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Susceptibility of Environmental Strains of *Rhizobium radiobacter* to Antimicrobial Agents

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Abstract: The objective of the present study was to evaluate the *in vitro* activities of antimicrobial agents against *Rhizobium radiobacter* strains, in order to identify the most effective antibiotics for the elimination and control of this bacterium from different biological systems. Carbenicillin, imipenem, meropenem, gentamicin, amikacin, polymyxin B, ciprofloxacin and levofloxacin were determined to be applicable for transformation of the plants with these bacteria. Carbenicillin, carbapenems and fluoroquinolones would be suggested for treatment of human infections.

Key words: *Rhizobium radiobacter* · Antibiotics · Transformation · Human infections

INTRODUCTION

Rhizobium radiobacter ('Agrobacterium' radiobacter, 'Agrobacterium tumefaciens') is a Gram-negative, oxidase-positive, non-fermenting bacterium predominantly found in soil.

R. radiobacter-mediated transformation is the method of choice for introducing foreign genes into plants [1]. During the development of a transformation protocol, it was noted that controlling *R. radiobacter* growth after gene delivery and increasing the efficiency of the somatic embryogenesis system were critical for transformation success. Typically, various antibiotics are used for counter-selecting *R. radiobacter*.

R. radiobacter, as well as *Rhizobium vitis*, causes crown gall disease of grapevine (*Vitis* spp.). Additionally, *R. radiobacter* occasionally has been associated with opportunistic infections in humans, including bacteraemia [2], endophthalmitis [3], peritonitis [4 Marta], meningitis [5], etc. Due to its low incidence and low-level virulence, there have never been clinical trials to determine the optimal therapy for *R. radiobacter* infections. Antibiotic treatment may be established, based on the analyses of a large group of isolates.

The objective of the present study was to evaluate the *in vitro* activities of antimicrobial agents against *R. radiobacter* strains, in order to identify effective antibiotics for the elimination of this bacterium from different biological systems.

MATERIALS AND METHODS

Thirteen strains of *R. radiobacter*, selected from the collection at the Institute of Viticulture and Enology (IVE), Pleven, Bulgaria, were included in the study (Table 1). They had been identified previously by phenotypic and genotypic methods. Species determinations were performed using metabolic characterisation in micro-titre plates as described by Cubero and López, 2001 [6] and Moore *et al.*, 2001 [10]. The grapevine strains of *R. radiobacter* were distinguished from *R. vitis* by PCR with specific primer pairs [11] and DNA sequence analyses [12].

Antibiotic susceptibility testing was performed by the disc diffusion method [13]. The *in vitro* activities of ten antimicrobial agents (carbenicillin, 100 mcg; ceftazidime, 30 mcg; aztreonam, 30 mcg; imipenem, 10 mcg; meropenem, 10 mcg; gentamicin, 10 mcg; amikacin, 30 mcg; polymyxin B, 300 UI; ciprofloxacin, 5 mcg; and

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Table 1: Origin data and phytopathogenic characteristics of *R. radiobacter* strains

Strain	Host	Year	Country of isolation	Pathogenic for			Reference
				Vitis	*Solanum*	*Datura*	
B-6	Tomato	1935	USA	+	+	+	6
C-58	Cherry	1958	USA	+	+	+	7
B-23	Grapevine	1967	Bulgaria	+	+	+	8
BT	Apple	1967	Bulgaria	+	+	+	8
1001	Grapevine	1973	Bulgaria	+	+	+	8
426	Grapevine	1974	Bulgaria	+	+	+	8
IVE-016	Grapevine	2000	Bulgaria	+	+	+	9
IVE-048	Soil	2004	Bulgaria	+	-	-	9
IVE-068	Grapevine	2004	Bulgaria	+	-	-	9
IVE-170	Grapevine	2007	Bulgaria	+	+	+	9
IVE-187	Grapevine	2007	Bulgaria	+	+	+	9
IVE-203	Grapevine	2007	Bulgaria	+	-	-	9
IVE-238	Grapevine	2008	Bulgaria	+	+	+	9

levofloxacin, 5 mcg) were determined. The Clinical and Laboratory Standards Institute criteria were used for interpretation of the results [13].

RESULTS AND DISCUSSION

All strains were observed to be susceptible to eight of the tested antibiotics: carbenicillin; imipenem; meropenem; gentamicin; amikacin; polymyxin B; ciprofloxacin; and levofloxacin. The activities of ceftazidime and aztreonam varied markedly between strains. In those cases, seven antibiotic sensitivity patterns were detected (Table 2), although no correlation was detected between the antibiograms and the phytopathogenicity of strains.

Among the antibiotics tested, imipenem, ciprofloxacin, meropenem, levofloxacin and carbenicillin demonstrated effective control over bacterial growth and produced the largest inhibition areas, with maximal zones of 52, 52, 50, 48 and 48 mm, respectively. Gentamicin, amikacin and polymyxin B were effective in controlling bacterial growth in this assay, although the zones of inhibition were smaller, with maximal zones of 40, 36 and 22 mm, respectively.

Ogawa and Mii, 2004 [14] performed screenings of highly active β-lactam antibiotics against *R. radiobacter*. Their findings are similar to the results reported in this study. The most active antimicrobial agent was the carbapenem meropenem (imipenem was not tested in their study) and the least active were the monobactams,

Table 2: *In vitro* activities of 10 antibiotics against *R. radiobacter* strains

Strain	CB[a]	CAZ	ATM	IMP	MEM	GM	AN	PB	CIP	LVX
B-6	S[b]	S	I	S	S	S	S	S	S	S
C-58	S	S	R	S	S	S	S	S	S	S
B-23	S	S	I	S	S	S	S	S	S	S
BT	S	I	I	S	S	S	S	S	S	S
1001	S	R	I	S	S	S	S	S	S	S
426	S	S	I	S	S	S	S	S	S	S
IVE-016	S	S	I	S	S	S	S	S	S	S
IVE-048	S	R	R	S	S	S	S	S	S	S
IVE-068	S	S	I	S	S	S	S	S	S	S
IVE-170	S	S	S	S	S	S	S	S	S	S
IVE-187	S	S	R	S	S	S	S	S	S	S
IVE-203	S	I	R	S	S	S	S	S	S	S
IVE-238	S	S	S	S	S	S	S	S	S	S

[a]CB, carbenicillin; CAZ, ceftazidime; ATM, aztreonam; IMP, imipenem; MEM, meropenem; GM, gentamicin; AN, amikacin; PB, polymyxin B; CIP, ciprofloxacin; LEV, levofloxacin;

[b]S, susceptible; I, intermediate; R, resistant

aztreonam and carumonam (carumonam was not tested in the present study). *R. radiobacter* strains were susceptible to carbenicillin, but resistant to ceftazidime. Islam *et al*., 2010 [15] determined the sensitivities of *R. radiobacter* to four agents: cefuroxime, kanamycin, tetracycline and rifampicin. Isolates were susceptible to cefuroxime (cephalosporin, 2nd generation) and kanamycin (aminoglycoside) and resistant to tetracycline and rifampicin. In the present study, a 3rd generation cephalosporin (ceftazidime) was tested and its activity was observed to vary. Two aminoglycosides (gentamicin and amikacin) exhibited very good activity. Other studies have reported higher levels of antimicrobial resistance of *R. radiobacter*. Mendes *et al*., 2010 [16] investigated 18 strains of human origin; 72.2% of which were susceptible to polymyxin B and only 44.4% to ceftazidime. An explanation of that resistance is the extensive use of antibiotics in the human population, resulting in a selective pressure for the emergence of resistant strains. There have been several recent case reports of *R. radiobacter* infections, caused by isolates resistant to aminoglycosides [3, 4].

In conclusion, the majority of strains isolated from soil and plants, in this study showed significant levels of susceptibility to the antimicrobials tested, suggesting that antimicrobial resistance of environmental strains of *R. radiobacter* is of little concern. Carbenicillin, imipenem, meropenem, gentamicin, amikacin, polymyxin B, ciprofloxacin and levofloxacin would be applicable for plant transformation with these bacterial strains. On the basis of the present study and listed references, carbenicillin, imipenem, meropenem, ciprofloxacin and levofloxacin also could be proposed for effective antibiotic treatment of human infections. Further studies will focus on clarifying the relationships between *in vitro* and *in planta* antibacterial activities, as well as the issues between antibacterial activities and side effects on plant tissues. The number of tested *R. radiobacter* strains should be expanded in order to recommend the most reliable therapy for human infections.

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