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Membrane Vesicles and β-Lactamase in *Erwinia herbicola*

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ABSTRACT

Erwinia herbicola 48 is a gram-negative phytopathogen. Electron microscopy of *E. herbicola* 48 washed cells indicated that it produced spherical tubular outer membrane vesicles under normal conditions, and larger ones after exposure to the aminoglycoside antibiotic, gentamicin. The addition of β-lactam antibiotic, cephradine to *E. herbicola* 48 culture, inhibited the membrane vesicle formation. Natural membrane vesicles produced by the washed cells (antibiotic-untreated) of this bacterium lysed a variety of gram-positive and gram-negative host bacteria. Vesicles produced by gentamicin-treated cultures had a higher lytic activity against the host bacteria tested, and also higher-than-normal β-lactamase activity (1.6-fold). Different β-lactam inducers resulted in only low levels of constitutive β-lactamase and insignificant amounts of the induced enzyme. *E. herbicola* 48 is susceptible to minimal inhibitory concentrations of β-lactam antibiotics, due to the low level of β-lactamase produced. Gentamicin-treated cultures exhibited higher resistance to amoxycillin, cefazolin, cefoperazone, ceftazidime and ceftriaxone.

Keywords: *Erwinia herbicola*, gentamicin-membrane vesicles (g-MVs), Bacteriolytic activity, β-lactamase induction, MIC.

INTRODUCTION

Many gram-negative bacteria produce external membrane vesicles (MVs) during growth. During their formation, MVs entrap several periplasmic components; for *Pseudomonas aeruginosa* these include alkaline phosphatase, phospholipase C, proelastase, protease and peptidoglycan hydrolase (Chatterjee & Das 1967; Devoe & Gilchrist 1973; Dorward *et al*. 1989; Wispelwey *et al.*1989; Kondo *et al.* 1993; Whitmire & Garon 1993; Kadurugamuwa & Beveridge 1995; Wai *et al.* 1995; Kadurugamuwa & Beveridge 1996; Li *et al.* 1996). Because several of these components are virulence factors (including the lipopolysaccharide contained in the MV membrane) MVs may be important during the initial phases of infection, as they concentrate such factors and convey them to host tissue (Kadurugamuwa & Beveridge 1995, 1997). The partitioning of peptidoglycan hydrolase into MVs lyses surrounding dissimilar bacteria in the donor bacterium's environment, releasing organic compounds for growth (Li *et al*. 1998). It has been demonstrated that MVs are small (30–50 nm) bilayered particles into which degradative enzymes are concentrated. It is possible that MVs play a predatory role in natural ecosystems, in which they are released by a parent bacterium and lyse surrounding cells, increasing available nutrients to the parent strain (Kadurugamuwa *et al.* 1998). Beveridge (1999) reported that MVs are spherical bilayered membranous structures (50 – 250 nm in diameter) released from the surface of gram-negative bacteria. Hayashi *et al.* (2002) concluded that an autolysin mutant of *Porphyromonas gingivalis* constructed and produced

elevated levels of MVs relative to parental strain, and they suggested that vesicle formation of this organism might be regulated by cell wall turnover.

MVs are able to attack gram-positive and gram-negative bacteria in a different manner. For gram-positive bacteria, MVs adhere to the surface of the cell wall, break open, and hydrolyze the peptidoglycan immediately under the adherence junction. The same mechanism occurs even if the cell wall has an S-layer. After the MVs have broken open, the peptidoglycan hydrolase can penetrate the lattice network of the S-layer and attack the underlying cell wall. In this way whether or not gram-positive cells possess an S-layer, MVs attack their surfaces through a single-hit route which produces a single large lesion in the cell wall. For gram-negative bacteria, MVs fuse into the outer membrane, releasing their contents into the periplasmic space for dispersal around the cell so that the peptidoglycan sacculus can be hydrolyzed at several points (Kadurugamuwa & Beveridge 1996; Kadurugamuwa *et al*. 1998).

Depending on the predatory role of the MVs and in a medical context, it is possible that MVs could have bacteriolytic activity against both gram-positive and gram-negative pathogens and that by increasing the production of these predatory MVs by gentamicin treatment (Kadurugamuwa & Beveridge 1995), the gentamicin-MVs (g-MVs) would have even more killing power against hard-to-kill clinical isolates, because of the associated antibiotic.

β-Lactamases are a diverse group of bacterial enzymes that vary in their abilities to hydrolyze β-lactam antibiotics and convert them into antimicrobially inactive agents (Medeiros 1997). Enzymes which destroy penicillin have been known almost as long as penicillin has been available for therapy. Abraham and Chain detected penicillin-destroying activity in extracts of *Escherichia coli* in 1940, but called the enzyme penicillinase, largely because the cephalosporins were unknown at that time and the enzymes were thought to be specific for β-lactam bond of the penicillin nucleus (Abraham & Chain 1940; Abraham *et al*. 1949). The frequency of β-lactamase production in gram-negative bacteria has increased considerably during the recent years. Expression of β-lactamase is the most common form of bacterial resistance to β-lactam antibiotics. β-Lactamase production by bacteria continues to be one of the main mechanisms of bacterial resistance to β-lactam antibiotics, and it seems likely to remain so (Weston *et al*. 1998; Nyfors *e*t *al*. 1999; Mealey 2001). β-Lactamases produced by gram-negative bacteria may be inducible or constitutive and, with few exceptions, are periplasmic (commonly referred to as cell-bound) (Richmond & Sykes 1973). It has been reported that β-lactamase can be also entrapped within the MVs produced by gram-negative bacteria and this may increase the breakdown of β-lactam antibiotics at the infection site (Beveridge 1999).

The aim of the present study is concerned with the study of MVs formation and β-lactamase production by *E. herbicola* 48 and whether a relation is found between them.

MATERIALS AND METHODS

Erwinia herbicola 48 was isolated from diseased *Phaseolous vulgaris* seedlings in Egypt and identified using El-Hendawy & Azab (1998). *Enterobacter cloacae* NCTC10005, *Escherichia coli* DH5, *Proteus vulgaris* 1753, *Serratia marcescens* HIM 307-2 and *Serratia marcescens* 921/79 LR were kindly provided by Professor H. H. Martin, Institute of Microbiology, TH Darmstadt, Germany. *Erwinia carotovora* NCPPB312 and *Erwinia carotovora* NCPPB671

were obtained from The National Collection of Plant Pathogenic Bacteria, Ministry of Agriculture, Fisheries and Food Plant Pathology Laboratory, Harpenden, UK. *Pseudomonas syringae* pv. *Syringae* Van Hall strain 347448 was obtained from the International Mycological Institute, Bakeham Lane, Egham, Surrey TW20 9TY, UK. *Bacillus cereus* 1080, *Bacillus subtilis* 1020, *E. coli* 1357, and *Pseudomonas solanacearum* 1274 were obtained from the culture collection of the Microbiological Research Center (MIRCEN), Faculty of Agriculture, Ain-Shams University, Cairo, Egypt.

Amoxycillin and ampicillin (E.I.P.I. Co., Egypt), cefamandol and cefazolin (Eli Lilly, Italia SPA), ceftazidime, cefuroxime, cephalexin and gentamicin (Glaxo Welcome, Egypt), ceftriaxone (TA3 Pharma Group, Egypt), cefoperazone (Phizer, Egypt), nitrocefin (Glaxo, Greenford, UK), Cephradine (Pharco, pharmaceutical, Alexandria, Egypt), and chloramphenicol (Roth, Karlsruhe, Germany) were commercially available. Cefotaxime (Hoechst AG, Frankfort, Germany) was kindly provided by Professor H. H. Martin, Institute of Microbiology, TH Darmstadt, Germany.

For transmission electron microscopy (TEM), *Erwinia herbicola* 48 was grown in nutrient broth (Oxoid, England) at 30 °C to the early stationary phase (OD_{600} = 1.0) in an orbital shaker incubator (Model OSFT-LS-R, refrigerated) at agitation rate of 150 rpm. The cells were harvested by centrifugation and washed twice with 0.05 M potassium- sodium phosphate buffered saline pH 7. The washed cells were resuspended in the same buffered saline and 20 µl suspension was placed on carbon-coated nickel grids and stained with 2% aqueous uranyl acetate, rinsed and examined with ZEISS EM10 transmission electron microscope operating under standard conditions.

Exposure of *E. herbicola* 48 cultures to cephradine and gentamicin were carried out according to the method described by Kadurugamuwa & Beveridge (1995), with modification. The bacterium was inoculated in nutrient broth and grown to the end of exponential phase at 30 °C and an agitation rate of 150 rpm in an orbital incubator shaker. Gentamicin and cephradine at final concentration of four times the MICs (cephradine, 8 µg ml^{-1} and gentamicin, 0.25 µg ml^{-1}) were added to the cultures separately and the growth was allowed to continue for one hour more but at a reduced agitation rate of 100 rpm to avoid the mechanical damage of the cells in the presence of the antibiotics. The cells were sedimented by centrifugation and washed twice with K-Na-phosphate buffered saline, then resuspended in the same buffered saline and kept until required.

To study bacteriolytic activity**,** cells of overnight cultures of the host bacteria were sedimented and washed twice with K-Na-phosphate buffered saline pH 7. 10^6 CFU of the washed cells suspended in 100 µl of the same buffered saline were spread on the surface of agar plates (15 g agar per litre of 0.05 M K-Na-phosphate buffered saline at pH 7). Early stationary phase cultures of gentamicin-treated and untreated *E. herbicola* 48 were prepared in nutrient broth; cells of 250 µl of these cultures were washed twice, resuspended in 100 µl phosphate buffered saline and loaded into wells previously made in the agar plates. The plates were incubated overnight at the appropriate temperature of the host organisms. Under these conditions, bacteriolysis of the host bacteria was seen after overnight incubation as clear zones around the wells.

Induction of β-lactamase by different β-lactam antibiotics was carried out using the method described by Lindberg & Normark (1987), with modifications. Log-phase culture (OD_{600} 0.7-0.8) of *E. herbicola* 48 was prepared in nutrient broth at 30 °C and agitation rate of 150 rpm. The culture was diluted two-fold by a prewarmed (30 °C) fresh nutrient broth

containing the tested concentration of β-lactam inducer, then the incubation was allowed to continue for two hours but at a reduced agitation rate (120 rpm) to avoid the mechanical damage of the cells in the presence of the antibiotics.

50 ml of the noninduced and induced cultures for β-lactamase production (by β-lactam inducers) and also for MVs formation (by gentamicin) were mixed rapidly with 2.5 mg chloramphenicol (final concentration of 50 μg ml^{-1}) (Gootz & Sanders 1983) and cooled in an ice-water bath to stop the β-lactamase synthesis. The cells were sedimented by centrifugation at 6000 rpm for 30 min and the cell sediments were washed twice with 5 ml ice-cold K-Na-phosphate buffer pH 7 and resuspended in 0.8 ml of the same buffer containing DNase I (Boehring, Manheim, Germany) activated with Mg Cl_2, and 0.2 ml of 50 mM EDTA containing 0.5 mg ml^{-1} lysozyme (Sigma, USA). The suspension was incubated at 37 °C for 30 min and frozen at -20 °C (Azab 1992). 4 ml ice-cold K-Na-phosphate buffer was added to the thawed suspension and the cells were sonicated by using the microtip of sonicator (Model UP 200 S) at 100 % amplification and 7 pulses each of 10 seconds and 30 seconds interruption for cooling in an ice-water bath. The cell debris was removed by centrifugation (Sigma, laboratory cooling centrifuge 1K15) at 4 °C for 1 hour at 15000 rpm and the clear supernatants (β-lactamase crude extracts) were transferred to polypropylene tube to avoid the adsorption of the enzyme by glass container (Joris *et al*. 1986) and stored at -20 °C.

For rapid detection of β-lactamase, 20 μl of cell free extract of the tested organism was mixed with 50 μl of nitrocefin (dissolved in dimethyl sulfoxide 1/25 of the total volume and then filled up to final volume with phosphate buffer) and incubated at 30 ºC. The change of yellow color of the mixture into pink color is a β–lactamase positive result.

β-Lactamase activity was determined by a spectrophotometric method (Minami *et al*. 1980), measuring the increase in absorbance of 100 μM nitrocefin as substrate at 482 nm in a recording spectrophotometer (Shimadzo, UV 240) at 30 °C. One unit of activity is the amount of enzyme that hydrolyzed 1 μmol of nitrocefin in one minute at 30 °C in 0.05 M K-Na-phosphate buffer pH 7.

The protein content of the enzyme crude extract was determined according to the method described by Lowry *et al.* (1951) with bovine serum albumin as standard.

The minimal inhibitory concentrations (MICs) of the used antibiotics for *E. herbicola* 48 were determined in nutrient broth by two-fold serial dilution technique according to DIN 58, 940, Part 5 (1975), yielding a final inoculum of $5x10^5$ CFU ml^{-1} in sterile 6x100 mm capped tubes. To determine the MICs of the used β-lactam antibiotics for gentamicin-treated culture, overnight culture of *E. herbicola* 48 was prepared in nutrient broth, and at the end of log phase, four times the MIC of gentamicin was added and then the culture was allowed to grow overnight. $5x10^5$ CFU ml^{-1} of this culture were used for MICs determination by the above mentioned technique.

RESULTS

Figure 1 shows that *E. herbicola* 48 grown under normal conditions (without antibiotic treatment) produced natural spherical MVs (n-MVs) (Fig. 1a). On the other hand, the addition of four times the MIC of gentamicin to the culture at the end of exponential phase induced the formation of tubular MVs (g-MVs) which were larger in size than natural ones (Fig. 1b). The addition of β–lactam antibiotic, cephradine, at a concentration of four times the MIC,

inhibited the formation of MVs by this bacterium (Fig. 1c) under the experimental conditions used.

The killing of different bacteria by *E. herbicola* 48 cells producing MVs is presented in Table 1. Both gram-positive and gram-negative bacteria tested were lysed by the action of MVs produced by *E. herbicola* 48. The gram-positive bacteria, *Bacillus cereus* 1080 and *B. subtilis* 1020 were highly affected compared to gram-negative hosts. Comparing to other gram-negative bacteria tested, the *E. herbicola* 48 MVs had a higher lysing activity against *Serratia marcescens* HIM 307-2, *Proteus vulgaris* 1753, *Pseudomonas syringae* pv. *Syringae* Van Hall strain 347448 and *E. coli* 1357. *Enterobacter cloacae* NCTC10005, *Erwinia carotovora* NCPPB312, *E. coli* DH5 and *Pseudomonas solanacearum* 1274 were also killed by the action of *E. herbicola* 48 MVs. The growth of *Erwinia carotovora* NCPPB671, and *Serratia marcescens* 921/97 LR were not affected by MVs of *E. herbicola* 48 under the experimental conditions used. On the other hand, the MVs produced by the washed cells of *E. herbicola* 48 exposed to gentamicin (g-MVs) had a higher lytic activity against the all host bacteria used compared to natural MVs (n-MVs). The growth of *Erwinia carotovora* NCPPB671 and *Serratia marcescens* 921/97 LR which were not affected by natural MVs were affected by gentamicin MVs (Table 1).

The rapid detection of β-lactamase indicated that *E. herbicola* 48 produced β-lactamase. When a drop of *E. herbicola* 48 cell-free extract was mixed with a drop of the chromogenic β-lactam substrate, nitrocefin, the yellow color of nitrocefin changed into pink within 10 min. The appearance of pink colour indicated that *E. herbicola* 48 produced the enzyme that hydrolyzed the substrate nitrocefin into the pink products.

To induce β-lactamase in *E. herbicola* 48, structurally different β-lactam antibiotics were used as inducers at different concentrations (Table 2). Concentrations higher than 20 μg ml^{-1} of amoxycillin, ampicillin, cefamandol, cefazolin, ceftazidime, cefuroxime and cephradine damaged the *E. herbicola* 48 cells, while concentrations higher than 2 μg ml^{-1} of ceftriaxone, 5 μg ml^{-1} of cefotaxime and 10 μg ml^{-1} of cefoperazone damaged the induced cells. On the other hand, low amounts of constitutive β-lactamase were produced by *E. herbicola* 48 (10 μU), and insignificant amounts of induced enzyme, using the different β-lactam inducers, were recorded. Compared to untreated culture, gentamicin-treated culture (induced for MVs formation) of *E. herbicola* 48 produced higher amounts (1.6-fold) of β-lactamase constitutively. The low insignificant levels of induced enzyme produced might be due to the absence of β-lactamase regulatory genes in the tested bacterium.

The susceptibility of *E. herbicola* 48 to different β-lactam antibiotics was studied by determining the MICs of amoxycillin, ampicillin, cefazolin, cefamandol, cefoperazone, cefotaxime, ceftazidime, ceftriaxone, cefuroxime, cephalexin and cephradine, using gentamicin-treated and untreated cultures (Table 3).

The determined MICs revealed that gentamicin-untreated *E. herbicola* 48 was susceptible to amoxycillin, cefazolin, cefuroxime, cefamandol, ampicillin, cephalexin and cephradine (Table 3). The MICs of cefoperazone, cefotaxime, and ceftriaxone were very low for *E. herbicola* 48, indicating that it was highly sensitive to these antibiotics. On the other hand, compared to untreated culture, gentamicin-treated *E. herbicola* 48 showed two-fold higher resistance to amoxycillin, cefazolin and ceftazidime, and higher resistance to cefoperazone and ceftriaxone.

DISCUSSION

Gram-negative cell walls have a dynamic feature that is not seen in their gram-positive counterparts: outer membrane vesicles are constantly being discharged from the cell surface during bacterial growth. The transmission electron microscopy indicated that the phytopathogenic gram-negative bacterium *E. herbicola* 48 produced spherical MVs. Compared to untreated cells, *E. herbicola* 48 exposed to gentamicin produced larger and tubular MVs. Similar observations were reported by Kadurugamuwa & Beveridge (1995, 1996) and Beveridge (1999). Transmission electron microscopy revealed that no MVs were produced by *E. herbicola* 48 cells exposed to the β-lactam antibiotic, cephradine. The inhibition of MVs formation by cephradine might be because β-lactam antibiotics bind to and inactivate specific targets on the outer surface of the bacterial cell membrane, the penicillin-binding proteins (PBPs), which have a role in MVs formation (Tipper & Wright 1979; Tomasz 1979; Yocum *et al.* 1980; Neu 1982; Tipper 1986; Kadurugamuwa & Beveridge 1995). The PBPs (transpeptidases, carboxypeptidases and endopeptidase) are involved in the terminal stages of assembling and reshaping the bacterial cell walls during growth and division (Blumberg & Strominger 1974; Spratt 1975, 1980).

The washed cells of *E. herbicola* 48 had a killing effect on most of bacteria tested (gram-positive and gram-negative). Their bacteriolytic potency on the gram-positive bacteria tested was higher than that on gram-negative. The killing effect was due to the MVs produced by *E. herbicola* 48 cells under insufficient nutrient conditions. These MVs contained peptidoglycan hydrolases which in turn hydrolyzed the peptidoglycan of the cell wall of the host bacteria. Kadurugamuwa & Beveridge (1996) and Li *et al.* (1998) also reported that MVs isolated from gram-negative bacteria hydrolyzed isolated gram-positive and gram-negative murein sacculi. The MVs produced by *E. herbicola* 48 washed cells exposed to gentamicin had a higher lytic activity against the host bacteria compared to those produced by untreated cells. This might be due to the formation of larger tubular MVs filled with enzymes and gentamicin which might act synergistically with the degradative enzymes enclosed. Similar conclusions were reported by Kadurugamuwa & Beveridge (1995, 1996) and Beveridge 1999. The bacteriolytic activity of *E. herbicola* 48 against bacterial pathogens may encourage us to try to use it for biological control of plant diseases caused by other bacteria.

E. herbicola 48 also produced β–lactamase enzymes that hydrolyze β–lactam antibiotics. Gentamicin-treated *E. herbicola* 48 (induced for MVs formation) produced higher constitutive β–lactamase activity (1.6-fold) compared to untreated culture. Also, it has been discovered that a chromosome-encoded β–lactamase in *Pseudomonas aeruginosa* can also be packaged into MVs. This raises the intriguing possibility that β–lactamase-containing MVs could be discharged from pathogens at their infection sites in tissue to increase the breakdown of β–lactam antibiotics in the local tissue environment (Beveridge 1999). Low levels of the enzyme were produced constitutively, and insignificant amounts (twice the constitutive levels) of the induced enzyme were obtained using different β-lactam inducers (penicillins and cephalosporins). The low level of β-lactamase production might be due to the absence of the β-lactamase regulatory genes (required for induction) in *E. herbicola* 48. Similarly, Azab (1992) reported that *Proteus vulgaris* U7 produced low β-lactamase level (constitutive, 6 μU and induced 20 μU) and after introduction of the β-lactamase regulatory genes, *ampC* and *ampR*, it produced higher level of the induced enzyme (116-fold). Because the β-lactamase level (which confers higher resistance to β–lactams) was low, *E. herbicola* 48 showed

different levels of susceptibility to all β–lactam antibiotics used, recording low MICs. On the other hand, gentamicin-treated *E. herbicola* 48 exhibited higher resistance compared to untreated culture, to some of antibiotics used, and this might be due to β-lactamase enclosed in the larger tubular MVs induced by gentamicin. Accordingly, we conclude that, *E. herbicola* 48 produced outer MVs that were active against some gram-positive and gram-negative bacteria, and low levels of β-lactamase. In addition, higher β-lactamase activity was accompanied with the induction of larger tubular MVs, and this indicates that the gentamicin-induced MVs entrapped larger amounts of β–lactamase from the periplasmic space of the gram-negative cells.

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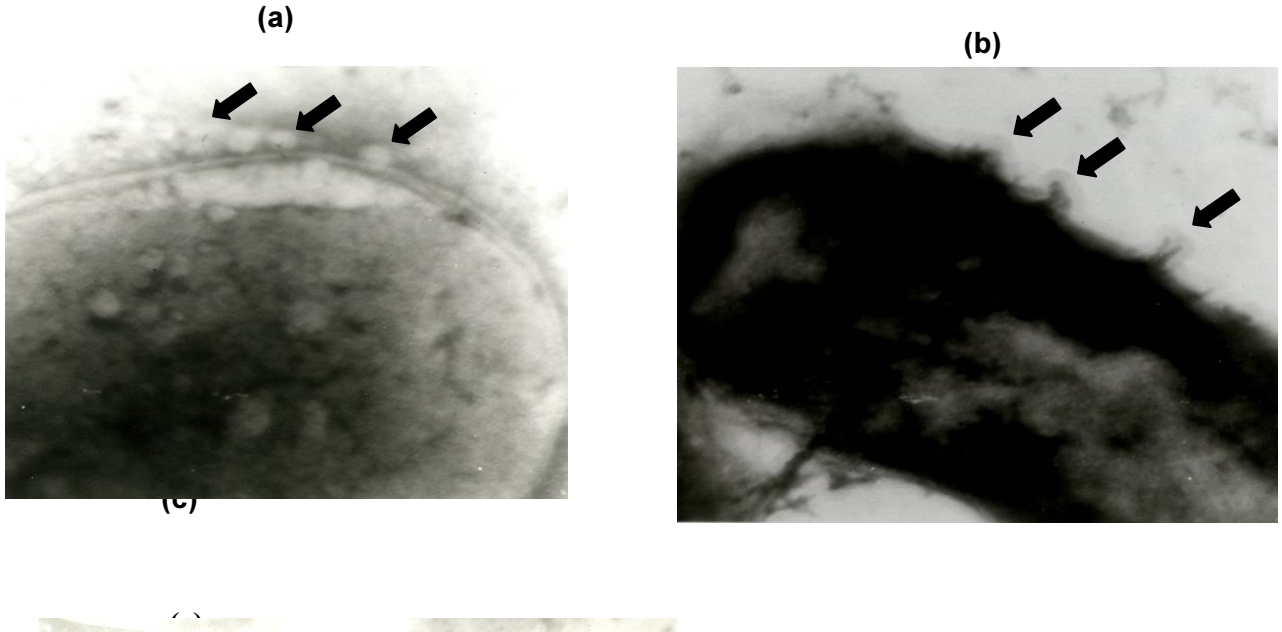

Fig. 1. Transmission electron micrograph showing the formation of MVs of *E. herbicola* 48: (a) spherical MVs; (b) tubular MVs after exposure to gentamicin; (c) inhibition of MVs formation after exposure to cephradine.

Table 1: Lytic effect of *E. herbicola* 48 MVs on different bacteria.

Host Bacteria		B. c. 1080	B. s. 1020	S. m. 307-2	P. v. 1753	P. s. 347448	E. c. 1357	E. c. 10005	E. c. 312	E. c. DH 5	P. s. 1274	E. c. 671	S. m. LR
Inhibition zone(mm)	n-MVs	24 ± 0.67	22 ± 1.1	20 ± 0.44	19.7 ± 0.89	19 ± 0.67	19 ± 1.8	17.7 ± 0.89	17.3 ± 1.1	16.3 ± 0.44	16 ± 0.05	0.0	0.0
	g-MVs	29 ± 0.89	27 ± 1.8	24.4 ± 0.67	22.5 ± 0.44	23 ± 0.67	22.8 ± 0.44	20.5 ±1.1	21.7 ± 0.89	20 ± 0.89	20 ± 0.67	5 ± 0.44	4 ± 0.67

B. c.1080, *Bacillus cereus* 1080; B. s.1020, *Bacillus subtilis* 1020; S. m. 307-2, *Serratia marcescens* HIM 307-2; P. v. 1753, *Proteus vulgaris* 1753; P. s. 347448, *Pseudomonas syringae* pv. *Syringae* Van Hall strain 347448 ; E. c. 1357, *E. coli* 1357; E. c. 10005, *Enterobacter cloacae* NCTC10005; E. c. 312, *Erwinia carotovora* NCPPB312; E. c. DH5, *E. coli* DH5; P. s. 1274, *Pseudomonas solanacearum* 1274; E. c. 671, *Erwinia carotovora* NCPPB671, S. m. LR, *Serratia marcescens* 921/79 LR.
values are ± 1 standard error of the mean

Table 2: Induction of β-lactamase in *E. herbicola* 48 by different β-lactam antibiotics

Inducer (μg/ml)	*Specific activity (μU/mg protein) at different concentration of the inducers									
	**Cons	0.25	0.5	1.0	2.0	5.0	10.0	15.0	20.0	30.0
Amoxycillin	10	11	13	13	12	15	15	15	15	D
Ampicillin	10	9	11	12	11	14	14	14	16	D
Cefamandol	10	8	10	12	12	15	17	17	18	D
Cefazolin	10	10	10	12	13	15	16	16	15	D
Cefoperazone	10	11	11	15	14	15	17	D	D	D
Cefotaxime	10	10	10	12	11	13	D	D	D	D
Ceftazidime	10	12	12	14	13	15	17	19	19	D
Ceftriaxone	10	12	13	15	20	D	D	D	D	D
Cefuroxime	10	10	12	12	14	17	17	17	15	D
Cephalexin	10	10	10	13	12	15	15	17	17	17
Cephradine	10	9	9	11	12	12	12	10	9	D

* : 0.1 mM nitrocefin as a substrate.
D: Cells were damaged.
**: Constitutive enzyme.

Table 3: Minimal inhibitory concentrations of different β–lactam antibiotics for gentamicin-treated and untreated *E. herbicola* 48.

Culture type	MICs of β-lactam antibiotics (μg ml^{-1})										
	AMX	AMP	CMD	CFZ	CPZ	CTX	CTZ	CRZ	CRX	CPX	CRD
untreated	1	4	2	1	<0.0625	<0.0625	0.125	<0.0625	1	8	8
g-treated	2	4	2	2	0.0625	<0.0625	0.250	0.0625	1	8	8

Abbreviations: AMX, amoxycillin; AMP, ampicillin; CMD, cefamandol; CFZ, cefazolin; CPZ, cefoperazone; CTX, cefotaxime; CTZ, ceftazidime; CRZ, ceftriaxone; CRX, cefuroxime; CPX, cephalexin and CRD, cephradine.
g-treated: gentamicin-treated.

الملخص العربي

حويصلات غشائية و بيتا-لاكتاميز في بكتيرة الإروينيا هربيكولا ٤٨

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الإروينيا هربيكولا ٤٨ بكتيرة سالبة الجرام وممرض نباتي. و قد تبين من فحص الخلايا المغسولة لبكتيرة الإروينيا هربيكولا٤٨ بالميكروسكوب الإلكتروني أن هذه البكتيرة تنتج حويصلات غشائية كروية الشكل وذلك في ظروف نمو طبيعية ولكن عند تعرض مزرعة هذه البكتيرة للمضاد الحيوي الچنتامايسين (من مجموعة الأمينوجليكوسيد) فإنها تنتج حويصلات غشائية أكبر في الحجم و ذات شكل أنبوبي، و عند إضافة المضاد الحيوي السيفرادين (من مجموعة بيتا-لاكتام) إلى مزرعة هذه البكتيرة فإنه قد تسبب في منع تكوين هذه الحويصلات.
الحويصلات الغشائية المنتجة بواسطة بكتيرة الإروينيا هربيكولا٤٨ في ظروف نمو طبيعية كان لها القدرة على قتل و تحليل أنواع مختلفة من البكتيريا الموجبة و السالبة الجرام المستخدمة كعائل ، و بالمقارنة بالحويصلات الغشائية المنتجة في ظروف نمو طبيعية فإن الحويصلات الغشائية المنتجة بعد تعرض المزرعة للچنتامايسين كان لها نشاط تحليلي أكبر ضد البكتيريا المستخدمة كعائل.
ومن ناحية أخرى فقد وجد أن هذه البكتيرة تنتج إنزيم البيتا-لاكتاميز و أن المزرعة المحثة لإنتاج حويصلات غشائية أظهرت نشاطاً أكبراً لهذا الإنزيم (١,٦ مرة) مقارنةً بالمزرعة الغير محثة.
كما أنه وجد أن هذه البكتيرة تنتج كمية قليلة من الإنزيم الغير محث و كميات لا يعتد بها من الإنزيم المحث بمضادات حيوية مختلفة من مجموعة بيتا-لاكتام.
و قد تم حساب أقل تركيز مثبط من المضادات الحيوية المستخدمة ضد هذه البكتيرة و تبين أنها حساسة لهذه المضادات الحيوية و هذا يرجع إلى قلة إنزيم البيتا-لاكتاميز المنتج، ولكن المزرعة التي تعرضت للچنتامايسين أظهرت بعض المقاومة للأموكسيسلين، السيفازولين، السيفوبرازون، السيفتازيديم و السيفترياكسون.