CHARACTERIZATION OF BACTERIAL STRAINS WITH BIOTECHNOLOGICAL POTENTIAL FROM A PHARMACEUTICAL INDUSTRY

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ABSTRACT

Microorganisms isolated in pharmaceutical industry are important in tracking sources of contamination and some may have biotechnological applications. Despite the difficulty in identifying environmental strains, some cases are theoretically innovative. *Metabacillus idriensis*, that presents strong antibacterial and antioxidant properties and ability to be used as a natural dye, and *Agrococcus terreus* that has bioremediation abilities against heavy metals have been isolated in an immunobiological industry. The aim of this study was to characterize the strains B6389 and B6455 with phenotypical and molecular methodologies. After incubation in Tryptone Soy Agar, the strains were analyzed in VITEK®2 (bioMérieux), MALDI Biotyper® (Bruker) and VITEK®MS (bioMérieux), according to the manufacturers' instructions. Both strains were incorrectly identified by VITEK®2. B6389 was not identified by none of the MALDI-TOF MS equipments, while B6455 was only identified by MALDI Biotyper®. The strains were analyzed by 16S rRNA full gene sequencing, using the MicroSEQ™ Full Gene 16S rDNA kit and identified as *Metabacillus idriensis* and *Agrococcus terreus*, respectively. The results show that many strains may be incorrectly identified over time, depending on the methodology used, which may result in strains with biotechnological potential not being identified and therefore being less studied.

Keywords: *Metabacillus idriensis. Agrococcus terreus.* Biotechnological potential. MALDI-TOF MS. 16S rRNA sequencing.

1 INTRODUCTION

The pharmaceutical industry has great concerns with the presence of contaminants in its production line, especially for sterile products. According to Good Manufacturing Practices, it is necessary to adopt microbiological control programs to obtain valuable information about microorganisms and to track the sources of contamination as well as to implement appropriate preventive and corrective actions [1,2].

The correct identification of microorganisms also allows the detection of species with biotechnological potential, such as *Metabacillus idriensis* and *Agrococcus terreus*. The species *M. idriensis* is considered a mesophilic, obligately aerobic, spore-forming human pathogen, originally isolated from a newborn with sepsis [3], which has strong antibacterial and antioxidant properties and also has the potential to be used as a dyeing agent for textiles, suggesting that it can be used as a natural dye substitute for synthetic dyes. Furthermore, it is estimated that the production of orange pigment has a yield of 400 µg of carotenoid/g of biomass. Furthermore, the pigment exhibited antibacterial activity against *Yersinia enterocolitica, Staphylococcus aureus* and *Escherichia coli* and antioxidant potential in the DPPH and ABTS assays [4].

Another example is *A. terreus,* an aerobic, mesophilic, and Gram-positive bacterium that was originally isolated from forest soil [11], which has bioremediation potential for heavy metals such as iron, copper, manganese, cobalt, silver, zinc, nickel and arsenic, which commonly accumulate in soils due to the dumping of industrial waste and sewage [5].

The aim of this study was to characterize two strains with potential biotechnology applications isolated from a pharmaceutical industry facility with phenotypical and molecular methodologies.

2 MATERIAL & METHODS

The strains B6389 and B6455 isolated from a pharmaceutical facility producing immunobiologicals had been previously characterized after Gram staining, micro and macroscopic observation, and further analysed by the VITEK®2 Compact System (bioMérieux, France), according to manufacturer`s instructions.

Stock cultures of these strains were prepared and maintained at <-70°C in 30% Difco™ Skim Milk (BD Biosciences, France) containing 30% glycerol (Merck KGaA,). For daily use, strains were seeded on Tryptone Soy Agar (TSA) plates and incubated at 30-35°C for 24-72 h.

Proteomic characterization by Matrix-Assisted Laser Desorption Ionization – Time of Flight Mass Spectrometry (MALDI-TOF MS) was performed using two systems: MALDI Biotyper® (Bruker Corporation, USA) and VITEK® MS RUO (bioMérieux, France), according to the manufacturers` instructions. Results were reported by MALDI Biotyper® as score values of 0.00-1.69 (no organism identification possible), 1.70-1.99 (low confidence species identification), and 2.00-3.00 (high confidence species identification). VITEK®MS RUO's confidence interval was reported as a percentage value, and the strains were considered identified when a percentage ≥75.0 was obtained. For analysis of the two strains, a pre-extraction step was required with 70% formic acid (v/v) or ethanol + 70% formic acid (v/v) + acetonitrile, after which the supernatant was applied, in duplicate, in a specific slide for each equipment and, after drying, 1 µL of alpha-cyano-4-hydroxycinnamic acid matrix (VITEK MS-CHCA; bioMérieux, France; Bruker Matrix HCCA, Bruker Corporation, USA) was added to the smear of each slide. The results were analyzed with the Saramis Premium program (version 4.0.0.14) for VITEK®MS and with the MBT Compass HT, version 4.1.100 for MALDI Biotyper® 2.0.

The full 16S rRNA gene sequencing was performed using the MicroSEQ™ Full Gene 16S rDNA kit (Thermo Fisher Scientific, USA) according to the manufacturer's instructions. Sequences were obtained using the 3500 Series Genetic Analyzer (Applied Biosystems, USA) and processed using the DNA Star LaserGene SeqMan software, version 7.0.0. The identification results were obtained from the website https://www.ezbiocloud.net/ (database update: August 23, 2023) [6]. All sequences were deposited at https://www.ncbi.nlm.nih.gov/ (database update: December 29, 2023). Identification at species level by 16S rRNA gene sequencing was considered valid when the identification percentage was ≥98.7 [7].

The alignment of 16S rRNA gene sequences was performed with BioEdit Sequence Alignment Editor software, version 7.0.5.3. [8]. A phylogenetic tree based on multiple alignments of 16S rRNA gene sequences was constructed using the Neighbor-joining and the ClustalW algorithm with the software MEGA 11 and Kimura two-parameter model with branch support based on 1,000 bootstrap replicates [9].

The two strains were deposited at the Coleção de Bactérias do Ambiente e Saúde (CBAS) hosted at Fundação Oswaldo Cruz (Fiocruz), Rio de Janeiro, Brazil (www.cbas.fiocruz.br). CBAS is affiliated with the World Federation for Culture Collections and registered as World Data Centre for Microorganisms 958 (http://www.wfcc.info/ccinfo/collection/by_id/958).

3 RESULTS & DISCUSSION

The strains B6389 and B6455 had been previously characterized as Gram-positive bacteria after Gram staining, micro and macroscopic observation, and further identified by VITEK®2 as *Bacillus sporothermodurans* and *Micrococcus luteus/Micrococcus lylae*, respectively (Table 1.).

Table 1 Results of identification of the strains by the VITEK ® 2 Compact System.

Strain	Source	Year	Morpho-dye characteristics	VITEK®2		
				Card selected	Result	Confidence (%)
B6389	Human Monitoring	2013	Gram-positive bacilli	BCL	*Bacillus sporothermodurans*	Very good identification (95%)
B6455	Not informed	2013	Small, irregular, coccoid to short rod cells, Gram-positive	GP	*Micrococcus luteus/ Micrococcus lylae*	Low discrimination

The proteomic and molecular characterization of the two strains are showed in Table 2.

Table 2 Results of MALDI-TOF MS and 16S rRNA sequencing analysis of the strains evaluated in the present study.

Strain	VITEK® MS (%)	MALDI Biotyper® (Score)	16S rRNA sequencing (% of similarity)
B6389	Not Identified (0.0%)	Not Identified (1.39)	*Metabacillus idriensis* (99.35%)
B6455	Not Identified (0.0%)	*Agrococcus terreus* (2.19)	*Agrococcus terreus* (99.51%)

Identification by VITEK®2 (Table 1) was considered incorrect after comparison with 16S rRNA sequencing. This occurrence may be related to the fact that the equipment database has mainly clinical species [10,11]. B6389 strain was incorrectly identified by VITEK®2 as *Bacillus sporothermodurans* with very good confidence and not identified by both MALDI-TOF MS equipment's (Table 2). B6455 strain was incorrectly identified by VITEK®2 as *Micrococcus luteus/ Micrococcus lylae* but was correctly identified by both MALDI Biotyper® as *Agrococcus terreus* (Table 2). The species *M. idriensis* was described by Ko et al [12] in 2006 and the species *A. terreus* was descried by Zhang in 2010 [4]. None of them were present in VITEK®2 database. This fact reinforces the necessity of constant improvement of manufacturers` database to provide correct identification. Due to this fact, many *M. idriensis* and *A. terreus* strains with potential biotechnological approaches may have been isolated along time and not selected to further studies due to incorrect identification. The two strains were subjected to 16S rRNA gene sequencing, providing fragments of 1374 and 1344 base pairs (bp) that were analyzed in the EzBioCloud database, in which the both were identified at the species level (Table 2).

Considering the results of 16S rRNA sequencing as true-positive results, the strains that were not identified by MALDI-TOF MS can be used to expand its databases to allow further identification of *A. terreus* and *M. idriensis* strains [13,14]. As the strains were deposited at the CBAS (Fiocruz), other researchers can requisite these strains to develop studies about their biotechnological applications.

4 CONCLUSION

The phenotypic methodologies, VITEK®2 and MALDI-TOF MS were not able to correctly identify the strain B6389 as *M. idriensis*. However, the B6455 strain could be identified as *A. terreus* by MALDI Biotyper®. This demonstrates the importance of genotypic techniques for the characterization of environmental isolates to further expand the MALDI-TOF MS databases to include these species.

Many strains with biotechnological properties may have been incorrectly identified over time, hindering the study of their industrial applications, what demonstrates the relevance of seeking increasingly appropriate methodologies for the species-level identification of environmental origin microorganisms.

In addition, encouraging deposits in biological collections, whose analysis are based on polyphasic taxonomy (phenotypic and genotypic methodologies to achieve the lowest taxonomic level of identification), is necessary for the authentication of the strains and to make them available to the scientific community, strengthening the Health Industrial Economic Complex.

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