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Tetracycline and oxytetracycline resistance determinants detected in *Bacillus cereus* strains isolated from honey samples

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ABSTRACT

The aim of this study was to investigate the presence of tetracycline and oxytetracycline resistance determinants in *Bacillus cereus* strains isolated from honey samples. Of a total of 77 isolates analyzed, 30 (39%) exhibited resistance to tetracyclines according to the results of a disk diffusion method. Resistant strains (n=30) were screened by PCR for the presence of the resistant determinants *tet*K, *tet*L, *tet*M, *tet*O, *tet*W, *otr*A and *otr*B and their MIC values for tetracycline, oxytetracycline and minocycline were assessed. According to the PCR results, 23 isolates (77%) presented at least one tetracycline or oxytetracycline resistance determinant. The *tet*K genotype was present in 10 isolates while the *tet*L, *tet*M, and *otr*A genotypes were present in 3, 2, and 5 isolates, respectively. In addition, 2 isolates of the *tet*K plus *tet*M genotype, 1 of the *tet*K plus *tet*L genotype, and 1 of the *tet*K plus *otr*A genotype were found. All isolates were *tet*W, *tet*O and *otr*B negatives. On the other hand, 7 isolates (23%) showed a tetracycline-resistant and/or minocycline-resistant phenotype (MIC) but did not carry any of the *tet* or *otr* determinants investigated in this study. This research has shown that *B. cereus* isolates from honey samples contain a variety of tetracycline and oxytetracycline resistance genes, including the *tet*K *and tet*L determinants which encode for efflux proteins, and *tet*M and *otr*A, which encode for ribosomal protection proteins. These findings indicate that strains isolated from honeys could represent a reservoir for tetracycline resistance genes. To our knowledge, this is the first report of tetracycline-resistant and oxytetracycline-resistant *B. cereus* strains carrying the *tet*K determinant, and also the first report of oxytetracycline-resistant and tetracycline-resistant *Bacillus* species carrying the *otr*A determinant.

Key words: *Bacillus cereus,* honey, tetracycline resistance determinants, *tet*K, *tet*M, *tet*L, *otr*A

RESUMEN

Detección de determinantes de resistencia a tetraciclina y oxitetraciclina en cepas de *Bacillus cereus* aisladas de muestras de miel. El objetivo del presente estudio ha sido investigar la presencia de diversos determinantes de resistencia a tetraciclina y oxitetraciclina en las poblaciones de *Bacillus cereus* presentes en la miel. De un total de 77 aislamientos evaluados, 30 (39%) resultaron resistentes a tetraciclina y/o minociclina de acuerdo con los resultados de las pruebas de difusión en disco. Dentro del grupo que presentó un fenotipo resistente, se investigó la presencia de los determinantes *tet*K, *tet*L, *tet*M, *tet*O, *tet*W, *otr*A y *otr*B por PCR y se determinaron los valores de CIM para tetraciclina, oxitetraciclina y minociclina. De acuerdo con los resultados obtenidos por PCR, 23 aislamientos (77%) presentaron al menos un determinante de resistencia a tetraciclina o a oxitetraciclina; el genotipo *tet*K se encontró en 10 de esos aislamientos, mientras que los genotipos *tet*L, *tet*M y *otr*A se hallaron en 3, 2 y 5 aislamientos, respectivamente. Ningún aislamiento presentó los genotipos *tet*W, *tet*O ni *otr*B. Adicionalmente, se encontraron los genotipos *tet*K plus *tet*M (2 aislamientos); *tet*K plus *tet*L (1 aislamiento) y *tet*K plus *otr*A (1 aislamiento). Por otra parte, 7 cepas (23%) resultaron resistentes a tetraciclina, oxitetraciclina y/o minociclina por CIM, pero no presentaban ninguno de los determinantes *tet* u *otr* estudiados. Estos resultados indican la existencia de un alto porcentaje de cepas de *B. cereus* aisladas de miel con genes de resistencia a tetraciclina y oxitetraciclina, incluyendo los determinantes *tet*K, *tet*L, *tet*M y *otr*A. Este estudio constituye el primer registro de la presencia del determinante *tet*K de resistencia a tetraciclina en *B. cereus*, como así también la presencia del determinante *otr*A dentro del género *Bacillus.*

Palabras clave: *Bacillus cereus,* miel, determinantes de resistencia a tetraciclina, *tet*K, *tet*M, *tet*L, *otr*A

INTRODUCTION

Tetracycline (TC) is a broad-spectrum antibiotic used in the treatment of bacterial infections in humans, animals, and insects. Therefore, bacteria from different ecosystems are exposed to this antibiotic.

There are three main mechanisms of resistance to tetracycline: energy-dependent efflux, protection of the bac-

terial ribosome, and enzymatic inactivation of the tetracycline molecule (17). The two first mechanisms are widely distributed among gram-negative and gram-positive bacteria. Resistance to TC is primarily due to the acquisition of *tet* determinants frequently associated with mobile elements (17, 18). Up to now, 39 different *tet* and *otr* genes have been reported (2, 8, 18, 22).

Oxytetracycline (OTC) is commonly used in apiculture for the prevention and control of American (AFB) and European Foulbrood (EFB) of honey bees, the two major bacterial diseases affecting the larval and pupal stages of honey bees (*Apis mellifera* L.) (19). Recently, oxytetracycline-resistant (OTC^r) *Paenibacillus larvae* isolates, the cause of AFB, have been detected and this phenotype correlated with the presence of two different plasmids carrying the *tet*L gene (12) and the *tet*K gene (5).

Aerobic spore-forming bacteria of the genera *Paenibacillus* and *Bacillus* are commonly found in honey (6, 10, 20). Qualitative examination of honeys revealed that the most prevalent species of *Bacillus* are *Bacillus cereus, B. megaterium, B. pumilus, and B. coagulans* (6, 20). *B. cereus* is an ubiquitous species commonly found in diverse habitats including several types of food. It has been involved in food poisoning cases due to the production of diverse toxins (1, 9). Tetracycline resistant (TC^r) strains of the *B. cereus* group (*B. cereus, B. thuringiensis, B. anthracis*) isolated from soil and animal manure samples were found to carry the *tet*L gene, while other isolates carried both the *tet*M and *tet*L genes (3). These genes can be mobilized in the presence of either conjugative plasmids or transposons (3).

Although the genotypic diversity of *B. cereus* recovered from honey has been reported (10), there is no information regarding the existence of *B. cereus* TC^r strains isolated from honeys or other apiarian sources. The aim of this study was to investigate both phenotypic and genotypic features of TC and OTC resistance in *B. cereus* strains isolated from honey samples.

MATERIALS AND METHODS

Bacterial strains

A total of 77 *B. cereus* isolated from honey samples were used in this work. Sixty six isolates were recovered from Argentinean honeys randomly collected from apiaries located in different geographical areas (6, 10). In addition, isolates obtained from commercial honeys from Mexico (n= 1), Brazil (n = 2), Italy (n= 2), USA (n= 6) and Chile (n= 1) and a reference strain (ATCC 11778) were also studied and are listed in Table 1. All isolates were frozen-stored at - 80 °C in tryptic-soy broth plus 20% glycerol (v/v) until used.

Antimicrobial susceptibility tests for tetracyclines

Resistance to tetracyclines was preliminary determined by the double disk test method described by Trzcinski and co-workers (21), using a 30 µg tetracycline disk, a 30 µg minocycline disk and a 5 µg tetracycline disk in a straight line, 9 mm apart with the minocycline disk in the center. Isolates were considered susceptible when showing an inhibition zone around the 30 µg TC disk and/or the 30 µg MN disk of ≥ 19 mm in diameter, intermediate for those showing 15 to 18 mm and resistant when the inhibition zone was ≤ 14 mm (13). When using 5 µg TC disks, the breakpoint values for susceptible, intermediate and resistant strains were ≥ 20 mm, 15 to 18 mm, and ≤ 14 mm respectively (5).

For those isolates showing diminished inhibition zones to tetracycline, and/or minocycline, the MICs of tetracycline (TC), oxytetracycline (OTC), and minocycline (MN) were determined by the agar dilution method according to the CLSI guidelines (14). Isolates were considered susceptible when their MICs were ≤ 4 µg/ml, intermediate for MICs of 8 µg/ml, or resistant when their MICs were ≥ 16 µg/ml (14). For quality control in susceptibility testing, *Escherichia coli* ATCC 2592*2 and B. cereus* ATCC 11778 were used (13, 14).

PCR analysis of tetracycline resistance genes

PCR analysis was performed only on those strains that showed resistant or intermediate values according to the double disk diffusion technique, and also on both ATCC reference strains.

Total DNA was extracted by a rapid procedure as described by Alippi and Aguilar (4). The presence of the tetracycline resistance determinants *tet*K, *tet*L and *tet*M was investigated by PCRs using specific primers according to Ng and co-workers (15). The presence of *tet*O and *tet*W tetracycline resistance determinants was examined according to Aminov *et al* (7). In addition, the presence of oxytetracycline resistance determinants *otr*A and *otr*B was evaluated according to Nikolakopoulou *et al.* (16).

RESULTS AND DISCUSSION

Of a total of 77 isolates analyzed, 30 (39%) exhibited resistance to tetracycline and/or minocycline according to the results of a double disk diffusion method (Table 1). TC, OTC and MN MIC values for the resistant isolates (n=30) and the ATCC reference strains (n=2) were assessed and are listed in Table 2.

Of the 30 isolates that exhibited a TC^r, OTC^r, and/or MN^r phenotype according to their MICs, 23 (77%) presented at least one tetracycline or oxytetracycline resistance determinant (Table 2). According to the PCR results, 10 *B. cereus* isolates were classified as *tetK* genotype, 3 as *tet*L, 3 as *tet*M and 5 as *otr*A (Figure 1). In addition, the *tet*K plus *tet*M genotype was found in 2 isolates (Bc22 and Bc 121); the *tet*K plus *otr*A genotype was found in 1 isolate (Bc14) and the *tet*K plus *tet*L genotype was found in 1 isolate (Bc 55). All isolates were *tet*W, *tet*O and *otr*B negative (Table 2).

On the other hand, 7 isolates (23%) were TC^r, OTC^r and/or MN^r but did not carry any *tet* or *otr* genes studied here (Table 2), which suggests that they carry other determinants. A similar situation was reported by Agersø *et al* (3) when working with isolates belonging to the *B. cereus* group, where 81 out of 88 TC^r isolates did not yield any positive amplicons for any of the *tet* genes examined. Other authors have also reported that not all gram-positive bacteria showing a TC^r phenotype have been shown to carry any of the already known *tet* genes (17).

All the isolates of the *tet*K, *tet*L, or *tet*M genotypes and the combinations of *tet*K/*tet*M, *tet*K/*tet*L and *tet*K/*otr*A were

Table 1. Geographical origin and designation of bacterial strains used in this study and susceptibility test results (disk diffusion) to tetracycline (TC) and minocycline (MN).

Strain		Geographical origin	Zone (mm) TC 5 µg[(1)]	Zone (mm) TC 30 µg[(1)]	Zone (mm) MN 30 µg[(1)]
ATCC 25922	*E. coli*	-	26 S	20 S	26 S
ATCC 11778	*B. cereus*	-	39 S	35 S	36 S
Bc 3	*B. cereus*	G. Chávez (Buenos Aires, Argentina)	34 S	44 S	47 S
Bc 8	*B. cereus*	Bragado (Buenos Aires, Argentina)	30 S	28 S	35 S
Bc 14	*B. cereus*	Laprida (Buenos Aires, Argentina)	16 I	10 R	22 S
Bc 15	*B. cereus*	Pte. Perón (Buenos Aires, Argentina)	26 S	20 S	22 S
Bc 16	*B. cereus*	Tandil (Buenos Aires, Argentina)	29 S	32 S	27 S
Bc 17	*B. cereus*	Punta Alta (Buenos Aires, Argentina)	23 S	23 S	31 S
Bc 18	*B. cereus*	Las Flores (Buenos Aires, Argentina)	27 S	57 S	37 S
Bc 19	*B. cereus*	La Plata (Buenos Aires, Argentina)	6 R	7 R	8 R
Bc 20	*B. cereus*	Pedro Luro (Buenos Aires, Argentina)	17 I	18 I	18 I
Bc 21	*B. cereus*	Pedro Luro (Buenos Aires, Argentina)	21 S	19 S	20 S
Bc 22	*B. cereus*	Pedro Luro (Buenos Aires, Argentina)	19 I	16 I	18 I
Bc 23	*B. cereus*	25 de Mayo (Buenos Aires, Argentina)	12 R	6 R	19 S
Bc 29	*B. cereus*	Pigüé (Buenos Aires, Argentina)	24 S	25 S	29 S
Bc 32	*B. cereus*	Berisso (Buenos Aires, Argentina)	20 S	25 S	21 S
Bc 35	*B. cereus*	Chacabuco (Buenos Aires, Argentina)	35 S	33 S	40 S
Bc 36	*B. cereus*	Santa Fe (Santa Fe, Argentina)	19 S	23 S	19 S
Bc 44	*B. cereus*	P. Luro (Buenos Aires, Argentina)	28 S	38 S	44 S
Bc 46	*B. cereus*	Ranchos (Buenos Aires, Argentina)	6 R	10 R	19 S
Bc48	*B. cereus*	P. Luro (Buenos Aires, Argentina)	14 R	10 R	19 S
Bc50	*B. cereus*	Chascomús (Buenos Aires, Argentina)	15 I	18 I	20 S
Bc55	*B. cereus*	Pigüé (Buenos Aires, Argentina)	6 R	7 R	22 S
Bc56	*B. cereus*	Henderson (Buenos Aires, Argentina)	19 S	24 S	22 S
Bc57	*B. cereus*	Henderson (Buenos Aires, Argentina)	20 S	24 S	19 S
Bc58	*B. cereus*	Gorina (Buenos Aires, Argentina)	9 R	14 R	22 S
Bc61	*B. cereus*	Brandsen (Buenos Aires, Argentina)	20 S	24 S	19 S
Bc62	*B. cereus*	Santa Fe (Santa Fe, Argentina)	24 S	30 S	31 S
Bc65	*B. cereus*	Cancún (México)	12 R	14 R	9 R
Bc6	*B. cereus*	Chivilcoy (Buenos Aires, Argentina)	24 S	23 S	30 S
Bc67	*B. cereus*	Pigüé (Buenos Aires, Argentina)	28 S	33 S	33 S
Bc69	*B. cereus*	La Plata (Buenos Aires, Argentina)	17 I	12 R	22 S
Bc70	*B. cereus*	Rauch (Buenos Aires, Argentina)	11 R	7 R	19 S
Bc71	*B. cereus*	La Plata (Buenos Aires, Argentina)	10 R	11 R	22 S
Bc72	*B. cereus*	P. Luro (Buenos Aires, Argentina)	24 S	21 S	31 S
Bc76	*B. cereus*	G. Lavalle (Buenos Aires, Argentina)	25 S	30 S	36 S
Bc77	*B. cereus*	La Plata (Buenos Aires, Argentina)	21 S	26 S	28 S
Bc81	*B. cereus*	Laprida (Buenos Aires, Argentina)	6 R	6 R	19 S
Bc8	*B. cereus*	Laprida (Buenos Aires, Argentina)	32 S	27 S	29 S
Bc83	*B. cereus*	La Plata (Buenos Aires, Argentina)	20 S	19 S	21 S
Bc85	*B. cereus*	La Plata (Buenos Aires, Argentina)	20 S	21 S	20 S
Bc86	*B. cereus*	G. Pueyrredón (Bs. As., Argentina)	26 S	21 S	24 S
Bc87	*B. cereus*	C. Suárez (Buenos Aires, Argentina)	26 S	21 S	24 S
Bc88	*B. cereus*	25 de Mayo (Buenos Aires, Argentina)	24 S	19 S	20 S
Bc89	*B. cereus*	T. Lauquen (Buenos Aires, Argentina)	12 R	14 R	22 S

Table 1. Continued

Strain		Geographical origin	Zone (mm) TC 5 µg[(1)]	Zone (mm) TC 30 µg[(1)]	Zone (mm) MN 30 µg[(1)]
Bc 93	*B. cereus*	Pedro Luro (Buenos Aires, Argentina)	18 I	15 I	14 R
Bc 96	*B. cereus*	25 de Mayo (Buenos Aires, Argentina)	27 S	21 S	26 S
Bc 97	*B. cereus*	C. Casares (Buenos Aires, Argentina)	25 S	20 S	23 S
Bc 98	*B. cereus*	T. Lauquen (Buenos Aires, Argentina)	25 S	20 S	21 S
Bc 99	*B. cereus*	Tapalqué (Buenos Aires, Argentina)	11 R	14 R	20 S
Bc 100	*B. cereus*	Tapalqué (Buenos Aires, Argentina)	25 S	20 S	23 S
Bc 101	*B. cereus*	Brandsen (Buenos Aires, Argentina)	19 S	18 I	19 S
Bc 102	*B. cereus*	Chascomús (Buenos Aires, Argentina)	19 S	25 S	21 S
Bc 103	*B. cereus*	Berisso (Buenos Aires, Argentina)	20 S	21 S	19 S
Bc 105	*B. cereus*	Berisso (Buenos Aires, Argentina)	16 S	22 S	20 S
Bc 106	*B. cereus*	Boston (USA)	14 R	14 R	23 S
Bc 107	*B. cereus*	Boston (USA)	7 R	14 R	23 S
Bc 108	*B. cereus*	Boston (USA)	6 R	7 R	19 S
Bc 109	*B. cereus*	Boston (USA)	14 R	14 R	14 R
Bc 110	*B. cereus*	9 de Julio (Buenos Aires, Argentina)	29 S	24 S	28 S
Bc 111	*B. cereus*	9 de Julio (Buenos Aires, Argentina)	21 S	33 S	38 S
Bc 113	*B. cereus*	Italy	29 S	32 S	35 S
Bc 114	*B. cereus*	Italy	19 S	32 S	35 S
Bc 116	*B. cereus*	Olavarría (Buenos Aires, Argentina)	24 S	20 S	23 S
Bc 118	*B. cereus*	Laprida (Buenos Aires, Argentina)	27 S	21 S	23 S
Bc 119	*B. cereus*	Laprida (Buenos Aires, Argentina)	18 I	16 I	17 I
Bc 120	*B. cereus*	Pta. Indio (Buenos Aires, Argentina)	25 S	21 S	23 S
Bc 121	*B. cereus*	P. de la Costa (Buenos Aires, Argentina)	10 R	6 R	14 R
Bc 122	*B. cereus*	San Vicente (Buenos Aires, Argentina)	37 S	34 S	40 S
Bc 123	*B. cereus*	Pehuajó (Buenos Aires, Argentina)	14 R	8 R	25 S
Bc 125	*B. cereus*	Buzios (Brazil)	21 S	25 S	25 S
Bc 127	*B. cereus*	Magdalena (Buenos Aires, Argentina)	21 S	19 S	22 S
Bc 128	*B. cereus*	Laprida (Buenos Aires, Argentina)	9 R	6 R	20 S
Bc 130	*B. cereus*	Laprida (Buenos Aires, Argentina)	24 S	25 S	26 S
Bc 13	*B. cereus*	USA	9 R	10 R	25 S
Bc 132	*B. cereus*	USA	6 R	11 R	27 S
Bc 133	*B. cereus*	Chile	8 R	12 R	24 S
Bc 134	*B. cereus*	Roque Pérez (Buenos Aires, Argentina)	22 S	24 S	29 S
Bc 135	*B. cereus*	Tigre (Buenos Aires, Argentina)	20 S	23 S	28 S
Bc 136	*B. cereus*	Mexico	31 S	26 S	28 S

[(1)]Diameter of inhibitory zones in mm (including disk). Breakpoints: For Tc 30 µg and Mn 30 µg: S: ≥ 19 mm, I: 15 to 18 mm, R: ≤14 mm. For Tc 5 µg: S: ≥ 20 mm, I: 15 to 19 mm, R: ≤ 14 mm

from Argentina, while the genotype *otr*A was found in isolates from both Argentina and the USA. The highest MICs for TC and OTC were observed in isolates of the *tet*K and/or *otr*A genotypes (Table 2).

All the strains that exhibited genotypes *tet*K and/or *tet*L were resistant or intermediate to TC and OTC but susceptible to MN, and those with genotype *tet*M were resistant or intermediate to TC, OTC and MN, as previously reported for the genus *Staphylococcus* (17, 21). By using the double disk test, the *tet*K and/or *tet*L genotypes and also the *tet*M genotype might be predicted for the *B. cereus* isolates in the same manner as that reported for *S. aureus* (21).

The MIC and zone size values obtained for reference strain *E. coli* ATCC 25922 were within the range of accep-

Table 2. Susceptibility test results (MICs) to tetracycline (TC), minocycline (MN), and oxytetracycline (OTC) (in mg/l), and resistance determinants for selected strains of *B. cereus* isolated from honeys.

Strain	Origin	MIC TC (mg/l)	MIC MN (mg/l)	MIC OTC (mg/l)	Tetracycline or oxytetracycline resistance determinants						
					*tet*k	*tet*L	*tet*M	*tet*W	*tet*O	*otr*A	*otr*B
ATCC 25922	-	1 S	0.5 S	0.5 S	-	-	-	-	-	-	-
E. coli											
ATCC 11778	-	<0.125 S	2 S	<0.125 S	-	-	-	-	-	-	-
B. cereus											
Bc 14	Argentina	8 I	4 S	64 R	+	-	-	-	-	+	-
Bc 19	Argentina	16 R	8 I	8 I	-	-	-	-	-	-	-
Bc 20	Argentina	8 I	8 I	8 I	-	-	+	-	-	-	-
Bc 22	Argentina	8 I	8 I	8 I	+	-	+	-	-	-	-
Bc 23	Argentina	16 R	1 S	16 R	-	-	-	-	-	-	-
Bc 46	Argentina	16 R	8 I	16 R	-	-	+	-	-	-	-
Bc 48	Argentina	16 R	4 S	16 R	+	-	-	-	-	-	-
Bc 50	Argentina	8 I	0.5 S	8 I	-	+	-	-	-	-	-
Bc 55	Argentina	32 R	4 S	>128 R	+	+	-	-	-	-	-
Bc 58	Argentina	16 R	1 S	16 R	-	-	-	-	-	-	-
Bc 65	Mexico	16 R	32 R	16 R	-	-	-	-	-	-	-
Bc 69	Argentina	8 I	2 S	8 I	-	+	-	-	-	-	-
Bc 70	Argentina	16 R	4 S	16 R	+	-	-	-	-	-	-
Bc 71	Argentina	128 R	2 S	128 R	+	-	-	-	-	-	-
Bc 81	Argentina	>128 R	4 S	128 R	+	-	-	-	-	-	-
Bc 89	Argentina	16 R	1 S	16 R	+	-	-	-	-	-	-
Bc 93	Argentina	8 I	16 R	8 I	-	-	+	-	-	-	-
Bc 99	Argentina	16 R	2 S	16 R	+	-	-	-	-	-	-
Bc 101	Argentina	8 I	2 S	8 I	+	-	-	-	-	-	-
Bc 106	USA	16 R	2 S	64 R	-	-	-	-	-	+	-
Bc 107	USA	16 R	2 S	16 R	-	-	-	-	-	-	-
Bc 108	USA	16 R	1 S	64 R	-	-	-	-	-	+	-
Bc 109	USA	16 R	64 R	128 R	-	-	-	-	-	+	-
Bc 119	Argentina	8 I	16 R	32 R	-	-	-	-	-	+	-
Bc 121	Argentina	64 R	16 R	64 R	+	-	+	-	-	-	-
Bc 123	Argentina	16 R	2 S	16 R	+	-	-	-	-	-	-
Bc 128	Argentina	64 R	1 S	32 R	+	-	-	-	-	-	-
Bc 131	USA	16 R	1 S	32 R	-	-	-	-	-	-	-
Bc 132	USA	128 R	2 S	128 R	-	-	-	-	-	+	-
Bc 133	Chile	16 R	0.5 S	128 R	-	-	-	-	-	-	-

Breakpoints:

MIC values: S: < 4 µg/ml I: 8 µg/ml R: > 16 µg/ml

tance for quality control (13, 14). *B. cereus* ATCC 11778 resulted susceptible to TC, OTC and MN and, as expected, did not produce any positive amplicons for any of the *tet* or *otr* genes tested here.

To our knowledge, this is the first report of TC^r and OTC^r *B. cereus* strains carrying *tet*K and/or *otr*A determinants. It is also the first report of *B. cereus* isolates from honeys carrying *tet*L or *tet*M determinants, although previously found in the *B. cereus* group (3). In addition, this is also the first register of a *Bacillus* species carrying the *otr*A determinant, only previously reported in streptomycete populations from environmental samples (16).

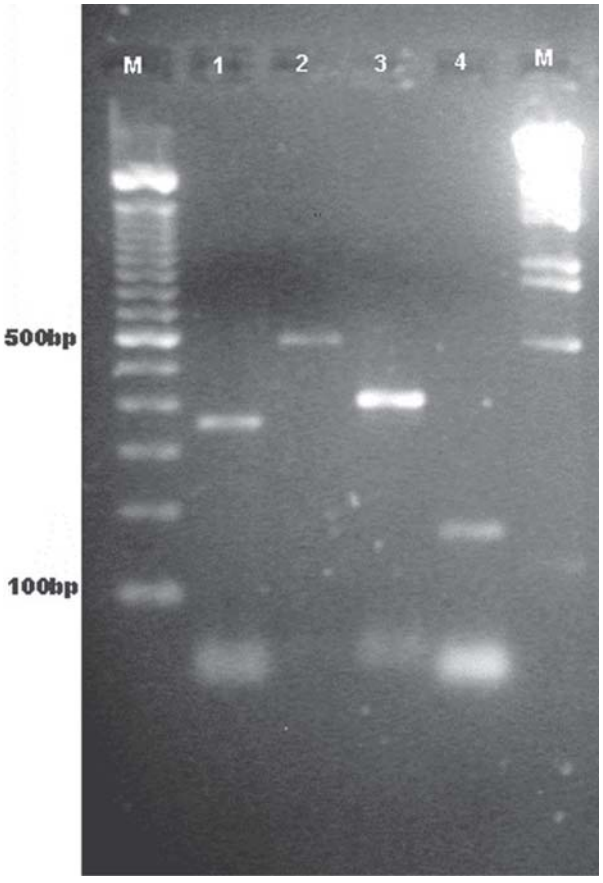

Figure 1. Agarose gel electrophoresis of PCR products amplified from tetracycline-resistant strains of *Bacillus cereus* isolates from honey samples. Lanes: M: molecular weight marker (100 bp ladder Promega), 1. Bc50 strain showing the 267 bp amplicon compatible with the *tet*L genotype, 2. Bc 108 strain showing the 778 bp amplicon corresponding to the *otr*A genotype, 3. Bc93 strain showing the 406 bp amplicon corresponding to the *tet*M genotype, 4. Bc 123 strain showing the 169 bp amplicon corresponding to the *tet*K genotype, and M: Molecular weight marker.

It is interesting to point out that 4 out of 6 isolates from the USA exhibited the *otr*(A) determinant while only 2 out of 23 Argentinean isolates from Laprida honeys, in Buenos Aires province, contained this determinant. OTC has been extensively used in many countries, including in the main honey producing areas of the USA and Argentina, to control bacterial diseases affecting honey bees. TC^r and OTC^r *P. larvae* isolates carrying the *tet*K (6) or *tet*M (11, 12) determinants in mobile elements have been recently found, which suggests that TC and OTC resistance could be transferred between gram-positive bacteria from the same ecological niche.

In conclusion, this study has shown that *B. cereus* isolates from honey samples contain a variety of TC and OTC resistant genes including the *tet*K and *tet*L determinants that encode efflux proteins, and *tet*M, and *otr*A that encode ribosomal protection proteins. This may indicate that strains isolated from honeys have access to and/or are a reservoir of resistance genes. Further studies will be needed to determine the genetic supports for these resistance genes in order to understand how they disseminate in gram-positive spore-forming bacteria from apiarian sources.

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