and antigenicity. Cell 182:1284–1294.e9. https://doi.org/10.1016/j.cell.2020.07.012.
7. Chen J, Wang R, Wang M, Wei G-W. 2020. Mutations strengthened SARS-CoV-2 infectivity. J Mol Biol 432:5212–5226. https://doi.org/10.1016/j.jmb.2020.07.009.
8. Pastorio C, Zech F, Noettger S, Jung C, Jacob T, Sanderson T, Sparrer KMJ, Kirchhoff F. 2022. Determinants of spike infectivity, processing, and neutralization in SARS-CoV-2 Omicron subvariants BA.1 and BA.2. Cell Host Microbe 30:1255–1268.e5. https://doi.org/10.1016/j.chom.2022.07.006.
9. Miller NL, Clark T, Raman R, Sasisekharan R. 2022. A structural dynamic explanation for observed escape of SARS-CoV-2 BA.2 variant mutation S371L/F. bioRxiv. 2022.02.25.481957. https://doi.org/10.1101/2022.02.25.481957.
10. Hemlali M, Chouati T, Ghammaz H, Melloul M, Alaoui Amine S, Rhoulam S, Touil N, Ennibi K, Oumzil H, Mohamed R, Hassan A, El Fahime E. 2022. Coding-complete genome sequences of a Delta subvariant (AY.33) of SARS-CoV-2 obtained from Moroccan COVID-19 patients. Microbiol Resour Announc 11:e0109921. https://doi.org/10.1128/mra.01099-21.
11. Johnson BA, Xie X, Bailey AL, Kalveram B, Lokugamage KG, Muruato A, Zou J, Zhang X, Juelich T, Smith JK, Zhang L, Bopp N, Schindewolf C, Vu M, Vanderheiden A, Winkler ES, Swetnam D, Plante JA, Aguilar P, Plante KS, Popov V, Lee B, Weaver SC, Suthar MS, Routh AL, Ren P, Ku Z, An Z, Debbink K, Diamond MS, Shi P-Y, Freiberg AN, Menachery VD. 2021. Loss of furin cleavage site attenuates SARS-CoV-2 pathogenesis. Nature 591:293–299. https://doi.org/10.1038/s41586-021-03237-4.
12. Garcia-Beltran WF, Lam EC, St Denis K, Nitido AD, Garcia ZH, Hauser BM, Feldman J, Pavlovic MN, Gregory DJ, Poznansky MC, Sigal A, Schmidt AG, Iafrate AJ, Naranbhai V, Balazs AB. 2021. Multiple SARS-CoV-2 variants escape neutralization by vaccine-induced humoral immunity. Cell 184:2372–2383.e9. https://doi.org/10.1016/j.cell.2021.03.013.