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Study of natural antagonists of potato cyst nematode "*Globodera* sp." in west Algeria: Nematological analysis

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Abstract

Potato (*Solanum tuberosum* L.) is among the most dominant crops in Algeria. However, it is confronted by many pests like potato cyst nematodes (PCN) *Globodera* sp. It is among the most important pests that can cause enormous damage and loss of yield each year. This study highlighted morpho-identification and PCN density in 4 different regions of west Algeria, including Tiaret, Saida, Mascara and El Bayadh as well as its association with some microorganisms (bacteria identified by Maldi-TOF-MS method) and fungi (identified by macroscopic and microscopic examinations) that might have biological control activity on PCN. Identification of 8 bacterial isolates by MALDI-TOF-MS technique revealed that 50 % of isolated bacteria belong to the genus *Bacillus*; *B. megaterium* for the isolates B15 with a score of 1.994 and B1 with a score of 1.882 and *B. cereus* for the isolate B20 the score value is 1.892 and 50 % to the genus *Pseudomonas* with different score values; P1 for a score of 2.294 identify as *P. fulva* P4 showed a score value of 1.974 to *P. corrugata,* P2 with a score of 2.148 *P. stutzeri* and P3 with a score of 2.009, *P. kilonensis*. Macroscopic and microscopic examination of fungal flora revealed high diversity: 7 orders were isolated, among which the most frequent were Trichocomaceae (30 %) and Pleosporaceae (22 %), while the less frequent were Pythiaceae (5 %) and Davidiellaceae (5 %). The genus *Aspergillus* recorded a frequency of 59.38 % and the genus *Penicillium* sp. was detected in 46.88 % of the cyst populations studied.

Keywords

Globodera sp.; *Solanum tuberosum* ; biological control; bacteria; fungi; Maldi-TOF-MS

Introduction

Potato is amongst the dominant crops in the world, ranked fourth after wheat, rice and maize (1). In 2020, its world production reached 359071403 tons (2). Algeria is one of the most potato producers in Africa, with more than 4.65 million tons over the year and 149465 ha of cultivated area. However, potato cultivation faces a substantial number of challenges o as a host plant for several pests and parasites (3).

One of the most threatening is potato cyst nematode (PCN) caused by *Globodera* sp. PCN round vermiform organism is a sedentary endoparasite regrouping 2 species *Globodera pallida* and *G. rostochiensis,* which are specific to Solanaceae, mainly potato *Solanum tuberosum* (4). PCN are typically temperate species and thus require a strategy to postpone develop-

ment until favorable conditions return after winter is complete.

After molting from J1 to J2 (Fig. 1), juvenile *G. rostochiensis* enter into immediate obligate diapauses when soil temperatures decrease. After the juvenile nematode hatches, it becomes dormant and remains in that state until it detects a signal or cue from the host. If no such stimuli are detected, the nematode continues to remain dormant until it detects a signal or cue. By utilizing diapauses and quiescence, PCN can survive in a dormant state in the soil for up to 20 years while waiting for a signal or cue from a host (5). PCN can also grow on tomatoes, eggplants and peppers as well as some weeds (6), causing yield losses of up to USD 80 billion annually.

Nearly all losses occur in potato-producing regions where few or no management measures are applied (7). Both species are classed as quarantine organisms due to their hazardous potential. In 1975, they were added to the European and Mediterranean Plant Protection Organization; the population density of the nematode as well as the type of cultivars, agricultural practices and environmental conditions was reported to a have huge impact on plant damage (8). A variety of methods and main strategies can be used to resolve this problem, such as the utilization of resistant cultivars and the chemical application of nematicide (9). Several nematicides have been withdrawn from the market, including Vydate® 10 g, which was banned in the UK in December 2020 due to its negative impacts on human health and the environment. It is thus, important to explore innovative alternatives as control measures. Mainly investigating natural products and resources (10). Inside and outside areas of PCN cysts, due to their specific habitats, harbor numerous microorganisms that can lead to cyst death and population decline, suggesting that they can be potential candidates for their use in biocontrol (11). Several germs, including bacteria and fungi, have shown antagonistic efficacy on nematodes and were used efficiently in biological control as an ecological and rational approach by controlling nematode populations in the soil and contributing to crop damage reduction or complementary way to physical and chemical measures for over 20 years (12). Bacteria are one of the major microorganism groups that can be used as effective biological control agents against PCN (13). They can benefit plant development in numerous ways, including the production of chemicals suppressing the pathogen's growth or lyse their cell walls (14, 15). The aim of this study is to perform nematological analysis of potato plots by morphometric

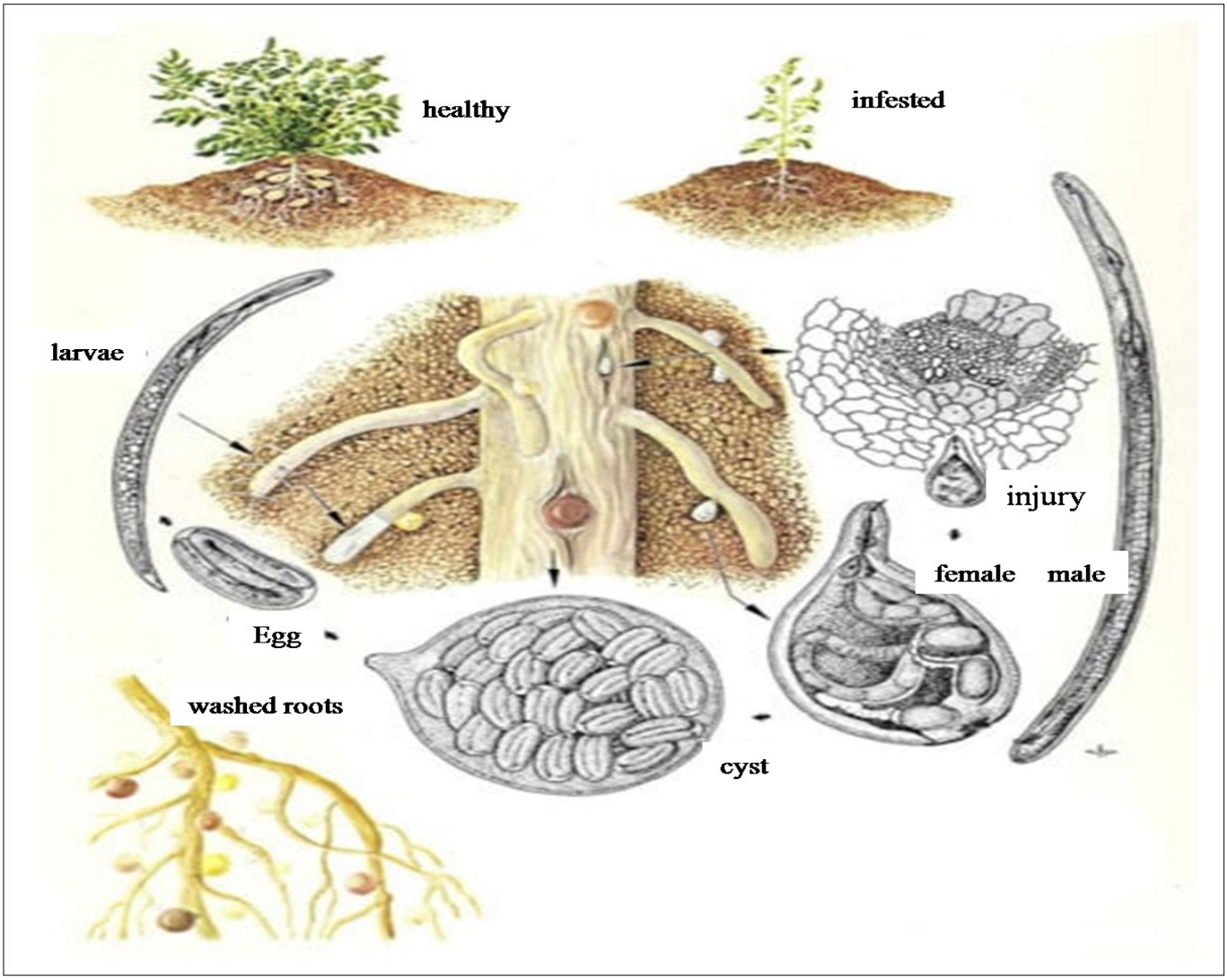


Fig. 1. Illustration of the life cycle of *Globodera rostochiensis* (modified after Charles S Papp, Exclusion and Detection, Plant Pest Detection Manual 5:1, California Department of Food and Agriculture, Division of Plant Industry, USA).

identification of *Globodera* sp. cysts and the isolation and identification of antagonistic bacteria and fungi from cysts.

Materials and Methods

Soil sampling

Study area

Surveyed region (Fig. 2) was composed of 4 producing regions: Mascara (Latitude: 35.3966400° Longitude: 0.1402700°), Saida (Latitude: 34.8303300° Longitude: 0.1517100), Tiaret (Latitude: 35.3710300° Longitude: 1.3169900) and El-Bayadh (Latitude: 33.6831800° Longitude: 1.0192700°) located in the west of Algeria. It is limited to the north by the Mediterranean, to the west by Morocco, to the south-west by Chott Chergui, to the south by the high plains of Sersou and to the east by the Monts de l 'Ouarsenis and the lower Chlef valley; physical assembly is characterized by a semi-arid Mediterranean climate with hot summer and temperate winter (16**).**

Soil preparation

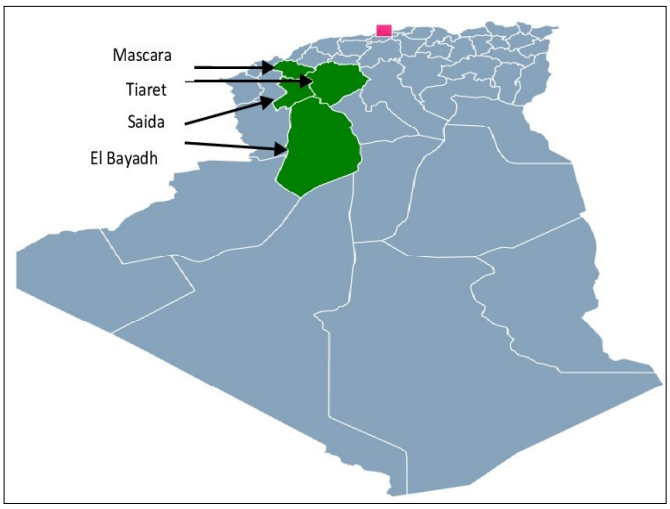


Fig. 2. Delimitation of the study areas.

A total of 64 elementary soil samples (4 samples per plot) from 16 mono potato plots of 3 to 5 ha were taken from 2017 to 2018, characterized by a minimum cultivation period of 10 years with 3 different varieties (Desire, Spunta, Kondor) belonging to the 4 regions (Table 1). Soil samples were collected using an auger from the upper layer of soil at 30 to 40 cm using the standard sampling technique (17). Elementary soil samples of each plot were mixed (1 kg) and 200 g of soil was taken and analyzed. They were transported to the laboratory in sealed and labeled plastic bags and dried for 48 h at room temperature.

Table 1. Geographical coordinates of the potato plots and the variety used.

Department	Variety of potato	Potato plot	Latitude	Longitude	Altitude
Tiaret	Desire	1	N3523147	E00136449	ALT950
		2	N3523325	E00136310	ALT943
		3	N3525225	E00135105	ALT945
		4	N3523105	E00136346	ALT942
Saida	Kondor	1	N3503035	E00040575	/
		2	N3505315	E00036965	/
		3	N3505352	E00037056	/
		4	N3506895	E00036187	/
Mascara	Desire	1	N351607	E 0000129	/
		2	N351632	E0012106.2	/
		3	N325700.2	E0012105.4	/
		4	N325700.8	E0012057.4	/
ELbayadh	Spunta	1	N325654.5	E0012100.6	/
		2	N325654.2	E0012106.2	/
		3	N325700.2	E0012105.4	/
		4	N325700.8	E0012057.4	/

Isolation and identification of Globodera cysts

Sixteen populations of cyst nematode potatoes from each plot (20 cysts per population) were considered for this study. Cyst nematode populations were collected from soil samples naturally infested with PCN. The cysts were isolated using the Fenwick scan method (18). This technique was based on flotation, which separates cysts from plant debris. Fenwick's apparatus was filled to the brim and analyzed soil was driven by a water stream through the sieve of 2 mm into a funnel, which dipped into the apparatus. Floating cysts were carried by overflow before their rehydration and retained by a sieve of 63 µm. The contents of the sieve were collected by spraying water into a filter paper carried by the funnel of an Erlenmeyer flask. After paper drains, cysts were examined under a binocular magnifying glass. The average density for the same plot or the same locality (N) was calculated using the following equation (19):

$$N = \frac{\text{number of full cysts extracted from all the samples}}{\text{total weight of all the samples}}$$

Identification of "*Globodera* sp." was based on morphometric characters of the vulval cone of cysts. The perineal region was examined after cyst cleaning in order to remove soil debris. Cysts were placed in a watch glass containing distilled water and purified using a fine brush.

Cysts were emptied of their contents (larvae and eggs) and peripheries were cut with a scalpel. Preparation was then covered with a slide to measure the morphometric criteria of the perineal region (10).

Morphometric criteria determination

Morphometric analysis of potato cyst nematode was performed on 320 cysts (20 cysts from 16 plots) from 16 Algerian populations 20; cysts were taken from each population. Cyst diameter, length and neck length were measured under a binocular magnifying glass using a micrometer scale. Perineal regions were examined under a light microscope by placing cysts in a drop of distilled water and making cross-sections with an ophthalmic scalpel at the level of the posterior third (20). Morphometric criteria of the perineal region were measured, including the diameter of the vulval slit, the distance between the anus and the vulval slit and the number of cutmarks between the anus and the vulval slit. Granek's ratio (distance between the anus and the vulvar window/diameter of vulvar window) was also calculated. The averages of morphometric criteria calculated were compared with that of earlier reports (21, 22), as summarized in Table 2.

Isolation of accompaniment micro-organism

Bacterial isolation

Table 2. Morphometric criteria evaluation of *G. pallida* and *G. rostochiensis*

Morphometric criteria	*G. pallida*		*G. rostochiensis*	
Length of body (L)	/	452-486	/	392-468
Width of body (W)	/	/	/	/
Length of neck	/	/	/	/
Number of streaks between the anus and the fenestra vulva	8-20 (<14)	12-17	12-31 (>14)	17-20
Reference	1	2	1	2

1: Fleming and Powers (1998); **2**: EPPO (2013).

Twenty cysts of different ages from each location were randomly selected under a microscope (magnification 15X) by excluding those that were small and damaged (10). They were disinfected in NaOCl solution (1 %) for 1 min and rinsed 5 times with sterile distilled water in a watch glass (23). Then, air dried at room temperature in a watch glass and emerged in 4 test tubes, 20 cysts per tube, containing 5 mL of nutrient broth under sterile conditions. Tubes were sealed and incubated at 35 °C/24 h. Isolation of bacteria by streak method was carried out on the surface of 5 petri plates comprising nutrient agar for each test tube (24). Petri plates were then incubated at 30 °C/72 h and examined each 24 h. The purity of the bacteria was established by successive subcultures of the isolated colonies. Bacterial colonies were subcultured on nutrient agar slant and stored at +4 °C.

Fungi isolation

Fungi were isolated by placing cysts in petri plates containing potato dextrose agar medium (20 g agar in 1 L of distilled water) at a rate of 5-10 cysts per petri plate. Four replicates were performed for each treatment. Plates were incubated at room temperature (27 °C) for 3-4 days. For microscopic observation, a very small amount of the fungus is stained with lactophenol blue for better cell and cell components visualization under a microscope. From the microscopic observation, the color, shape, appearance and arrangement of the fungus structures were identified (25). Cultures were preserved by serial transfer. Cultures must be checked frequently for contamination. Inoculum is transferred from an actively growing fungus culture to petri dishes wrapped with parafilm to reduce drying containing PDA medium (26).

Identification of microorganisms

Matrix Assisted Laser Desorption Ionization Time of Flight Mass Spectrometry (MALDI-TOF-MS Biotyper, Bruker Germany) MALDI-TOF MS relies on detecting the mass-to-charge ratio, abbreviated as m/z, of the ribosomal proteins of bacteria to generate a unique mass spectrum quickly and efficiently (27). The identification is determined by comparing the spectrum with the spectra of reference strains, aiming to find the closest match (28). Results were analyzed using software associated with the device by comparing the correspondence of reliability index between the spectrum of analyzed bacterium and database spectra. Profile matching was expressed on a Log scale with score values ranging between 0 to 3. Score values were interpreted according to the manufacturer's instructions, where score values of 2.3–3.000 indicate highly probable species-level identification. Score values between 2.00– and 2.299 refer to genus identification and probable species-level identification and score values ranging between 1.70– and 1.999 are designated as probable genus-level identification.

Fungi identification

Microscopic identification

The plate streaking procedure was conducted in a biohazard safety cabinet. A sterile inoculation loop was employed to streak the fungus over the surface of the PDA. The loop was allowed to glide over the surface of the medium. The handle was held at the balance point, with sweeping movements, to prevent damage and tearing of the agar surface. After inoculation, the petri plate was sealed with parafilm and incubated at room temperature for 4-5 days. The streaking method was repeated until a pure culture of each culture was obtained (25).

Fungi frequency

The distribution of fungal isolates is estimated by the frequency ratio (FR). It was calculated using the following equation:

Results

Isolation of Globodera cysts

$$FR = \frac{\text{total number of isolation of an isolate}}{\text{total number of isolates}} X\ 100$$

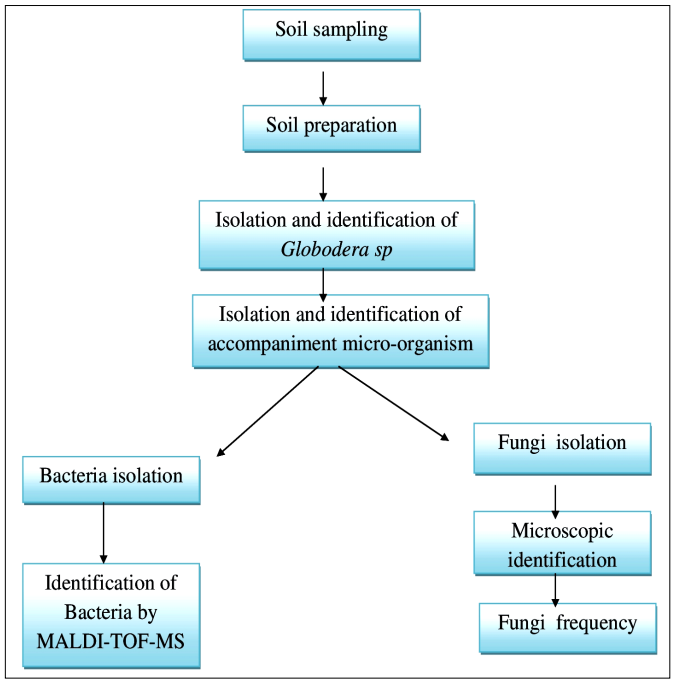


Fig. 3. Graphical representation of procedures involved in the study.

The nematological analysis revealed that all 16 soil samples of the surveyed potato plots were contaminated with *Globodera* cysts and infestation frequencies and empty cysts varied with region and soil sample (Table 3). The level of soil infestation by *Globodera* nematodes is often expressed as the number of cysts per soil unit (500 g). Soil samples 1, 2, 3, 5, 7, 9, 10, 15 and 16 showed dominance of *G. pallida.* While, *G. rostochiensis* was frequently found in soil samples 4, 6, 8, 11, 12 and 13. *G. pallida* is the dominant species colonizing the majority of studied plots. Moderate to high % of empty cysts were observed in the survived plots, ranging from the lowest % in the region of Saida (33.33 %) to the highest % in the region of El Bayadh (75.14 %). The frequencies of the infestation vary between 25 % and 75 % depending on the region.

Morphometric identification of Globodera sp.

Morphometric analysis allowed the first specific identification of different isolates of potato cyst nematodes. The 2 species *G. pallida* and *G. rostochiensis,* were present as a mixture in various inspected plots (Fig. 4).

In each isolate, some regions showed morphological characteristics of *G. pallida* and others of *G. rostochiensis*. Globally, measurements and averages were calculated in concordance with those of nematodes. Morphometric examination of considered nematode specimens showed that among 320 analyzed cysts, 197 (61.56 %) cysts were identified as *G. rostochiensis* and 123 (38.44 %) cysts as *G. pallida*. The morphological characteristics of cysts are listed in Table 4. Values of morphological characteristics overlapped and showed differences between the 2 PCN species. For perineal region of cysts, slite variation was recorded for number of cuticular ridges between fenestra and anus from the 4 studied regions (13.24 ± 0.47 µm for *G. pallida* and 20.01 ± 0.58 µm for *G. rostochiensis*), significant variance was observed between the 2 species concerning the measurement of the cysts; length (476.25 ± 38.16 µm for *G. pallida* and 391 ± 20.04 µm for *G. rostochiensis*), with (455 ± 38.40 µm for *G. pallida* and 388 ± 17.96 µm for *G. rostochiensis*)and the length of neck (72.2 ± 6.68 µm for *G. pallida* and 50.67 ± 5.49).

Identification of microorganisms

Bacteria identification by MALDI-TOF-MS typin

Table 3. Density of *Globodera* sp. cysts in soil samples (per 500 g).

Region	Soil samples	Number of G.p	number of G.r	Number of empty cyst	Rate of empty cysts (%)	Infestation rates of plots (%)
Tiaret	1	9	5	9	64.28	75
	2	13	10	15	65.22	
	3	5	3	3	37.50	
	4	6	8	10	71.43	
Saida	5	3	2	3	60.00	25
	6	1	3	3	75.00	
	7	4	0	2	50.00	
	8	1	5	2	33.33	
Mascara	9	6	3	5	55.55	50
	10	6	2	6	75.00	
	11	4	7	7	63.63	
	12	3	9	5	41.66	
ElBayadh	13	2	2	2	50.00	25
	14	4	1	3	60.00	
	15	3	0	2	75.14	
	16	3	2	2	40.00	
Total	16	69	62	79	/	/

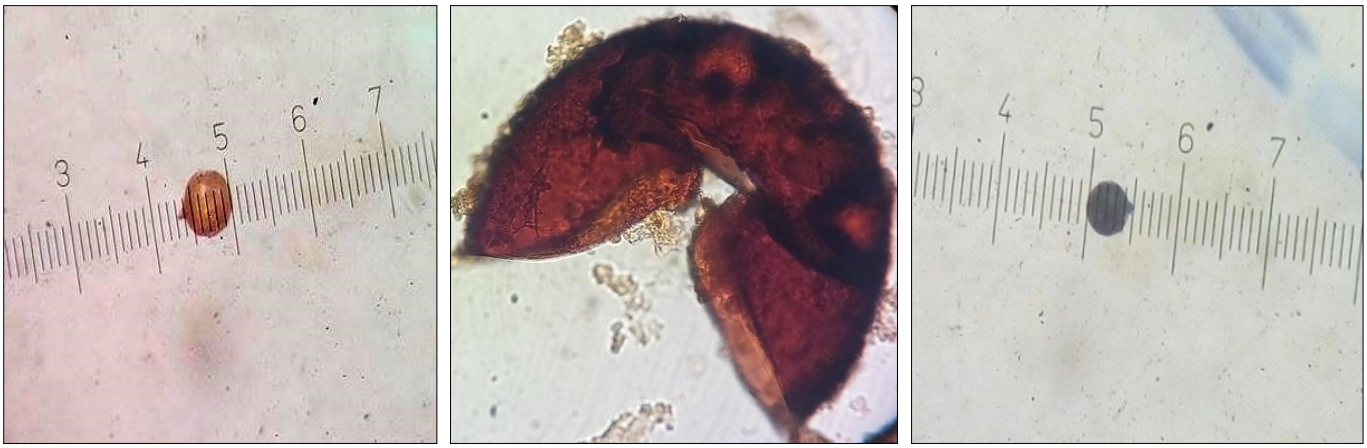


Fig. 4. *Globodera* sp. cysts (**a**): cyst of *Globodera pallida*; (**b**): empty cyst; (**c**): cyst of *Globodera rostonchiensis.*

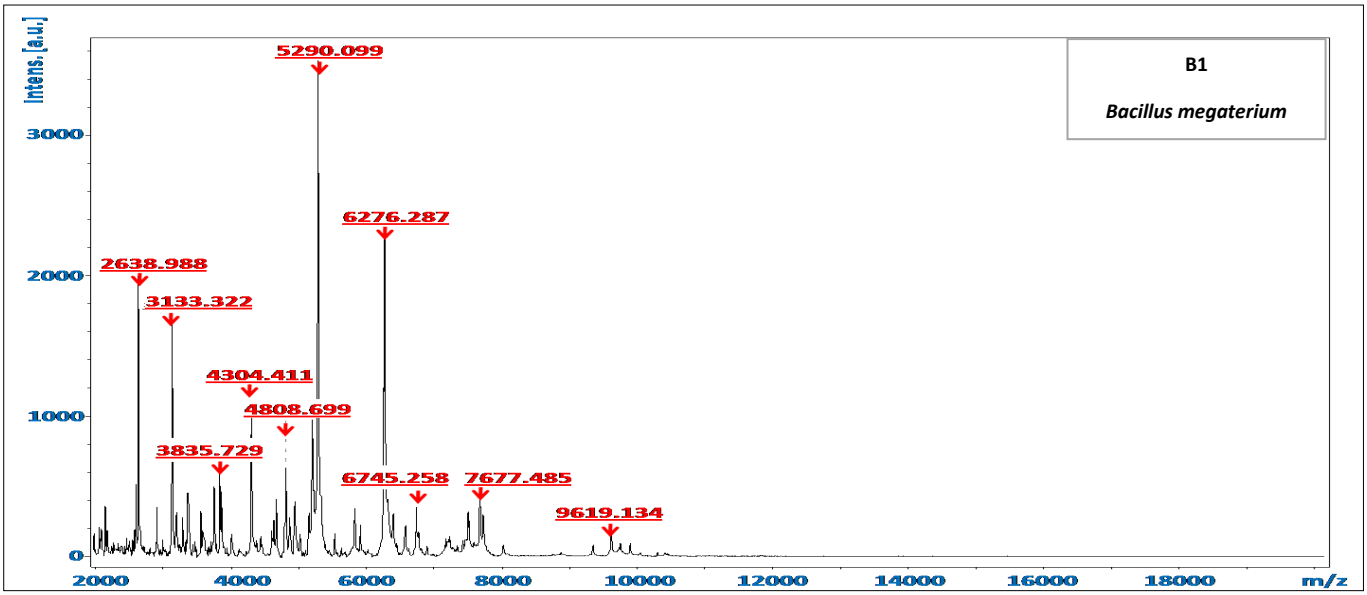
Table 4. Morphological characteristics of *G. pallida* and *G. rostenchiensis*.

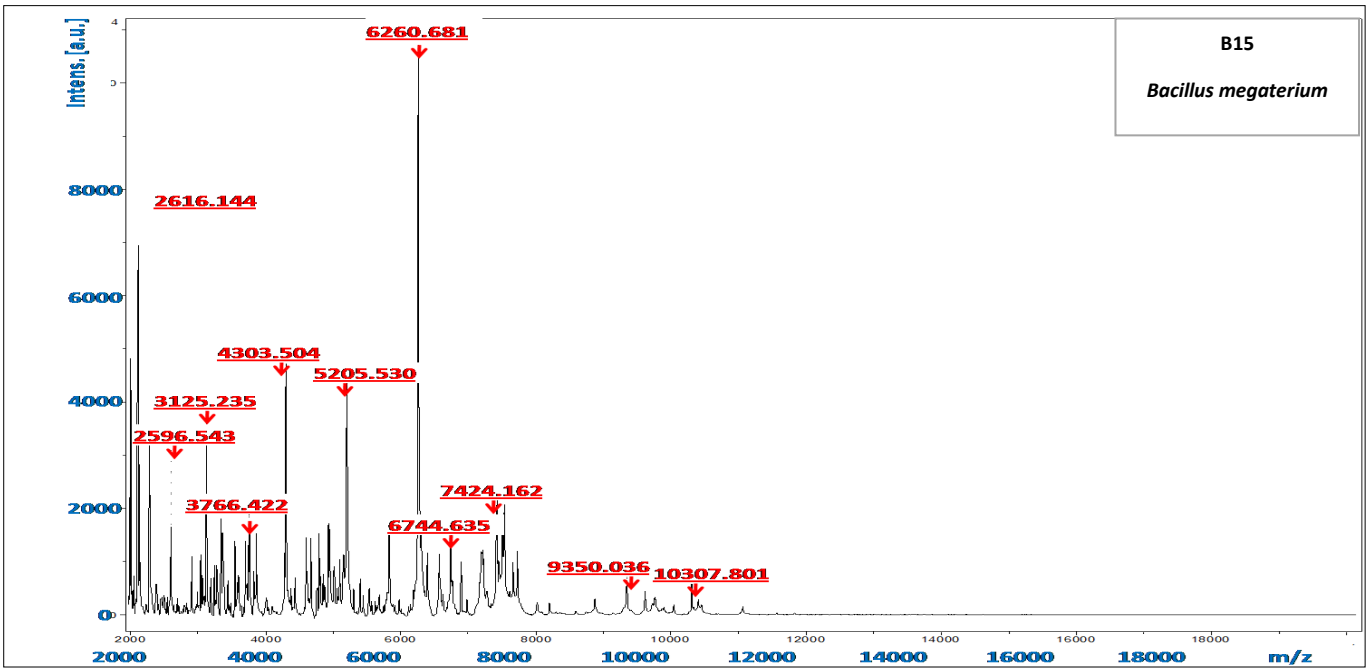
Region	Species	L (µm)	W (µm)	N (µm)	NSAFV
Mascara	*G. pallida*	425.00* ± 96(300-630)**	400.00* ± 75(360-690)**	63.00* ± 24 (20-88)**	13.50* ± 2.50 (9-19)**
	G. rostochiensis	403.0* ± 54(300-720)**	380.00* ± 55 (310-690)**	54.00* ± 25 (20-90)**	20.42* ± 3.70 (13-30)**
Tiaret	*G. pallida*	500.00* ± 14 (320-780)**	440.00* ± 160 (300-700)**	71.50* ± 21 (10-100)**	12.55* ± 3.90 (8-20)**
	G. rostochiensis	400.00* ± 52 (320-580)**	370.00* ± 110 (300-620)**	50.50* ± 23 (20-100)**	19.20* ± 4.00 (13-30)**
Saïda	*G. pallida*	510.00* ± 92 (300-640)**	500.00* ± 76 (320-680)**	77.00* ± 27.1 (45-160)**	13.31* ± 3.00 (8-19)**
	G. rostochiensis	400.00* ± 101 (390-620)**	412.00* ± 110 (330-680)**	43.00* ± 20.5 (30-195)**	20.00* ± 2.80 (12-29)**
ElBayadh	*G. pallida*	470.00* ± 107 (300-620)**	480.00* ± 101 (340-680)**	77.30* ± 25 (39-125)**	13.60* ± 2.20 (9-20)**
	G. rostochiensis	361.00* ± 54 (300-580)**	390.00* ± 81 (300-580)**	55.20* ± 27 (20-130)**	20.43* ± 3.70 (14-30)**

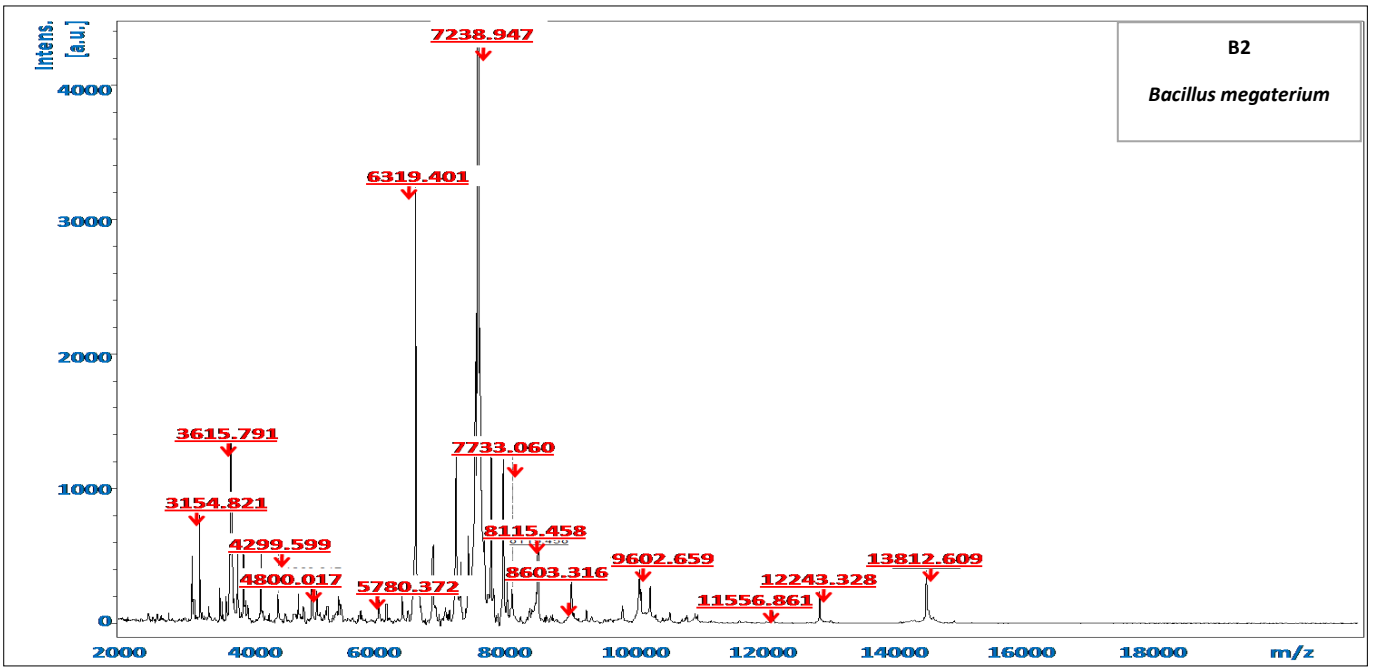
*: average; **: minimum and maximum values; **L**: Length of cyst; W: Width of cyst; **N**: Length of neck; **NSAFV**: Number of streaks between the anus and the fenestra vulva.

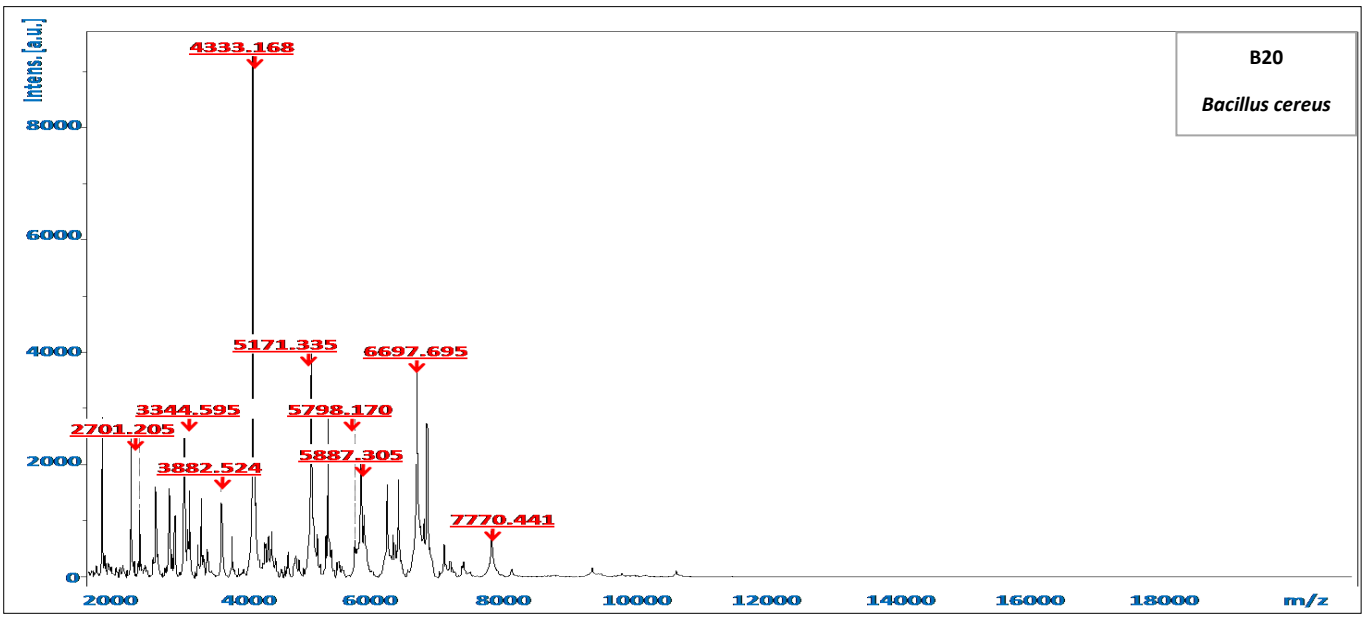
MALDI-TOF-MS spectra

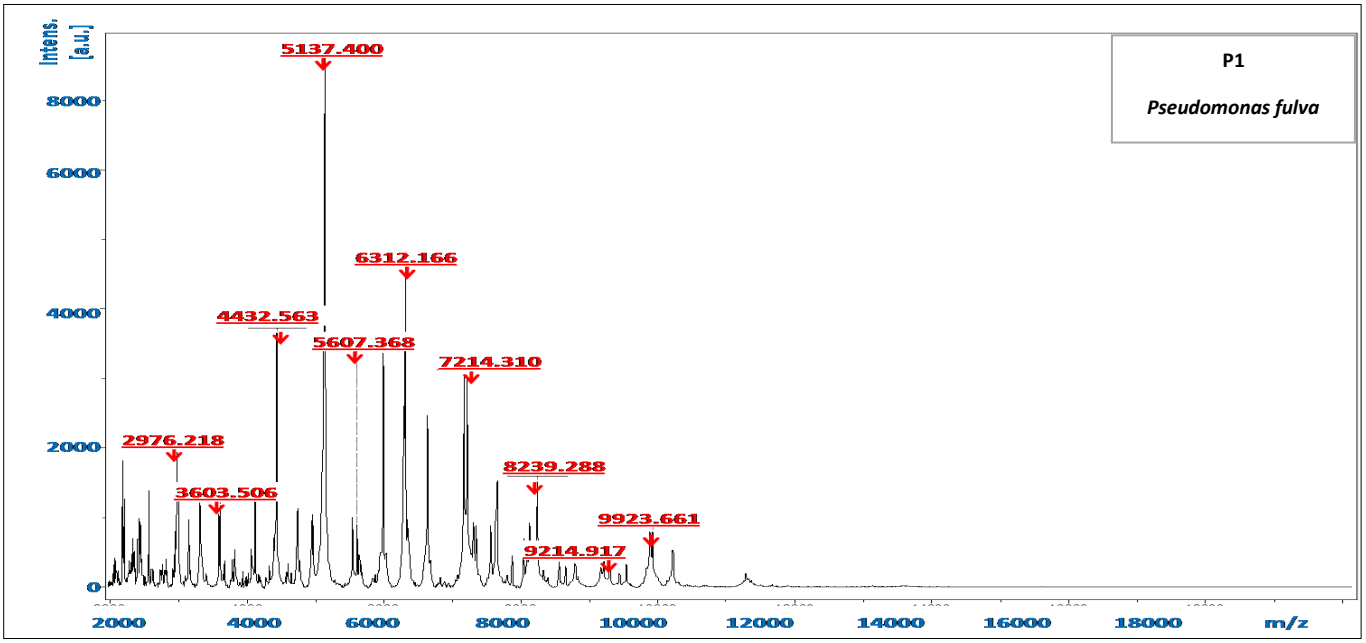
Eight species of bacteria were analyzed using MALDI-TOF-MS and the mass spectra obtained from bacterial isolates of *Bacillus* (B1, B2, B15 and B20) and *Pseudomonas* (P1, P2, P3 and P4) were shown in Fig. 5. These spectra were significantly different. A single mass spectral fingerprint was produced for each isolate in the mass range m/z 200 to 17000. The majority of ions detected were greater than 200 Da. The mass spectra obtained from bacterial isolates of *Bacillus* and *Pseudomonas* revealed also diversity among the *B. megaterium* isolates (B1 and B15). B1 signals were observed near 5300 m/z for the molecular peak and 6300 m/z for the base peak. Meanwhile, B15 signals appeared near 6300 m/z and 4300 m/z for the molecular and the base peak, respectively. Their spectrum exhibited peaks of low intensity (fragment peaks) in an almost identical mass range between 2500-5000 m/z and 6500-10000 m/z for B1. Whereas, for B15 it was between 2500-5500 m/z and 6500-11000 m/z. B2 showed no identification. Moreover, protein profiling of B2 was not successfully performed. MALDI-TOF-MS was then concluded as not appropriate for the identification of B2. B20 strain's

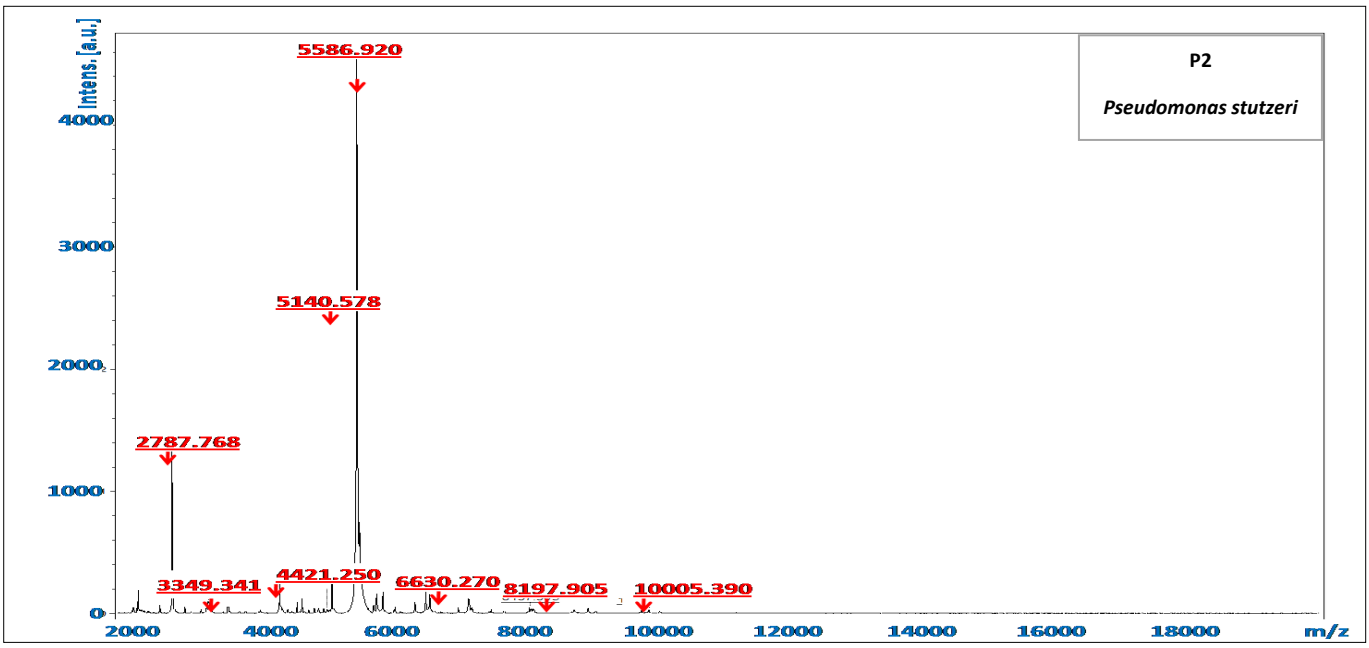


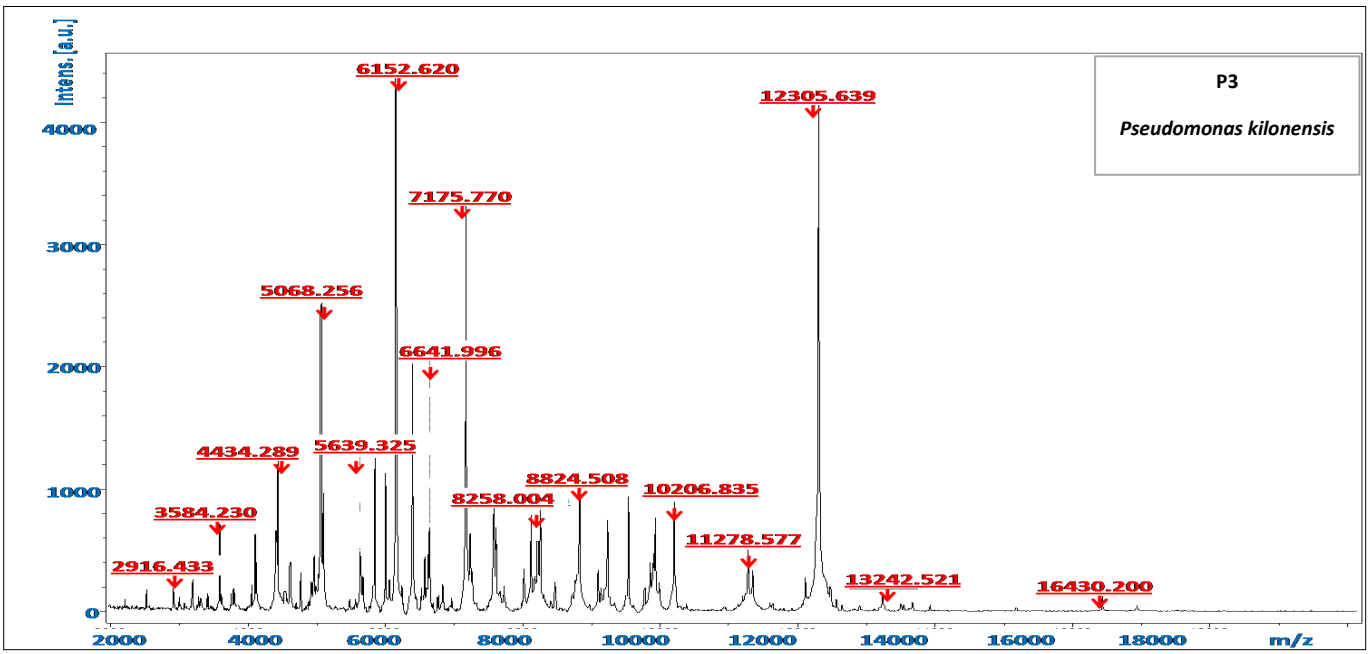

B15
Bacillus megaterium
Intens. [a.u.]
6260.681
2616.144
4303.504
5205.530
3125.235
2596.543
7424.162
3766.422
6744.635
9350.036
10307.801
m/z


B2
Bacillus megaterium
Intens. [a.u.]
7238.947
6319.401
3615.791
7733.060
3154.821
8115.458
4299.599
9602.659
13812.609
4800.017
8603.316
12243.328
5780.372
11556.861
m/z


B20
Bacillus cereus
Intens. [a.u.]
4333.168
5171.335
6697.695
3344.595
5798.170
2701.205
5887.305
3882.524
7770.441
m/z







protein profile showed that it matched with *B. cereus,* and it gave signals near both 4400 m/z for the molecular peak

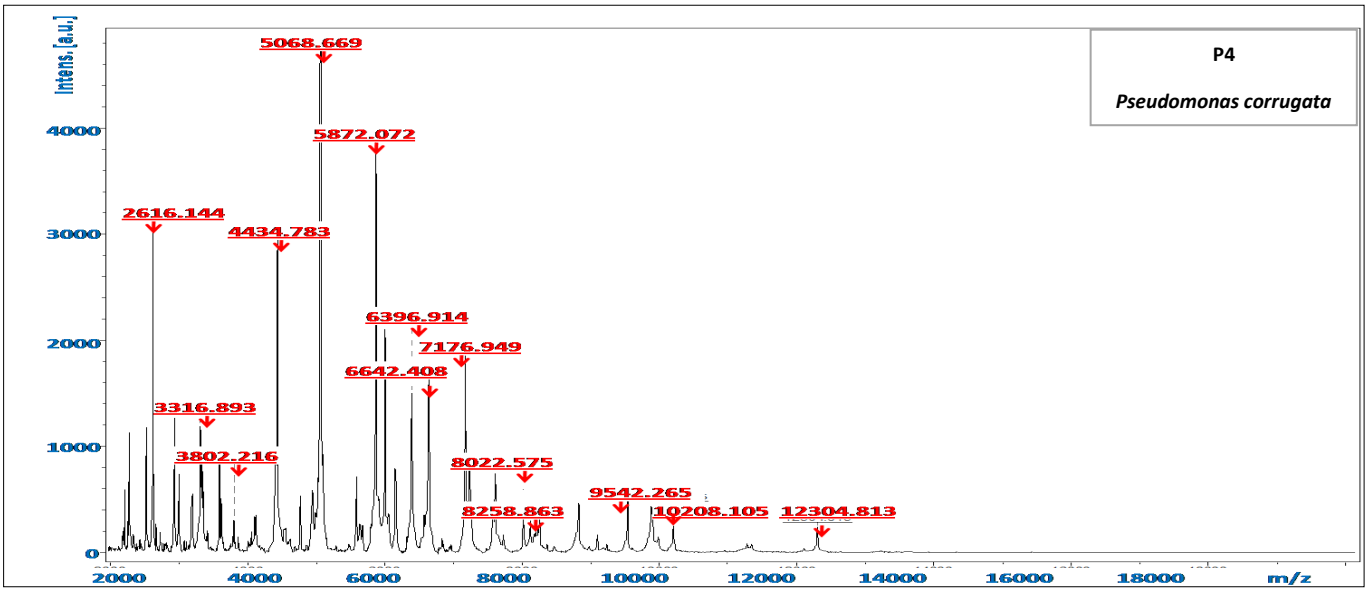


Fig. 5. Mass spectra profiling of the isolated strains using MALDI-TOF-MS typing.(**B1**: *Bacillus megaterium,* **B15**: *Bacillus megaterium,* **B2**: *Bacillus megaterium,* **B20**: *Bacillus cereus,* **P1**: *Pseudomonas fulva,* **P2**: *Pseudomonas stutzeri,* **P3**: *Pseudomonas kilonensis,* **P4**: *Pseudomonas corrugata*).

and 5200 for the base peak, as well as other low-intensity peaks ranging between 2500-4000 and 5500-8000 m/z. On the other hand, the spectra of P1, P2, P3 and P4 indicated that all of them belong to the genus *Pseudomonas* with various spectrums for 4 different species. P1 *P. fulva* signals were observed near 5200 m/z for the molecular peak and 6400 m/z for the base peak. For P2 *P. stutezeri,* the molecular peak was near 5600 m/z and 5200 m/z for the base peak and for the low-intensity peaks, ranging between m/z 2500-5000 and 6500-10000 for both spectrums. The spectrums of P3 *P. kilonensis* and P4 *P. corrugata* revealed a wider range of peaks than the other spectrums; reaching 17000 m/z for P3 and 13000 m/z for P4. The signals of P3 were near 6200 m/z and 12000 m/z for the molecular and the base peaks respectively. P4 revealed signals near 5000 m/z for the molecular peak and 5900 m/z for the base peak.

MALDI-TOF-MS typing

MALDI-TOF-MS was selected as a rapid and reliable technique for the identification and differentiation of 8 bacterial isolates. Three isolates were identified as *Bacillus* spp. (B1, B15 and B20) and 4 isolates as *Pseudomonas* spp. (P1, P2, P3 and P4) (Table 5). Identification scores for the three *Bacillus* spp. isolates were less than 2, which was considered probable genus identification. Two isolates were identified as *Bacillus megaterium,* representing different score values: 1.994 for isolate B15 and 1.882 for isolate B1. Isolate B20, representing a score value of 1.892 was identified as *Bacillus cereus*. Identification scores for three *Pseudomonas* spp. isolates were higher than 2, which were considered secure genus identification and probable species identification. Isolate P1 was identified as *Pseudomonas fulva,* revealing a score value of 2.294; isolate P2 was identified as *Pseudomonas stutzeri,* showing a score value of 2.148 and isolate P3 was identified as *Pseudomonas kilonensis* characterized by score value of 2.009. Isolate P4 was identified as *Pseudomonas corrugata,* showing a score value of 1.974, which corresponds to probable genus identification. One isolate, B2, showed no identification with a score of 1.648, corresponding to not reliable identification.

Table 5. MALDI-TOF-MS identification of bacteria isolated from cysts of *Globodera* sp.

Isolate code	ID	MALDI-TOF-MS score value
B1	*Bacillus megaterium*	1.882
B2	*Bacillus megaterium*	1.648
P1	*Pseudomonas fulva*	2.294
P2	*Pseudomonas stutzeri*	2.148
P3	*Pseudomonas kilonensis*	2.009
B15	*Bacillus megaterium*	1.994
B20	*Bacillus cereus*	1.892
P4	*Pseudomonas corrugata*	1.974

Fungi identification

Microscopic examination revealed 173 fungal isolates belonging to 12 genera: *Penicillium, Rhizoctonia, Chaetomium, Alteraria, Mucor, Aspergillus, Ulocladium, Fusarium, Epicoccum, Stemphylium, Cladosporium* and *Rhizopus.*

Fungi frequency

Fungi distribution by orders and families

The distribution of fungi in *Globodera* sp. cysts by order and family were summarized in Fig. 6. Fungal flora associated with cysts of *Globodera* obtained from different regions revealed 7 orders, among which 39.34 % belongs to Trichocomaceae, 26.31 % to Pleosporaceae, 3.5 % to Nectriaceae, 15.8 % to Mucoraceae, 4.05 % to Davidiellaceae, 3.5 % to Chaetomiaceae and 7.5 % to Ceratobasidiaceae.

Fungi distribution by genera

The most frequent genus, *Aspergillus,* recorded a frequency of 22 %, followed by *Penicillium,* which was detected in 17.34 % of the cyst populations of *Globodera*. The third genus, *Alternaria,* was common at 15.02 % (Fig. 7). A frequency of 10 % was recorded for *Rhizopus* and 5.8 % for *Mucor.* The frequency of *Rhizoctonia* was 7.5 %, while the genus *Cladosporium* was 4.05 % of the population studied. A frequency of 3.5 % was recorded for both the *Chaetomium* and *Fusarium*.

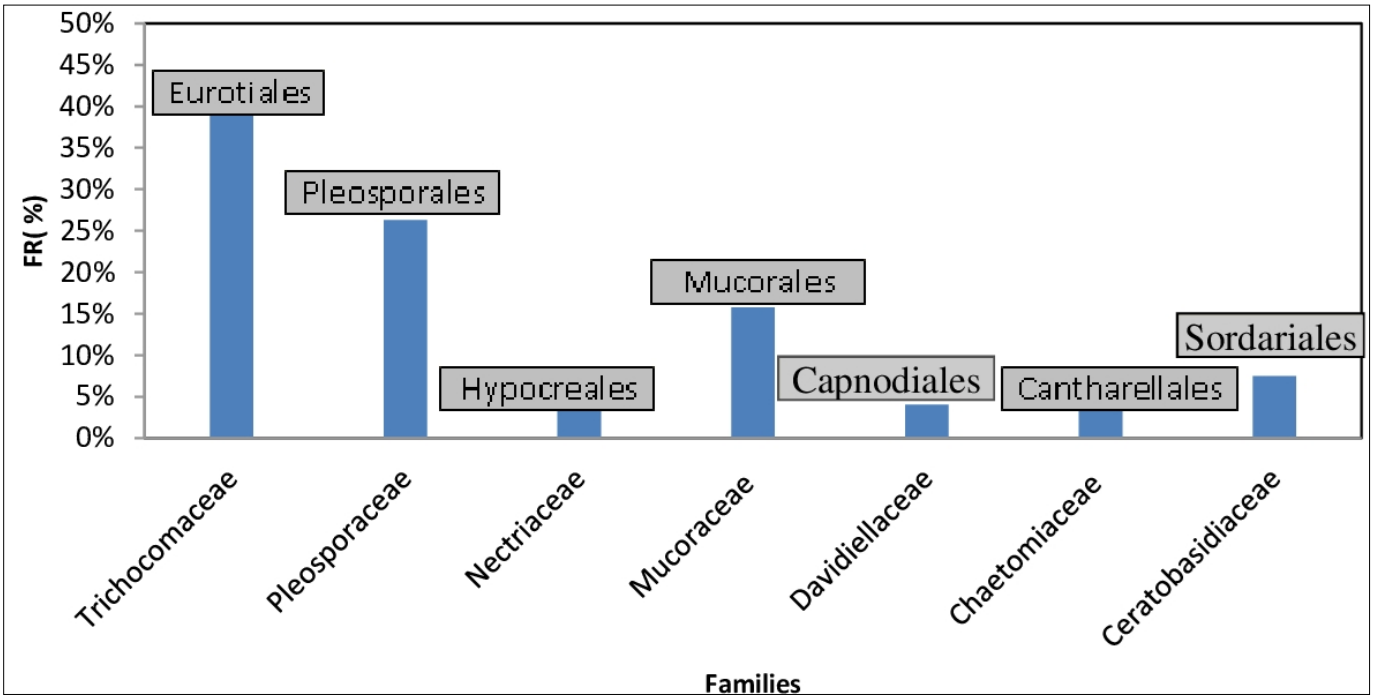


Fig. 6. Frequency of fungi isolated from *Globodera* sp. cysts by orders and families in the 4 surveyed regions. FR: frequency ratio.

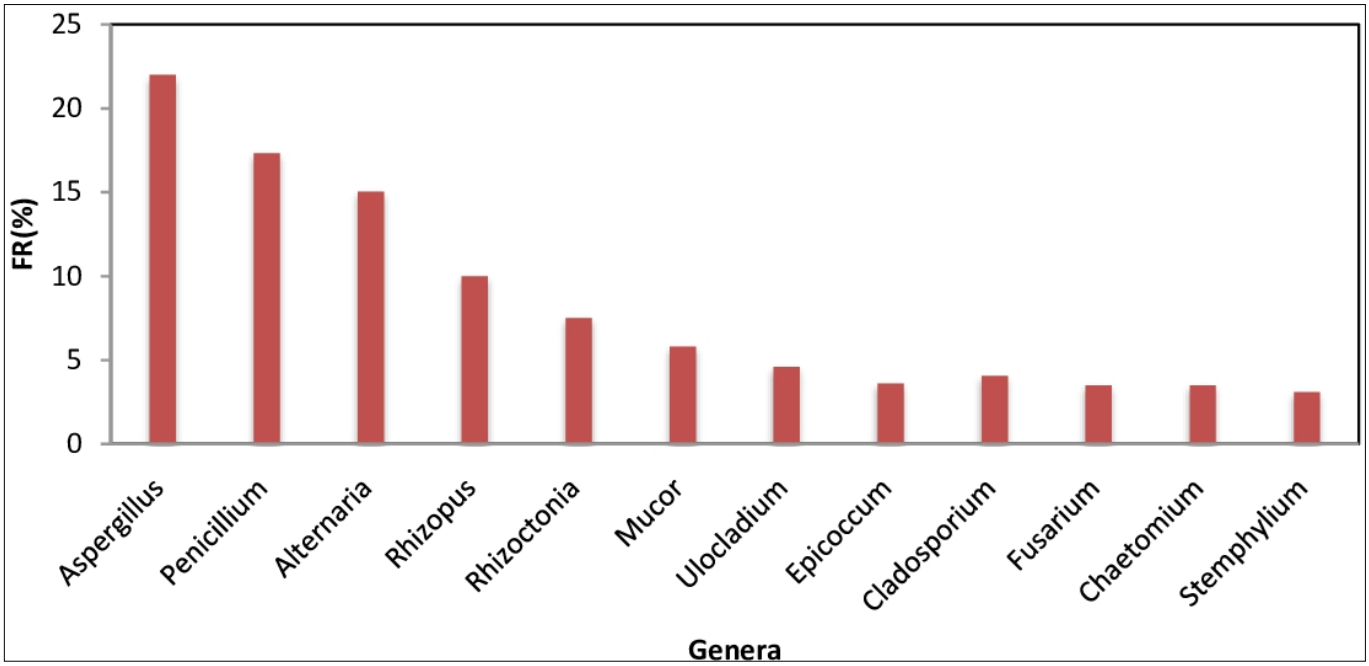


Fig. 7. Distribution of fungi isolated from *Globodera* sp. cysts by genera in the 4 surveyed regions. FR: frequency ratio.

Discussion

Nematological analysis of *Globodera* sp. cysts in potato plots as well as the isolation and identification of accompanying bacteria and fungi from cysts, seems to be of great importance in biological control against PCN and sustainable agriculture. Current results showed that the majority of potato plots in the studied regions (Mascara, Saida, Tiaret and El Bayadh) were infested with *Globodera* sp. These infestations were caused by 2 *Globodera* sp. with slit dominance of *G. pallid* over *G. rostochiensis*. The edaphoclimatic conditions and the ongoing presence of host plants within the plots would account for the significant infestations observed in this area. The diffusates released in the soil by the host stimulate egg hatching, but not all eggs hatch (60-80 %); in comparison, only approximately 5 % hatch in water. Some eggs do not hatch for several years (29). Some soil bacteria can produce nematicidal compounds (29) such as quinones (30), terpenoids (31), peptides (32), pyrans (33) and furans (34) and are candidates of nematode biological control agents (35). The genera *Pseudomonas* and *Bacillus* (36-38) have shown great potential for the biological control of nematodes. In this study, bacteria isolated from *Globodera* cysts belonging to *Pseudomonas* and *Bacillus* genera, 8 isolates were subject to a profound analysis using a more recent technique, MALDI TOF mass-spectrometry, a straightforward and swift strategy. It is based on the comparison of the protein spectrum of the studied specimen to a database of reference spectra (39). It is statistically more effective at the genus and species level for the identification of gram-

negative *Bacilli* (40). MALDI-TOF demonstrates superior genus identification capabilities, resulting in a lower rate of unidentified bacteria (41), the identification accuracy is known to tolerate varying growth conditions (42). The fluorescent *Pseudomonas* strains, when used as microbial inoculants, have been proven to protect plants from infection by soil-borne phytopathogens. They produce and excrete metabolites that are inhibitory to soil-borne plant pathogens like 2,4-diacetyl phloroglucinol, which is a phenolic compound with broadspectrum antifungal, antibacterial, antihelminthic, nematicidal and phytotoxic activity. It has gained special interest due to its formation by a diverse spectrum of *pseudomonas* employed for biological root disease prevention (43, 44). Under *in vitro* conditions, exposure to diacetyl phloroglucinol-producing *P. fluorescens* F113 lowered the capacity of *G. rostochiensis* to hatch and decreased the % of mobile juveniles of the potato cyst nematode (45). *Pseudomonas* species have the ability to produce other compounds like siderophores (46) and ammonia (47), which may inhibit the egg-hatching, juvenile survival and root penetration of plant nematodes (48). *Pseudomonas putida* can reduce *Globodera rostochiensis* population, densities by 40.7-42.2 % compared to the untreated population, resulting in an increase in plant growth parameters (49).

The genus *Bacillus*, due to its great reproduction and abundance, is one of the most important biocontrol agents and has been researched in the control of nematodes and other pathogens (50). Biocontrol mechanisms provided by rhizobacteria such as *B. cereus, B. megaterium* and others include root colonization, sporulation and parasporal crystal formation.

Bacterial isolates that inhibited egg hatching of the potato cyst nematodes were mostly from the genus *Bacillus*. In greenhouse experiments, coating seed with *Bacillus* isolates, particularly *B. cereus*, greatly reduced J2s infection on wheat roots. This could imply that these bacterial isolates can diminish the ability of *H. avenae* to infect roots. Rhizoshperic *Bacillus* from sugar beet also reduced the hatch of the potato cyst nematode, *Globodera rostochinensis* and *G. pallid* (51). *Bacillus cereus* strain S2 can synthesize sphingosine, which can be used to induce reactive oxygen buildup, damage nematode genital areas and prevent nematode reproduction (52). The mode of action of these bacteria is double: direct effects on egg hatching and nematode motility and indirect effects such as altering root secretions and inducing resistance, making roots less attractive to nematodes (53). One mechanism of bionematicides involves producing an enzyme that breaks down the eggshell and cyst wall of GCN (54), using it as an energy and carbon source. The eggshells of *G. rostochiensis* contain 59 % protein and 9 % chitin (55). Proteases, chitinases and lipases are degrading enzymes that play a crucial role in the degradation process. The genus *Bacillus* is known for producing protease and chitinase enzymes, which renders them a suitable candidate for a bionematicide (52, 56, 57). Hence, the possible activity of hydrolytic enzymes of isolates might be involved in the penetration process to help bacteria kill the juveniles (58, 59). According to one report (60), *Bacillus megaterium* is a promising choice for nematode biocontrol because it reduces penetration of *Meloidogyne graminicola.* It was reported that *B. megaterium* reduced by 50 % penetration of both *M. chitwoodi* and *Pratylenchus penetrans* in potatoes compared to non-treated rice plant roots (61). Another isolate of *Bacillus megaterium* reduced *M. graminicola* root penetration and migration by 40 to 60 % (62). According to another report, *Bacillus megaterium* strain Sneb207 prolonged the developmental stage of soybean cyst nematode (63). In the Sneb207 treatment, both J3 and J4 showed increased lengths as compared to the control indicating that Sneb207 inhibited nematode development. Thus, *B. megaterium* strain Sneb207 has the capacity to decrease SCN penetration and development as well as it influenced cyst formation in soybean roots and the proportion of female and nematodes (64).

Current findings revealed that diversified fungi were isolated from *Globodera* sp. cysts belonging to 7 orders. Numerous major classes of commercial chemicals, including many antibiotics used in medicine, have been produced by fungi, which are known to have a wide diversity of metabolic pathways (65). In this study, 12 genera were identified from the cysts of *Globodera*'s populations from several potato-producing regions, such as opportunistic fungi: *Fusarium*, *Penicillium*, *Aspergillus*, Zygomycetes (*Rhizopus* and *Mucor*) and fungi belonging to Dothideomycetes (*Ulocladium*, *Cladosporium, Epicoccum* and *Stemphylium*), Agaricomycetes (*Rhizoctonia*), Sordariomycetes (*Chaetomium*) and Deuteromycetes (*Alternaria*). Both *Aspergillus* and *Penicillium* have secondary metabolites with significant nematicidal activity like gliotoxin, fumagillin, brefeldin A, α ß-dehydrocurvularin, 8ß-hydroxy-7-oxocurvularin (66). There are studies indicating that after being infested by certain opportunistic fungi, nematode viability was significantly decreased (67). The use of fungi in biological control against *Globodera* sp. has shown to be effective. In this study, *Aspergilus* and *Penicillium* recorded higher frequencies. According to the findings of an experiment, exposure to *Aspergillus niger* F22 culture filtrate lowered the rates of J2 viability and egg hatching (68). The formation of oxalic acid as a nematicidal metabolite was linked to this impact. In a study conducted under laboratory conditions, adding *Penicilium oxalicum* to soils decreased the quantity and rate of J2 from PCN hatching (69). They noticed that *P. oxalicum* had a nematecidal effect on *G. pallida* cysts in the soil. The genus *Alternaria* a produced variety of biologically active compounds that possess significant nematicidal activity, such as brefeldin A isolated from *Alternaria carthami, A. zinniae* (70). Symmetric 16-membered macrodiolide helmidiol was produced by *Alternaria alternata* (71). Other fungi like *Rhizoctonia solani* had antagonistic effect on the population of *G. rostochiensis* when plants were treated with a combination of *G. rostochiensis* and *R. solani* (72). *Epicoccum nigrum* and *Epicoccum purpurascens* also contained the compound flavipin, which may prevent the hatching of the soybean cyst nematode *Heterodera* as well as the juvenile mobility of *Meloidogyne incognita in vitro* (66). *Chaetomium globosum* is one of the most prominent fungi in the environment (73). Several investigations have demonstrated *C.*

globosum's nematicidal ability. A study demonstrated that both *Heterodera* glycines and *M. incognita*'s egg hatch were inhibited by *C. globosum* culture broth filtrate (74). Additionally, it has been shown that *Fusarium* inhibited growth of *G. rostochiensis* by producing toxic metabolites such as beauvericin, enniatin A, enniatin B, cyclosporin A and 4,15-diacetylnivalenol that decreased nematode activity and the second-stage juvenile's (J2) attraction to roots (75). Several other secondary metabolites that have nematicidal activities include acetic acid, beauvericin and brefeldin A (66).

Conclusion

Emerging results have proposed that most bacteria and fungi isolated from the PCN had nematicidal properties, which can explain the high number of empty cysts. Different species of bacteria belong to 2 genera: *Bacillu*s and *Pseudomonas*, which, according to previous studies, are effective for the suppression of growth and infestation of nematodes. This current investigation also showed that numerous fungi were associated with cysts of PCN. Twelve genera were isolated from cysts of PCN. However, some of them were the subject of many studies and showed inhibitory action on the mobility of infective larvae (L2) of cyst nematodes.

While including biological control agents in integrated pest management for nematode pests should be optimized to maximize their safe and lucrative usage, additionally, increasing awareness campaigns for the knowledge of farmers is critical for making these biological methods familiar and simple to use, paving the door for their wider adoption.

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Authors' contributions

BAY was carrying out the study, correcting and writing the manuscript; KR supervise the study and MS corrected the manuscript. The authors contributed equally to the present study. All the authors read and approved the final manuscript, verify that the Text, Figures and Tables are original.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interests to declare.

Ethical issues: None.

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