Identification of quinoa seed bacterial endophytes and selection of strains for biofertilization of quinoa crops

Paula García-Fraile[1,2,3], José David Flores Félix[1,2,3]; Fernando Sanchez-Juanes[4], Raúl Rivas[1,2,3], Pedro F. Mateos[1,2,3], Álvaro Peix[2,3,5], José M. Igual[2,3,5], Encarna Velázquez[1,2,3], Eustoquio Martínez-Molina[1,2,3]

[1]Departamento de Microbiología y Genética, Universidad de Salamanca, Salamanca, Spain. [2]Instituto Hispanoluso de Investigaciones Agrarias (CIALE), Salamanca, Spain. [3]Unidad Asociada USAL-IRNASA (CSIC), Salamanca, Spain. [4]Instituto de Investigación Biomédica de Salamanca (IBSAL), Complejo Asistencial Universitario de Salamanca, Universidad de Salamanca, CSIC, 37007 Salamanca, Spain . [5]Instituto de Recursos Naturales y Agrobiología, IRNASA-CSIC, Salamanca, Spain

INTRODUCTION

Quinua, quínoa or quinua (from the quechua kinwa or kinuwa), whose scientifc name is Chenopodium quinoa Willdenow, is a plant of the family Amaranthaceae, subfamily Chenopodiaceae native to the Andean region of South America. Its seeds, which do not contain gluten, have higher nutritional value than cereals because of its high protein content and its balanced content among proteins, carbohydrates and fats. Coming from America, in recent years its consumption has became popular in European countries, where its cultivation has been recently introduced. In Spain, quinoa is mainly produced in the region of Andalucía, but its cultivation is beginning to be tested in other regions such as Castilla y León.

So far, there are no studies about quinoa endophytic bacteria and their potential as biofertilizers for this plant. In this study we have isolated and identify the endophytic bacteria of seeds from quinoa obtained after the cultivation of this plant in a soil from Ciudad Rodrigo located at the province of Salamanca, in the Castilla y León region.

MATERIALS AND METHODS

The isolation of endophytic bacteria from quinoa seeds was performed according to Verma et al., (2017).

The samples preparation, to perform MALDI-TOF MS analysis was performed as previously described (Ferrerira et al., 2011).

The 16S rRNA gene sequencing was performed as described by Carro et al. (2012).

The microcosms assays were performed as was previously described by García-Fraile et al. (2012)

Table 1. Identification by MALDI-TOF MS of strains isolated from quinoa seeds

CRCQ 1	*Bacillus subtilis*	1.758
CRCQ 2	*Bacillus subtilis*	1.774
CRCQ 3	*Bacillus licheniformis*	1.98
CRCQ 4	*Bacillus atrophaeus*	1.862
CRCQ 5	not reliable identification	1.661
CRCQ 6	*Staphylococcus capitis*	2.314
CRCQ 7	*Staphylococcus hominis*	2.255
CRCQ 8	*Staphylococcus capitis*	2.417
CRCQ 9	*Staphylococcus hominis*	2.264
CRCQ 10	*Pantoea agglomerans*	2.001
CRCQ 11	*Pantoea agglomerans*	2.007
CRCQ 12	*Pantoea agglomerans*	2.005
CRCQ 15	*Bacillus megaterium*	2.152

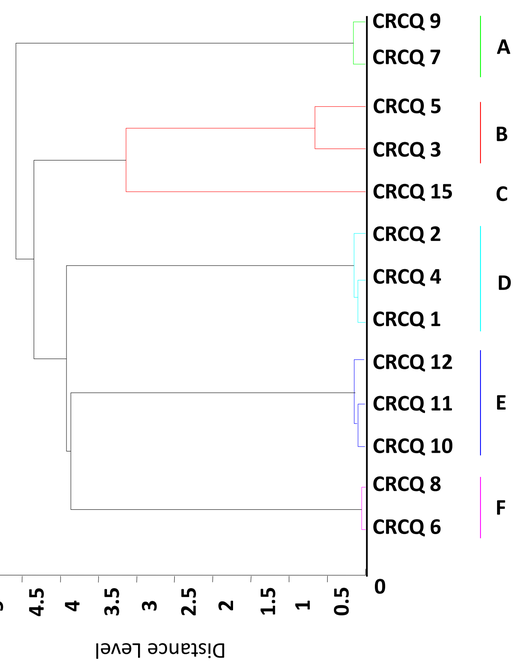

Figure 2. Dendrogram obtained with correlation average and UPGMA

Table 2. Identification by MALDI-TOF MS and 16S rRNA gene sequencing of quinoa seeds strains

Strains	MALDI-TOF MS group	MALDI-TOF MS matching species	Score values	Closest species 16s rRNA gene	Similarity (%)
CRCQ 7, CRCQ 9	A	*Staphylococcus hominis*	> 2.2		
CRCQ 3, CRCQ 5	B	Not identified	< 2.0	*Bacillus licheniformis*	100
CRCQ 15	C	*Bacillus megaterium*	2.152		
CRCQ 1, CRCQ 2, CRCQ 4	D	Not identified	< 2.0	*Bacillus siamensis*	99.9
CRCQ 10, CRCQ 11, CRCQ 12	E	*Pantoea agglomerans*	1.7-2.0		
CRCQ 6, CRCQ 8	F	*Staphylococcus capitis*	> 2.3		

Strains marked in blue ink were selected as representative of MALDI-TOF MS groups which were not identified by this methodology

Table 3. Effectiveness of selected strains on quinoa plants in microcosm conditions

Treatment	Shoot) dry weight (g)/ plant (± S.E.)	Weigth of 250 seeds (mg) (± S.E.)
Aldearrubia soil		
Uninoculated fertilized plants	0.333 (±0,02) a	598.3 (±34.7) a
SECRCQ04	0.206 (±0,02) b	659.3 (±13.38) a
SECRCQ05	0.281 (±0,10) a	658.7 (±5,60) a
Barbadillo soil		
Uninoculated fertilized plants	0.267 (±0,22) a	560.0 (±11.5) a
SECRCQ04	0.257 (±0,36) a	632.3 (±13.38) a
SECRCQ05	0.251 (±0,24) a	583.0 (±30,60) a

Values followed by the same letter in each treatment are no significantly different from each other at P=0.05 according to Fisher's Protected LSD (Least Significant Differences). S.E.= Standard Error

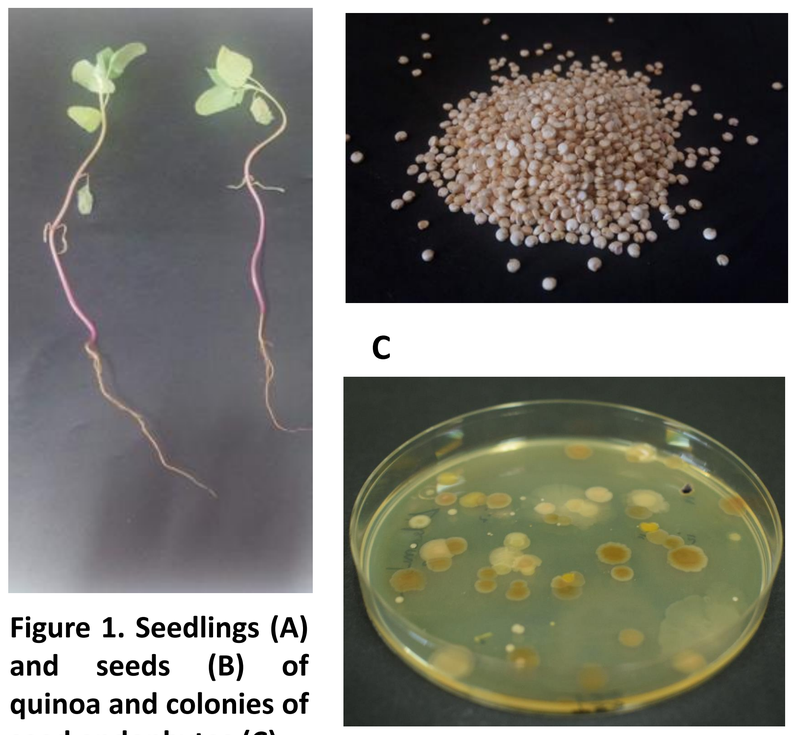

Figure 1. Seedlings (A) and seeds (B) of quinoa and colonies of seed endophytes (C)

RESULTS

From the quinoa seeds 12 bacterial strains were isolated on TSA (Figure 1). The results of the identification using MALDI-TOF MS (Table 1) showed that the isolated strains were classified at genus level (score values higher than 1.7) within the Gram negative genus *Pantoea* and the Gram positive genera *Bacillus* and *Staphylococcus*. The strains were identified as species level (score values higher than 2.0) as *Pantoea agglomerans, Bacillus megaterium*, *Staphylococcus capitis* and *Staphylococcus hominis*.

The spectra of isolated strains were mathematically analysed and the obtained dendrogram is recorded in figure 2. The strains were distributed into 6 groups with distance levels higher than 1 (figure 2 and Table 2).

The strains not identified at species level by MALDI-TOF MS belonging to the groups B and D were identified by 16S rRNA gene sequence analysis (Table 2). The strains CRCQ04 and CRCQ05 representative of groups B and D, respectively, presented 100% and 99.9% similarity with the type strains of *B. licheniformis* and *Bacillus siamensis*, respectively.

These two strains were inoculated on quinoa plants in a microcosms assay using two soils collected in the Salamanca province and the results showed that despite the dry weight per plant was lower in the inoculation treatments in comparison to the fertilized control, the weight of the collected seeds was similar or even higher in the two inoculated treatments. These results suggest that the biofertilization could be a reliable alternative to the chemical fertilization for quinoa crops. Nevertheless, these results should be confirmed in field assays before to design biofertilizers for quinoa crops.

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