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SHORT COMMUNICATION

First report of *Staphylococcus* isolates identified by genomic analysis from rhizospheric soils of *Capsicum annuum* L. cv Piquillo

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Abstract

The genus *Staphylococcus* comprises many species which can be isolated from many sources and could display plant growth-promoting properties. Moreover, *Capsicum* species are important export crops in Peru, which have gained greater interest in recent years. Therefore, the objective of this research was to identify *Staphylococcus* isolates from rhizospheric soil samples of *C. annuum* cv. Piquillo in La Libertad, Peru. Bacterial isolates were identified by genomic analysis targeting the 16s rRNA gene. Bacteria were isolated from samples by serial dilutions and cultured in solid medium agar plates. Then, genomic DNA extraction from pure and morphologically distinct isolates, 16s rRNA gene amplification, sequencing and bioinformatic analysis were performed. We found four bacterial isolates from the genus *Staphylococcus* not previously reported in *C. annuum* rhizospheric soils: Isolate Ca2 and Ca5 which both match to *Staphylococcus* sp., isolate Ca6 to *Staphylococcus arlettae* and isolate Ca7 to *Staphylococcus xylosus*. Further studies to assess these isolates' impact on crops as well as their potential applications in other fields of research such as antimicrobial development, food processing and pesticide biodegradation are recommended.

Keywords: *Staphylococcus*; 16s rRNA gene; *Capsicum annuum* L.; rhizospheric soils.

1. Introduction

The genus *Staphylococcus* currently comprises more than 80 species and subspecies according to the website of the List of Prokaryotic names with Standing in Nomenclature by Leibniz Institute DSMZ-German Collection of Microorganisms and Cell Cultures GmbH (https://lpsn.dsmz.de/genus/staphylococcus, consulted on March of 2020). Bacteria belonging to this genus are generally Gram-positive, nonmotile, aerobes, catalase-positive, oxidase-negative and able to grow between 18 and 40 °C (Oh *et al.*, 2019; Schleifer and Bell, 2015). Regarding the phylogeny, the genus *Staphylococcus* belongs to the *Staphylococcaceae* family and the *Bacillales* order, which shares with species of different genera such as *Bacillus*, *Listeria*, *Paenibacillus* as well as *Macrococcus*, *Enterococcus*, *Streptococcus*, and *Lactobacillus* all as part of the phylum *Firmicutes* (De Vos, 2015; Schleifer and Bell, 2015).

Besides the importance of *Staphylococcus* as a pathogenic agent of humans and several animals species, from fish to cattle, they could also act as infective agents of plants under certain conditions as described in *Arabidopsis thaliana* (Canovas *et al.*, 2016; Oh *et al.*, 2019; Prithiviraj *et al.*, 2005). Moreover, apart from the conventional sources of staphylococci mainly recovered from clinical samples extracted from human and animal skin, glands, and mucous membranes, staphylococci can be isolated from diverse sources such as air, water and soil (Correia *et al.*, 2019; Kozajda *et al.*, 2019; Mandal *et al.*, 2015; Schleifer and Bell, 2015).

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The importance of staphylococci go beyond their role as pathogens due to their capacity to produce antimicrobial peptides (Bastos *et al.*, 2009), their role in meat fermentation processes (Stavropoulou *et al.*, 2018) and mainly because of the beneficial effects on plants of some strains that inhabit the rhizosphere, as described for mangrove trees and *Leptochloa fusca* (Holguin, 1992; Shahid et al., 2019). These strains could be considered as rhizobacteria owning attributes related to plant growth promotion such as the capacity to fix nitrogen as well as to produce phytohormones and specific compounds (Holguin, 1992; Shahid *et al.*, 2019). *Capsicum annum* L. is an important crop in Peru. Members of the genus *Capsicum* belong to the Solanaceae family and are native of subtropical and tropical areas in America (Tripodi and Kumar, 2019). The cultivar Piquillo is one of five domesticated crops of *C. annuum* which is widely cultivated and studied along with its two variants: hot pepper as a spice crop and sweet pepper as a vegetable crop (Jindal *et al.*, 2020; Khoury *et al.*, 2020). *C. annuum* cv. Piquillo is an important export crop in Peru which sends this commodity as raw material and other derivate products to the European and American market (Agraria.pe, 2019; MINAGRI and ProInversion, n.d.). In recent years, along with the promulgation of the Plan of sustainable development of species of the *Capsicum* genus 2018-2028: peppers and chilies, devised by The Ministry of Agriculture of Peru, greater interest has been taken into this crop (MINAGRI, 2017). Therefore, the objective of this research was to identify *Staphylococcus* isolates from rhizospheric soil samples of *C. annuum* cv. Piquillo.

2. Materials and methods

Bacteria were isolated from rhizospheric soil samples of cultivated *Capsicum annuum* L. cv Piquillo areas under open-field conditions in January 2018 in Viru, La Libertad, Peru (08°24'18" S, 78°51'18" W). Samples were collected and kept under refrigeration during transportation to the laboratory and stored at 4 °C. Then, morphologically distinct bacteria were isolated by serial dilutions and cultured in Tryptic soy agar (TSA) and Reasoner's 2A (R2A) agar plates at 28 °C for up to five days. Isolates were labeled as Ca2, Ca5, Ca6 and Ca7. After, bacterial genomic DNA extraction from each pure isolate, 16s rRNA gene amplification, sequencing and bioinformatic analysis were performed according to the protocol described by Belgini *et al.*, 2014. Briefly, genomic DNA was extracted following the CTAB method and DNA integrity and concentration were estimated through electrophoresis in 0.8 % agarose gel stained with SYBR Safe 10.000x in DMSO (Invitrogen). DNA obtained was used in polymerase chain reaction (PCR) reactions for amplification of 16S rRNA using the primers 10f (5'-GAGTTTGATTCAGGCCCTG-3') and 1100r (5'-GTTGTGAGGGTTGGGG-3'). PCR products were purified using mini-columns (GFX PCR DNA and Gel Band Purification Kit, GE Healthcare) and subjected to sequencing in an automated sequencer ABI 3500XL Applied Biosystems™. Partial 16S rRNA consensus sequences were obtained using the BIOEDIT software (Hall, 1999) and were compared with those of reference type strains available in EZBioCloud (https://www.ezbiocloud.net/) and RDP (Ribosomal Database Project, Wisconsin, USA https://rdp.cme.msu.edu/) databases. The sequences were aligned using the CLUSTAL X program (Thompson *et al.*, 1997) and analyzed with MEGA 7 software (Kumar *et al.*, 2016). The evolutionary distances were calculated using the Kimura DNA substitution model (Kimura, 1980) and the phylogenetic reconstruction was done using the neighbor joining (NJ) algorithm (Saitou and Nei, 1987), with bootstrap values calculated from 1,000 replicates.

3. Results and discussion

We identified four bacterial isolates from the genus *Staphylococcus* not previously reported in *C. annuum* rhizospheric soils: Isolate Ca2 and Ca5 which both match to *Staphylococcus* sp., isolate Ca6 to *Staphylococcus arlettae* and isolate Ca7 to *Staphylococcus xylosus*.

The presence of *Staphylococcus* in rhizospheric soils could be explained as bacteria from that genus have been described as ubiquitous, being isolated from many sources such as beach sand, sea water, fresh water, plant surfaces and products, feeds, meat and poultry, dairy products, dust, air and soil (Schleifer and Bell, 2015). Nonetheless, studies reporting *Staphylococcus* present in rhizospheric soils are still scarce. López *et al.* (2009) found a mangrove rhizosphere-associated *Staphylococcus* sp., Leiva *et al.* (2013) reported a phosphate-solubilizing *S. vitulinus* present in the rhizosphere of cacao, and Shahid *et al.* (2019) identified two *Staphylococcus* strains which promoted the growth of maize under salt stress.

Table 1
Morphological description and molecular identification of *Staphylococcus* isolates from rhizospheric soil samples of *C. annuum* cv Piquillo. Cell and colony morphology of each isolate are described. Closest GenBank (www.ncbi.nlm.nih.gov/) match's accession number and similarity percentage are shown. +ve. Gram-variable

Isolate	Cell morphology	Colony morphology	Closest GenBank match	Similarity (%)	Molecular ID
Ca2	Gram-positive cocci. +ve	Whitish, even surface and circular regular edges.	AY953148	99.76	*Staphylococcus* sp.
Ca5	Gram-positive cocci	Whitish, even surface and circular regular edges.	AY953148	99.85	*Staphylococcus* sp.
Ca6	Gram-positive cocci. +ve	Translucent and shiny, even surface and curved regular edges.	AB009933.1	100	*Staphylococcus arlettae*
Ca7	Gram-positive cocci	Cream-colored, even surface and circular regular edges.	MRZO01000018	99.73	*Staphylococcus xylosus*

Regarding *S. arlettae* and *S.xylosus*, the former has been isolated from poultry and goats, but has also been reported in cardamom rhizosphere as a bacteria with bioremediation potential to degrade fipronil residues (At *et al.*, 2019; Schleifer and Bell, 2015); the latter, is able to grow in habitats that contain only an inorganic nitrogen source and thus could be more free-living than other staphylococci; besides, it has been isolated from beach sand, natural waters, marsh grass, and plant products, and has also been found in the rhizosphere of potato (Berg *et al.*, 2005; Schleifer and Bell, 2015).

Studies concerning rhizospheric microbiome profiling, isolation and identification of rhizobacteria from *C. annuum* report bacteria mainly from *Gammaproteobacteria* and *Bacilli* classes, *Serratia* and *Bacillus* genera (Asaff-torres *et al.*, 2017; González *et al.*, 2017). Particularly, *S. aureus* has been reported in the rhizosphere of cultivated *C. chinense* (Chinakwe *et al.*, 2019). Despite the above, we have not found reports of *Staphylococcus* present in rhizospheric soils of *C. annuum*. The isolates found in the present research could have the potential to promote plant growth or to be utilized for ecological, industrial and agricultural purposes, therefore, further studies to assess their role in the rhizosphere and their effect on *Capsicum* and other crops are suggested.

4. Conclusions

Not previously reported *Staphylococcus* sp., *Staphylococcus arlettae* and *Staphylococcus xylosus* isolates from *C. annuum* rhizospheric soils were identified. These isolates could have potential applications in agriculture. Further studies to determine their ecological role in the rhizosphere as well as their potential applications in antimicrobial development focusing on antimicrobial peptide production, food processing regarding meat fermentation processes and pesticide biodegradation as reported for fipronil residues in soils are recommended.

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