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Herbaspirillum Species: A Potential Pathogenic Bacteria Isolated from Acute Lymphoblastic Leukemia Patient

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Abstract *Herbaspirillum* species, colonized the plant rhizosphere, also called rhizobacteria, are plant growth-promoting bacteria. Recently we isolated *Herbaspirillum* from blood cultures of acute lymphoblastic leukemia (ALL) and identified by PCR and gene sequencing. *Herbaspirillum* may be a potential pathogenic bacteria. Although the exact role that these species play in ALL patients is unknown, their differentiation from other species has serious implications for clinical care and patient well-being.

Dear editor

The acute lymphoblastic leukemia (ALL), is a severe leukemia, or cancer of the white blood cells characterized by excess lymphoblasts. ALL causes damage and death by crowding out normal cells in the bone marrow or by metastasis [1, 2]. The ALL was also an important predisposition of hospital-acquired diseases that are called nosocomial infections [3, 4]. Recently, the impact of nosocomial infections on human health has increased dramatically. For example, in Germany, approximately 1 million nosocomial infections occur per year, of which about 40,000 were fatal [5]. The nosocomial infections causing bacteria mainly includes *Pseudomonas aeruginosa*, *Enterobacter*, *Staphylococcus*, and so on. Previous reports indicated that the *Herbaspirillum* species may be also a potential pathogenic bacteria for human [5, 6]. *Herbaspirillum* species, which mainly colonized the plant rhizosphere, also called rhizobacteria, are plant growth-promoting bacteria (PGPB), capable of enhancing growth and yield of many plant species, several of agronomic and ecological significance [5, 7].

At March 2008, we identified *Herbaspirillum* species in culture of blood specimen isolated from an ALL patient. We presented the case, and discussed the implications of the identification of these species in this patient.

Case Report

A 48-year-old woman with ALL received chemotherapy in *The Affiliated People's Hospital of Jiangsu University*. During the chemotherapy, the patient was administered recombinant human granulocyte colony stimulating factor (G-CSF) to increase the number of white blood cell (WBC). 2009 Jan 21 at 17:30, the patient suddenly felt chill, fever (40.3°C), without the other symptoms. The pulmonary respiration became crude without bubble. The neutrophilic granulocyte was 4.65 × 10^9/l (93%) among WBC (5.0 × 10^9/l). The patient self-reported that she

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drank sugarcane juice before fever. The doctor prescribed hemoculture and anti-infective therapy with cefmetazole and gatifloxacin. After 2 days, the patient recovered; aerobic culture of blood was positive.

A gram-negative bacilli was detected (Fig. 1a). However, isolate could not be identified using the VITEK-32 GNI$^+$ card (bioMérieux, Hazelwood, MO, USA). The results were as following: oxidase test (+), dynamia (+), glucose fermentation test (−), lactose acid test (−), maltose acid test (−), mannitol acid test (+), sucrose acid test (−), xylose acid test (+), rhamnose test (−), galactosidase test (−), esculin hydrolysis test (−), urease test (+), hydrogen sulfide test (−), ukrainian acid decarboxylation test (−), lysine decarboxylation test (−), arginine hydrolysis test (−), citrate utilization test (+), malonate salts using test (+), glucose oxidation test (+), polymyxin B (+). The MIC [8] showed that the bacteria was resistant to ampicillin, gentamicin, tobramycin, norfloxacin, and aztreonam; whereas the bacteria was susceptible to piperacillin, piperacillin/tazobactam, sulperazon, cefazolin, cefotaxime, cefoperazonem, ceftriaxone, ceftazidime, cefepime, amikacin, ciprofloxacin, oxygen difloxacin, and imipenem.

The isolates' DNA was prepared by boiling (picked from a plate grown overnight) at 100°C for 15 min in 20 μl of lysis buffer. After lysis, 180 μl of distilled water was added, and the DNA solutions were stored at −20°C. The complete sequence of the 16S rRNA gene was amplified by PCR. The PCR product was sequenced by Shanghai Sangon Biological Engineering Technology and Service Co., Ltd and the sequence was given in Supplementary data. The sequences were compared to sequences available in the GenBank database (http://www.ncbi.nlm.nih.gov). A similarity level of 99% with sequences of *Herbaspirillum* species was found in the NCBI database. To further identify the relationship between the bacterial isolate and *Herbaspirillum* species, the phylogenetic tree was generated by minimum-evolution (ME) methods (Fig. 1b).

Herbaspirillum species, one of the rhizobacteria, could promote plant growth as well as provide a defense against plant pathogens [9, 10]. The other striking feature is their common ability to degrade a wide range of environmental pollutants. Whereas, *Herbaspirillum* species also can colonize humans, cause severely debilitated, immunocompromised or suffering from cystic fibrosis (CF), or HIV infections and become a potential opportunistic pathogenic bacteria [11, 12]. Additionally, this bacteria was multi-drug resistant. Recently, with the development of biotechnological applications in the biological control of plant pathogens and bioremediation of xenobiotics, more risk assessment needs to be carried out on these products to

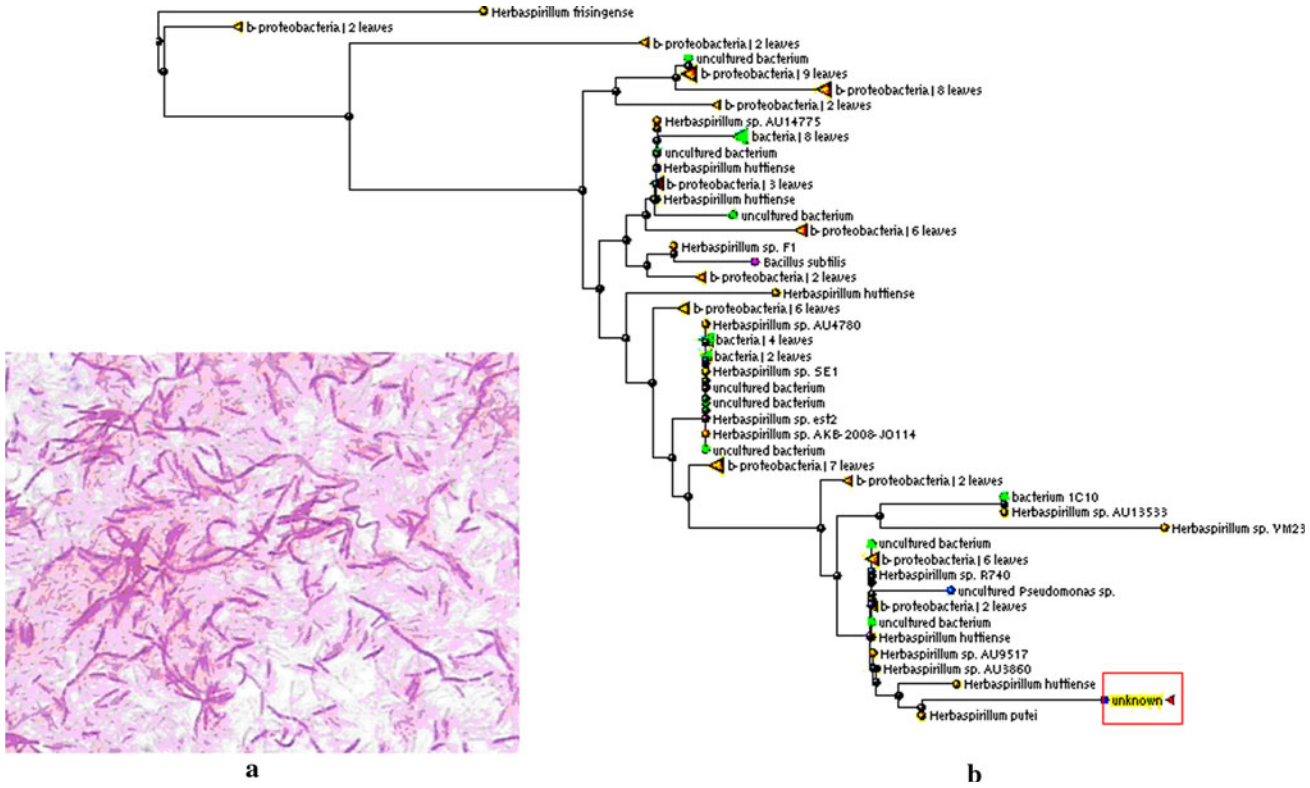

Fig. 1 The isolates and phylogenetic analysis. **a** The bacteria were dyed by Gram's stain; **b** ME Phylogenetic tree generated by using blast-based alignment of the 16S rRNA sequences of isolates

ensure that they do not inadvertently pose a threat to human health [5].

In summary, we identified *Herbaspirillum* in culture of blood from an ALL patient. Although it is not clear what the role of the *Herbaspirillum* in ALL, it may be a potential important opportunistic pathogen. Therefore, it is urgent to establish the control strategies for this opportunistic pathogen. It is especially necessary to develop alternative therapy and to establish new models for pathogenicity testing and epidemiological prediction, especially to understand their pathogenicity in both their natural hosts and humans. This is the first report on the occurrence of *Herbaspirillum* species associated with ALL in China.

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Conflict of Interest Statement The authors declare that they have no conflict of interest.

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