

'*Lysinibacillus saudimassiliensis*' sp. nov., a new bacterial species isolated from air samples in the urban environment of Makkah, Saudi Arabia

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Abstract

We report here the main characteristics of '*Lysinibacillus saudimassiliensis*' strain 13S34_air^T (CSUR = P1222), a new species of the *Lysinibacillus* genus that was isolated from air samples in the city environment of Makkah, Saudi Arabia, during the pilgrim period of Hajj 2012.

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As a part of a wider culturomics [1] and metagenomics study [2] in Saudi Arabia, we isolated a new bacterium, strain 13S34_air^T, from two air samples in the urban environment of Makkah, Saudi Arabia, during the pilgrim period of Hajj 2012. For each air sample, a volume of 1 m³ was collected with a FCC-IV biological air sampler (AES Laboratories, Combourg, France) mounted with a nutrient agar plate containing the antifungal agent amphotericin (Majed Al-Buqami Co. BMC, Riyadh, Saudi Arabia) according to the manufacturer's instructions. No identification was obtained for the strain 13S34_air^T using our systematic matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) screening on a MicroFlex spectrometer (Bruker Daltonics, Bremen, Germany). Strain 13S34_air^T was cultured in 5% sheep's blood-enriched Columbia agar (bioMérieux, Marcy l'Etoile, France) for 2 days in an aerobic atmosphere at 37°C. Growth was observed only in aerobic conditions, and no growth occurred in anaerobic conditions. On Columbia agar, strain 13S34_air^T colonies were opaque and round, with a greyish color, and their size varied

between 4 to 6 mm in diameter. The strain 13S34_air^T is a Gram-positive, obligate aerobic, endospore-forming, rod-shaped, catalase-positive and oxidase-negative bacterium. To test for spore formation, bacteria were heated at 80°C for 30 minutes and then were spread on blood-enriched Columbia agar. A positive result was taken after an overnight incubation at 37°C. Growth was observed in the range of 0.5 to 5% NaCl, with optimum growth at 0.5% NaCl. Observation under a light microscope showed bacterial motility.

The complete 16S rRNA gene was sequenced using fD1-rP2 primers as previously described and using a 3130-XL sequencer (Applied Biosciences, Saint Aubin, France) [3]. The strain 13S34_air^T exhibited a 96.4% sequence similarity with *Lysinibacillus sphaericus* (NR115724), which was the phylogenetically closest species with standing in nomenclature (Fig. 1). Consequently, it putatively classifies the strain 13S34_air^T as a new member of the genus *Lysinibacillus* within the family Bacillaceae in the phylum Firmicutes. The genus *Lysinibacillus* was first proposed in 2007 by Ahmed et al. [4] by the characterization of the type species *Lysinibacillus boronitolens* and the transfer of *Bacillus fusiformis* to *Lysinibacillus fusiformis* comb. nov. and *Bacillus sphaericus* to *Lysinibacillus sphaericus* comb. nov. In 2012, an emended description of the genus *Lysinibacillus* was proposed by Jung et al. [5]. To date, the genus includes 21 species (<http://www.bacterio.cict.fr/c/lysinibacillus.html>); they are mostly environmental bacteria that are distributed primarily in soil [5].

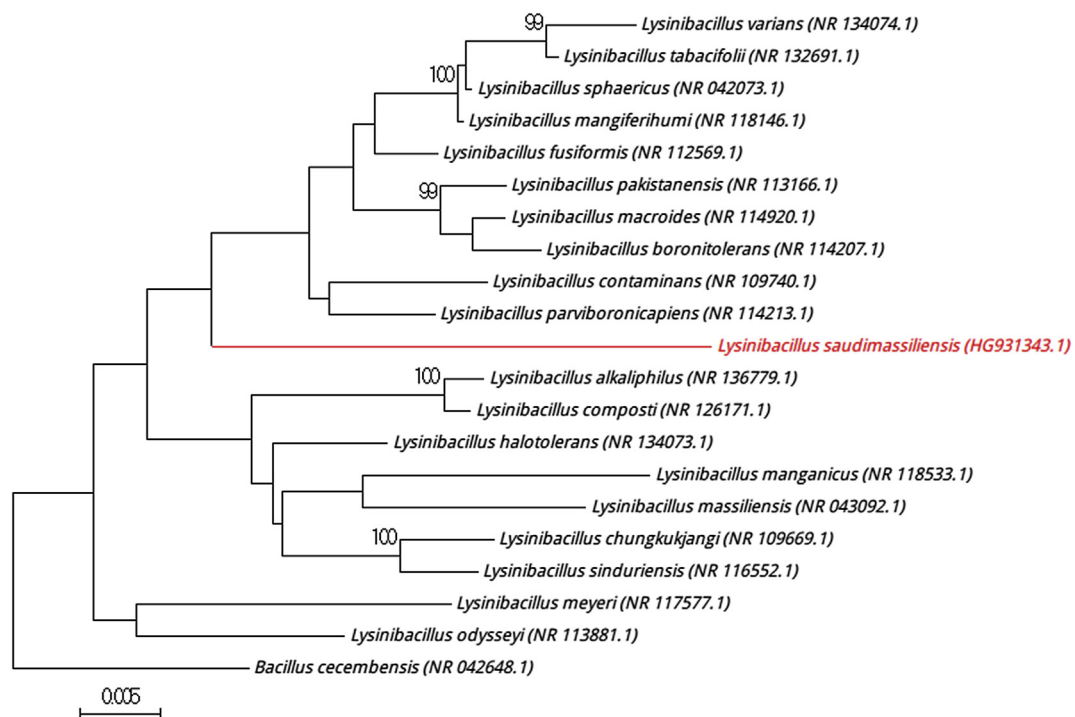


FIG. 1. Phylogenetic tree highlighting position of '*Lysinibacillus saudimassiliensis*' relative to other phylogenetically close members of *Lysinibacillus* genus. Numbers at nodes are percentages of bootstrap values obtained by repeating analysis 500 times to generate majority consensus tree. Only values >95% are displayed. Scale bar represents 0.5% nucleotide sequence divergence.

Strain 13S34_{air}^T exhibited a 16S rRNA gene sequence divergence >1.3% with *L. sphaericus*, the closest related species with standing in nomenclature, which classifies it as a new representative of the *Lysinibacillus* genus isolated from air samples in the urban environment of Makkah. As a result, we propose the creation of '*Lysinibacillus saudimassiliensis*' sp. nov., and the strain 13S34_{air} as the type strain.

MALDI-TOF MS spectrum

The MALDI-TOF MS spectrum of 13S34_{air}^T is available online (<http://www.mediterranee-infection.com/article.php?laref=256&titre=urms-database>).

Nucleotide sequence accession number

The 16S rRNA gene sequence of the strain 13S34_{air}^T was deposited in GenBank under accession number HG931343.1.

Deposit in a culture collection

Strain 13S34_{air}^T was deposited in the Collection de Souches de l'Unité des Rickettsies (CSUR, WDCM 875) under number PI222.

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Conflict of Interest

None declared.

References

- [1] Angelakis E, Yasir M, Azhar El, Papadioti A, Bibi F, Aburizaiza AS, et al. MALDI-TOF mass spectrometry and identification of new bacteria species in air samples from Makkah, Saudi Arabia. *BMC Res Notes* 2014;7:892.
- [2] Angelakis E, Yasir M, Bachar D, Azhar El, Lagier JC, Bibi F, et al. Gut microbiome and dietary patterns in different Saudi populations and monkeys. *Sci Rep* 2016;6:32191.
- [3] Safont M, Angelakis E, Richet H, Lepidi H, Fournier PE, Drancourt M, et al. Bacterial lymphadenitis at a major referral hospital in France from 2008 to 2012. *J Clin Microbiol* 2014;52:1161–7.
- [4] Ahmed I, Yokota A, Yamazoe A, Fujiwara T. Proposal of *Lysinibacillus boronitolerans* gen. nov. sp. nov., and transfer of *Bacillus fusiformis* to

Lysinibacillus fusiformis comb. nov. and *Bacillus sphaericus* to *Lysinibacillus sphaericus* comb. nov. Int J Syst Evol Microbiol 2007;57:1117–25.

- [5] Jung MY, Kim JS, Paek WK, Styrak I, Park IS, Sin Y, et al. Description of *Lysinibacillus sinduriensis* sp. nov., and transfer of *Bacillus massiliensis* and

Bacillus odysseyi to the genus *Lysinibacillus* as *Lysinibacillus massiliensis* comb. nov. and *Lysinibacillus odysseyi* comb. nov. with emended description of the genus *Lysinibacillus*. Int J Syst Evol Microbiol 2012;62: 2347–55.