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Cutaneotrichosporon (*Cryptococcus*) *cyanovorans*, a basidiomycetous yeast, isolated from the airways of cystic fibrosis patients

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ABSTRACT

Cystic fibrosis (CF) patients are colonized with a multitude of bacteria and fungi. From respiratory samples of two CF patients in our institute, a difficult to identify yeast was isolated repeatedly. This yeast was eventually identified as *Cutaneotrichosporon* (*Cryptococcus*) *cyanovorans* by internal transcribed spacer (ITS) and ribosomal large subunit (LSU) sequencing. *C. cyanovorans* is a basidiomycetous yeast originally reported as environmental isolate from South African soil and has not been described before as clinical isolate from CF patients.

1. Introduction

The airways of cystic fibrosis (CF) patients are progressively colonized with micro-organisms, such as *Staphylococcus aureus*, *Pseudomonas aeruginosa* and species belonging to the *Burkholderia cepacia* complex. Among the fungi, *Aspergillus fumigatus* and *Candida* spp. are frequently encountered. Colonization with most of these micro-organisms leads to chronic pulmonary infection, which is a major cause of morbidity and mortality. Many other bacteria and fungi are isolated from the airways of CF patients, although often less frequently. In our institute, respiratory samples from a large part of the Dutch CF population are frequently cultured. In this study, we report the repeated isolation of *Cutaneotrichosporon* (*Cryptococcous*) *cyanovorans* from the airways of two CF patients. *C. cyanovorans* was described in 2012 as a basidiomycetous yeast isolated from South African soil, contaminated with cyanide [1].

2. Cases

2.1. Patient 1

This patient is a 37 year old female with CF (homozygous deltaF508) and exocrine pancreatic insufficiency. She experienced repetitive lower tract infections with *Haemophilus influenzae*, *Stenotrophomonas maltophilia*, *Escherichia coli*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa*. Also *Aspergillus fumigatus* and *Candida albicans* were cultured from the airways. In 2014, a sputum sample (day 0) was positive for a yeast that could not be identified with matrix assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS), using the MALDI Biotyper (Bruker, Bremen, Germany). Several subsequent sputum samples were also positive for this yeast, up to day 1075. The yeast grew on a malt agar, containing chloramphenicol, colistin and vancomycin, after 1–2 days of culture at 37 °C in ambient air. Two of these isolates (from sputum samples obtained on day 957 and day 1069) were identified by internal transcribed spacer (ITS) and ribosomal large subunit (LSU) sequence analysis showing full agreement with sequences of *C. cyanovorans* isolates CBS 11948 (GenBank Accession No. JF 680900, JF 680899, respectively), CBS 11949 (JF 680898, JF 680897, respectively) and CBS 11950 (JF 680902, JF 680901, respectively) from the Westerdijk Institute collection that were obtained from South African soil [1]. The antifungal susceptibility of one isolate was tested with a microdilution method (Yeastone, Trek Diagnostic Systems, UK). The minimal inhibitory concentrations (MICs) for the echinocandins were above 8 µg/ml (Table 1). The MICs for fluconazole and flucytosine were 8 and > 64 µg/ml respectively, with lower MICs for voriconazole, itraconazole, posaconazole and amphotericin B.

Due to progressive lung function decline, she underwent bilateral

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Table 1
In vitro susceptibility of *C. cyanovorans* as determined by microdilution.

Antifungal agent	*C. cyanovorans* (strain K314) Patient 1 MIC (μg/ml)	*C. cyanovorans* (strain G2100) Patient 2
Fluconazole	8	8
Itraconazole	0.25	0.5
Voriconazol	0.25	0.5
Posaconazole	0.5	1
Anidulafungin	> 8	> 8
Micafungin	> 8	> 8
Caspofungin	> 8	> 8
Flucytosine	> 64	> 64
Amphotericin B	1	1

lung transplantation in 2017 (day 1094). Since *A. fumigatus* was cultured before transplantation, the patient was treated for six months after transplantation with voriconazole. No systemic antifungal therapy was given in the years before lung transplantation. Patient is doing well, has no signs of transplant rejection and bronchoscopy performed four months after transplantation (day 1230) showed no evidence of fungal infection. She has never been to South Africa.

The patient regularly consumed several herbal teas. None of the tea leaves were positive for *C. cyanovorans* in culture (data not shown).

2.2. Patient 2

This patient is a 20 year old female CF patient (homozygous deltaF508) with exocrine pancreatic insufficiency and CF related liver disease. During earlier childhood cultures were frequently positive for *H. influenzae*, *S. aureus*, including MRSA, Enterobacteriaceae spp., *P. aeruginosa* (no colonization), *S. malthophilia*, *A. fumigatus*, and *Candida* spp. During these years she suffered multiple infectious exacerbations, responding well to (mostly) oral antibiotic courses.

In 2012, a throat swab (day 0) was positive with an unidentifiable yeast by MALDI-TOF MS. In the following years (up to day 1064), several throat swabs, sputum samples and bronchoalveolar lavage (BAL) were also positive with an unidentified yeast. The yeast grew on malt agar after two days at 37 °C. Two isolates were further analysed (from BAL on day 463 and a throat swab on day 777) and were shown to be *C. cyanovorans* based on ITS and LSU sequencing. The antifungal susceptibility profile was comparable to that of the other patient (Table 1). There were no specific symptoms or changes in the clinical course during the presence of *C. cyanovorans* (such as growth, weight gain, lung function, etc.). Her lung function remained quite well preserved, forced expiratory volume in one second (FEV1) around 70%, when she entered adulthood. Maintenance therapy consisted of flucloxacilline and she did not use antimycotics. She has never travelled to South Africa.

The three South African reference strains and clinical isolates were used to create reference mass spectra for the Westerdijk Institute MALDI-TOF MS in-house database. A dendrogram based on these spectra shows clustering of the patient strains (K314, patient 1 and G2100, patient 2) with the reference strains and clear separation from other yeasts (Fig. 1).

3. Discussion

This is the first report of *C. cyanovorans* cultured from the airways of CF patients. As far as we know, there are no earlier publications about

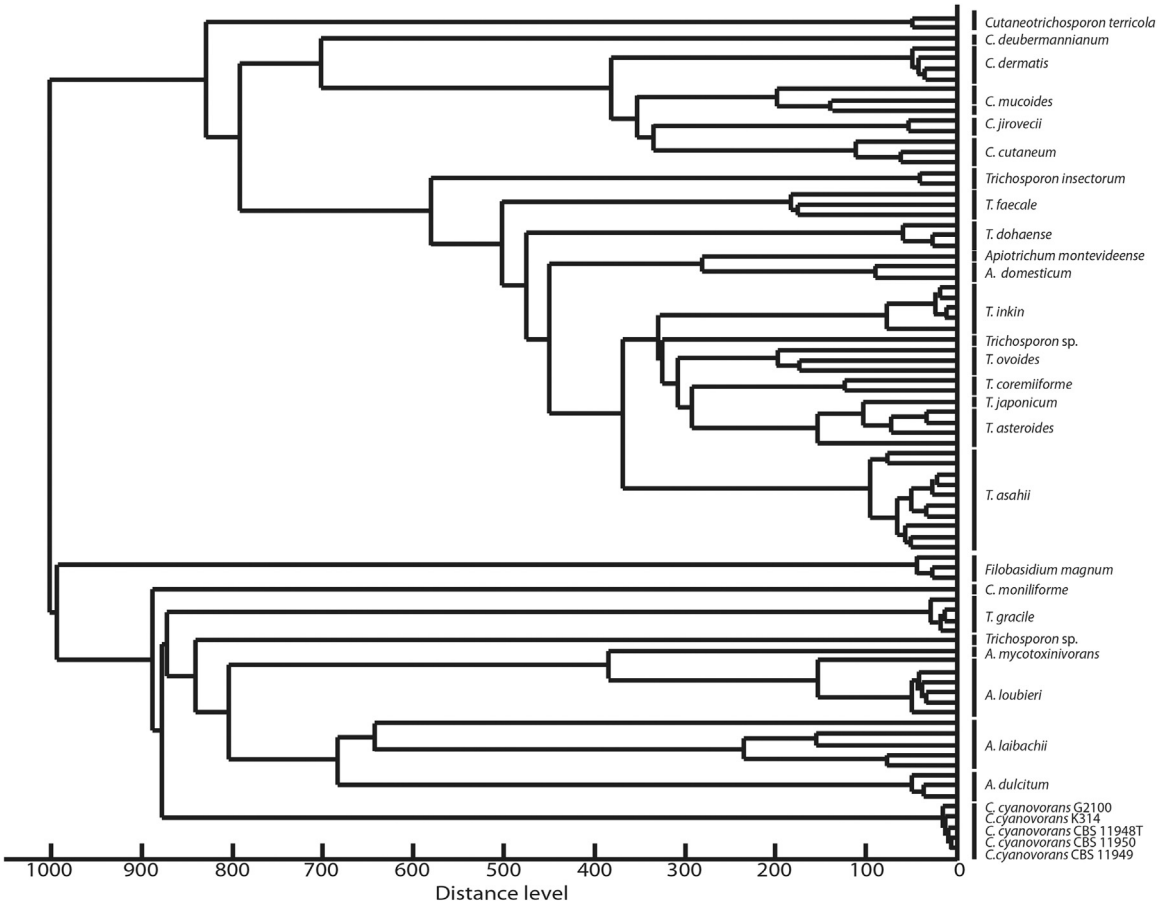

Fig. 1. MALDI-TOF MS based dendrogram.

C. cyanovorans as clinical isolate in any patient group, except an unpublished abstract in 2013 with *C. cyanovorans* cultured from a diabetic patient with rheumatoid arthritis and granulomatous lung nodules [2].

C. cyanovorans has recently been described as environmental yeast and is difficult to identify [1]. *C. cyanovorans* is currently not present in the commercial Bruker MALDI-TOF MS databases and only after ITS and LSU sequencing it was possible to identify the isolates correctly. Future investigations are needed to determine whether *C. cyanovorans* could be more often isolated from CF patients (or any other patient group), and if so to what extent. The expansion of the MALDI-TOF MS databases with *C. cyanovorans* could facilitate such studies. The clinical significance of *C. cyanovorans* cultured from CF patients remains unknown at present, since the numbers reported here are too small to draw conclusions.

In vitro, *C. cyanovorans* can grow in the presence of cyanide, using it as a source of carbon or nitrogen [1]. Interestingly, in BAL or sputum of CF patients, substantial levels of cyanide have been found [3,4]. In these studies, the presence of cyanide seemed to be associated with the presence of *Pseudomonas aeruginosa* and neutrophilic inflammation [3,4]. Possibly, the microenvironment in the lung could be advantageous for *C. cyanovorans* in some CF patients, although we were not able to perform a measurement of pulmonary cyanide levels in our patients.

It is unknown how these patients acquired *C. cyanovorans*. Both have never been to South Africa, the original place of discovery of the yeast. It is likely that the habitat of *C. cyanovorans* extends beyond South Africa and that the strains were acquired in other parts of the world. Alternatively, the patients might have been exposed to products imported from regions where *C. cyanovorans* is endemic. The herbal tea leaves of patient 1 were negative for *C. cyanovorans*, but there may be other, as yet unidentified, products as source of exposure.

In conclusion, this is the first study reporting *C. cyanovorans* as clinical isolate in two CF patients. Additional studies are needed to determine whether more colonized patients can be found, if cyanide could be a factor contributing to a favorable pulmonary environment and if colonization has clinical implications.

Conflict of interest

There are none.

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