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Genomic DNA extraction from *Mycobacterium avium*

In 1 collection

Tetsuya Komatsu[1], Kenji Ohya[2], Justice Opare Odoi[3], Shota Suganuma[2], Kotaro Sawai[2], Takayuki Wada[4], Tomotada Iwamoto[5], Fumito Maruyama[6]

[1]Aichi Prefectural Chuo Livestock Hygiene Service Center, Okazaki, Aichi, Japan;
[2]Faculty of Applied Biological Sciences, Gifu University, Gifu, Gifu, Japan;
[3]United Graduate School of Veterinary Sciences, Gifu University, Gifu, Gifu, Japan;
[4]Graduate School of Human Life Science, Osaka City University, Osaka, Osaka, Japan;
[5]Department of Infectious Diseases, Kobe Institute of Health, Kobe, Hyogo, Japan;
[6]Office of Academic Research and Industry-Government Collaboration, Hiroshima University, Higashi-Hiroshima, Hiroshima, Japan

Tetsuya Komatsu
Aichi Prefectural Chuo Livestock Hygiene Service Center, Oka...

ABSTRACT

Mycobacterium avium subsp. *hominissuis* (MAH) is one of the most important agents causing non-tuberculosis mycobacterial infection in human and pigs. Genome analysis on MAH of human isolates has been proceeding, however, those of pigs are limited despite its potential source of infection to human. Here, we isolated MAH from pig lymph nodes or livers and obtained their genomes.

MATERIALS

TE: Tris-EDTA Buffer; 10 mM Tris-Cl (pH 8.0), 1 mM EDTA; sterilize by autoclaving.
Phenol/chloroform/isoamyl alcohol; 25:24:1

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1 Suspend colonies on 7H11 agar into 300 µl of TE.

2 Centrifuge at 5,000 *g* for 10 min.

3 Discard supernatant, followed by suspension in 300 µl of acetone.

4 Centrifuge at 5,000 *g* for 10 min.

5 Discard acetone, followed by suspension in 200 µl of TE.

6 Add lysozyme at the final concentration of 100 µg/ml.

7 Incubate at 37°C overnight.

8 Add proteinase K at the final concentration of 0.2 mg/ml.

9 Incubate at 50°C for 3 hrs.

10 Add 1/10 volume of 3 M NaOAc.

11 Add equal volume of phenol/chloroform/isoamyl-alcohol and shake vigorously.

12 Centrifuge at 12,000 *g* for 10 min.

13 Transfer aquatus phase to a new tube.

14 Add equal volume of 2-propanol.

15 Centrifuge at 12,000 *g* for 10 min.

16 Remove supernatant and wash with 70% ethanol.

17 Suspended in 400 µl of TE, followed by adding RNase at the final concentration of 100 µg/ml.

18 Incubate at 37°C for 30 min.

19 Repeat steps 10 to 16.

20 Suspended in 100 µl of TE.