Plant Cell Rep (2005) 23:736–743
DOI 10.1007/s00299-004-0885-9

Yoichi Ogawa · Masahiro Mii

Evaluation of 12 β-lactam antibiotics for *Agrobacterium*-mediated transformation through in planta antibacterial activities and phytotoxicities

Received: 13 April 2004 / Revised: 27 August 2004 / Accepted: 30 August 2004 / Published online: 5 October 2004

Abstract The antibacterial activities of 12 β-lactam antibiotics against *Agrobacterium tumefaciens* strains LBA4404 and EHA101 living in tobacco (*Nicotiana tabacum* L.) leaf tissues, and their phytotoxicities to tobacco leaf tissues were evaluated. All β-lactams at minimum bactericidal concentration (MBC) or higher showed weak bactericidal activities against agrobacteria persisting in tobacco leaf tissues. The β-lactams evaluated were classified into two major groups according to their inhibitory effect on shoot regeneration of tobacco leaf tissues: (1) highly phytotoxic drugs, and (2) moderately phytotoxic drugs. According to these results, suitable kind and concentration of β-lactam antibiotics were evaluated for *Agrobacterium*-mediated transformation.

Keywords *Agrobacterium tumefaciens* · β-Lactam antibiotics · Bactericidal activity · Phytotoxicity

Introduction

In the last decade, many transgenic plants have been obtained by using *Agrobacterium*-mediated transformation for crop improvement and genetic research. For *Agrobacterium*-mediated transformation, suppression and elimination of agrobacteria are necessary to minimize the risk of interfering growth and regeneration of the potentially transformed plant tissues, and to reduce the hazard of releasing genetically engineered agrobacteria into the environment. β-Lactam antibiotics such as carbenicillin and cefotaxime have commonly been utilized for this purpose, because this class of antibiotics lyse and kill the bacteria by specifically interfering with the biosynthesis of the prokaryotic peptidoglycan component of the bacterial cell wall by binding to penicillin-binding proteins (PBPs) (Ghuysen 1997; Demain and Elander 1999; Asbel and Levison 2000). On the other hand, stimulating effects on plant cell growth, organogenesis and embryogenesis have also been reported for both carbenicillin and cefotaxime, as listed by Nauerby et al. (1997), as well as for other classes of antibiotics (Pollock et al. 1983; Young et al. 1984; Okkels and Pedersen 1988).

Many β-lactam antibiotics, which are classified into penicillin, cephalosporin, cephamycin, oxacephem, monobactam and carbapenem, are currently being developed to enhance and expand antibacterial activity (Demain and Elander 1999). Recently, several researchers successfully introduced a penicillin derivative, ticarcillin, alone or in combination with a β-lactamase inhibitor, clavulanic acid (Timentin; SmithKline Beecham, Brentford, UK) into the *Agrobacterium*-mediated transformation procedure for several plant species (e.g., Schroeder et al. 1993; Frary and Earle 1996; Aida et al. 1999). Other β-lactam antibiotics have also been applied to plant genetic transformation, e.g., Augmentin (SmithKline Beecham) (Vergauwe et al. 1996), cefoxitin (mefoxin) (Hammerschlag et al. 1997), cephaloridin (Enomoto et al. 1990) and sulbenicillin (Zhang et al. 2000). Although it is important to understand both the antibacterial activity against *Agrobacterium tumefaciens* strains and the phytotoxicity of these β-lactam antibiotics in *Agrobacterium*-mediated transformation, only a few such detailed characterizations have been published (Okkels and Pedersen 1988; Shackelford and Chlan 1996).

In a previous study, we evaluated in vitro antibacterial activities of 12 β-lactams against two common strains of *A. tumefaciens*, LBA4404 and EHA101, by two parameters—minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) (Ogawa and Mii 2004). In this study, we evaluated in planta antibacterial

Communicated by J.C. Register III

Y. Ogawa · M. Mii (✉)
Laboratory of Plant Cell Technology, Faculty of Horticulture,
Chiba University,
648 Matsudo, Matsudo, Chiba, 271-8510, Japan
e-mail: miim@faculty.chiba-u.jp
Fax: +81-47-3088720

Present address:
NEDO Laboratory of Applied Plant Genomics,
Kazusa DNA Research Institute,
2-6-7 Kazusa-Kamatari, Kisarazu, Chiba, 292-0818, Japan

Table 1 β-Lactam antibiotics used in the present study. *MBC* Minimal bactericidal concentration

Antibiotic	Class	MBC (mg l^{-1})[a]		Trade name and manufacturer
		LBA4404	EHA101	
Carbenicillin	Penicillin	6.25	50	Sigma-Aldrich, St Louis, Mo.
Piperacillin	Penicillin	12.5	400	Pentcillin (Toyama Chemical, Tokyo, Japan)
Ticarcillin	Penicillin	3.13	25	Monapen (Fujisawa Pharmaceutical, Osaka, Japan)
Cefotaxime	Cephalosporin	0.78	50	Claforan (Aventis Pharma, Frankfurt, Germany)
Ceftazidime	Cephalosporin	25	200	Modacin (Glaxo Wellcome, London, UK)
Cefbuperazone	Cephamycin	0.39	100	Tomiporan (Toyama Chemical, Tokyo, Japan)
Cefminox	Cephamycin	3.13	50	Meicelin (Meiji Seika Kaisha, Tokyo, Japan)
Moxalactam	Oxacephem	1.56	6.25	Shiomarin (Shionogi, Osaka, Japan)
Flomoxef	Oxacephem	3.13	200	Flumarin (Shionogi, Osaka, Japan)
Aztreonam	Monobactam	100	400	Azactam (Eisai, Tokyo, Japan)
Carumonam	Monobactam	800	800	Amasulin (Takeda Chemical Industries, Osaka, Japan)
Meropenem	Carbapenem	0.20	0.20	Meropen (Sumitomo Pharmaceuticals, Osaka, Japan)

[a] Ogawa and Mii (2004)

activities of these β-lactam antibiotics against *A. tumefaciens* LBA4404 and EHA101, as well as the side effects of the antibiotics on shoot regeneration from tobacco leaf explants. We also discuss the possibilities for application of various β-lactam antibiotics in *Agrobacterium*-mediated transformation.

Materials and methods

β-Lactam antibiotics

The 12 β-lactam antibiotics used in the present study are listed in Table 1. The quantitative bactericidal activities (MBCs) of these β-lactams against agrobacterial strains reported previously (Ogawa and Mii 2004) are also listed in the table.

Bacterial strains

Disarmed *A. tumefaciens* strains LBA4404 (Hoekema et al. 1983) and EHA101 (Hood et al. 1986) were used in the present study without an introduced binary plasmid. Strain LBA4404 has a chromosomal background of an octopine strain Ach5 and carries disarmed helper Ti plasmid, pAL4404. Strain EHA101 has a chromosomal background of a nopaline strain C58 and carries a disarmed helper plasmid pEHA101. Agrobacteria were maintained at −80°C as glycerol stocks, and were cultured routinely on LB (10 g l^{-1} tryptone, 5 g l^{-1} yeast extract, 5 g l^{-1} NaCl, pH 7.0) agar plate at 28°C.

Inoculation and regeneration of tobacco leaf explants with *A. tumefaciens*

Bacterial cells were harvested by centrifugation from an overnight culture of agrobacteria, and resuspended in liquid MS medium (Murashige and Skoog 1962) to A_{600}=0.2 [ca. 5×10^8 colony-forming unit (cfu) ml^{-1}]. Leaf explants (ca. 7 mm square segments) prepared from in vitro plantlets of tobacco (*Nicotiana tabacum* L.) 'Wisconsin 38' were submerged in inoculum solution for 5 min; after blotting excess inoculum on a sterile paper towel, explants were then placed onto regeneration medium [MS basal medium containing 0.1 mg l^{-1} 1-naphthaleneacetic acid (NAA), 1.0 mg l^{-1} 6-benzylaminopurine (BAP), 30 g l^{-1} sucrose, 2.5 m*M* 2-(*N*-morpholino)-ethanesulfonic acid (MES), pH 5.8, solidified with 8 g l^{-1} agar]. After 3 days of cocultivation, explants were transferred to regeneration medium supplemented with various concentrations of β-lactams and without selective antibiotics for transformed cells. For EHA101, 2-fold serially increased concentrations were made for each β-lactam and applied at 1–32× MBC (up to 3,200 mg l^{-1}), while moxalactam and meropenem were applied at 1–128× MBC. For LBA4404, 2-fold serial concentrations of cefotaxime (1–32× MBC), cefbuperazone (1–32× MBC), moxalactam (1–8× MBC) and meropenem (1–128× MBC) were applied. Explants were cultured under the 24 h photoperiod at 30–50 μmol m^{-1} s^{-1} and at 25°C, and subcultured every 2 weeks onto fresh β-lactam-supplemented regeneration medium. Explants that showed overgrowth of agrobacteria were removed from the plates.

Antibacterial activities of 12 β-lactams in planta

Six weeks after cocultivation, explants were homogenized with 2 ml phosphate-buffered saline (PBS; 0.43 g gl^{-1} KH_2PO_4, 1.48 g l^{-1} Na_2HPO_4, 7.2 g l^{-1} NaCl, pH 7.2) using motor and pestle after weighing and counting the number of shoots. Homogenates were appropriately diluted with PBS, and then 100 μl portions of homogenates were spread over LB agar plates. The plates were incubated at 28°C for 3 days or more for colony formation. Explants cocultivated for 3 days (just before antibiotic treatment) were also examined in order to count the number of viable agrobacteria. The number of persisting agrobacteria was defined as cfu per gram fresh weight (FW) of explant.

Effect of β-lactams on shoot regeneration from tobacco leaf tissues

Shoot regeneration from tobacco explants with or without β-lactam treatment was observed 6 weeks after cocultivation. Phytotoxicity was defined as shoot formation rate, which was determined as the percentage of the number of regenerated shoots per explant on antibiotic-treated explants with or without inoculation with agrobacteria, against that on control explants.

Data analysis

In all experiments, five (for EHA101) or six (for LBA4404) explants per treatment were placed individually onto 90×20 mm plates containing 25 ml medium. Each experiment was repeated three times. Significant differences among the data were obtained from one-factor ANOVA followed by Fisher's PLSD. Arcsin transformation and logarithmic transformation were applied for the data in percentile value and in exponential value, respectively, before statistical analyses, although the data before transformation were represented.

Results and discussion

Suppression of agrobacterial overgrowth on explants inoculated with EHA101 by β-lactams

When tobacco leaf explants were inoculated with *A. tumefaciens* EHA101 and cultured on media containing β-lactam antibiotics at or above the MBC, overgrowth of EHA101 on the explants and surrounding medium was observed with six β-lactams, i.e., cefotaxime, cefbuperazone, cefminox, moxalactam, flomoxef and meropenem, typically at 1–2× MBC of each antibiotic (Fig. 1). These results are consistent with those obtained by Falkiner (1988, 1990) and Barrett and Cassells (1994), who recommended use of 2–4× MBC of antibiotics to prevent bacterial growth in plant tissue culture. Although meropenem is the most active antibiotic against both LBA4404 and EHA101 in vitro (Table 1; Ogawa and Mii 2004), it allowed agrobacterial overgrowth in 100% of explants inoculated at 1–8× MBC (0.20–1.56 mg l^{-1}). However, meropenem could suppress overgrowth of EHA101 at 6.25 mg l^{-1} (32× MBC), which is the lowest effective concentration of those evaluated here. The antibacterial activity of many of the β-lactams was slightly affected by factors such as inoculum size, culture medium, and pH of the medium (Fukasawa et al. 1992). Thus, the loss of in planta antibacterial activity of meropenum at relatively high MBC might be due to an interaction between this compound and plant tissues, such as β-lactam permeability or uptake.

In planta bactericidal activities of β-lactams against EHA101

Under the present inoculation conditions, ca. 2.5×10^9 cfu gFW^{-1} viable EHA101 cells were detected in the explant after 3 days of cocultivation. Persistence of agrobacteria (up to 2.8×10^9 cfu gFW^{-1}) was observed in explants cultured on β-lactam-containing agar plates after 6 weeks of culture, even when no agrobacterial overgrowth was observed on explants visually (Fig. 2). Although persisting EHA101 cells in planta decreased to 10^6–10^8 cfu gFW^{-1} over the wide range of β-lactam concentrations tested, bactericidal activity of β-lactams was not complete and was only weakly correlated with antibiotic concentration. Obvious in planta bactericidal activity was observed only for ceftazidime and cefbuperazone, which decreased the agrobacterial survival rate to below 10^6 cfu gFW^{-1} at 2× MBC and 4 × MBC, respectively. The poor correlation between concentration and in planta antibacterial activities against EHA101 observed for other antibiotics might be affected by multiple factors, e.g., the physiological state of agrobacteria persisting in plant tissue known as "latent infection" (Hayward 1974; Cooke et al. 1992), the presence of high numbers of agrobacterial cells in inoculated leaf tissue, the stability of the β-lactams at the temperature and light regimes adopted for culture, and the efficiency of uptake of β-lactams from gelled medium to plant tissue.

Survival of agrobacteria was observed in both leaf explants and regenerated shoots after 6 weeks of β-lactam treatment. The persistence of agrobacteria in transgenic plants has been reported when carbenicillin, ticarcillin or cefotaxime were used at 125–500 mg l^{-1} (van der Hoeven et al. 1991; Matzk et al. 1996; Barrett et al. 1997). In addition, the bactericidal activities of β-lactams against *A.*

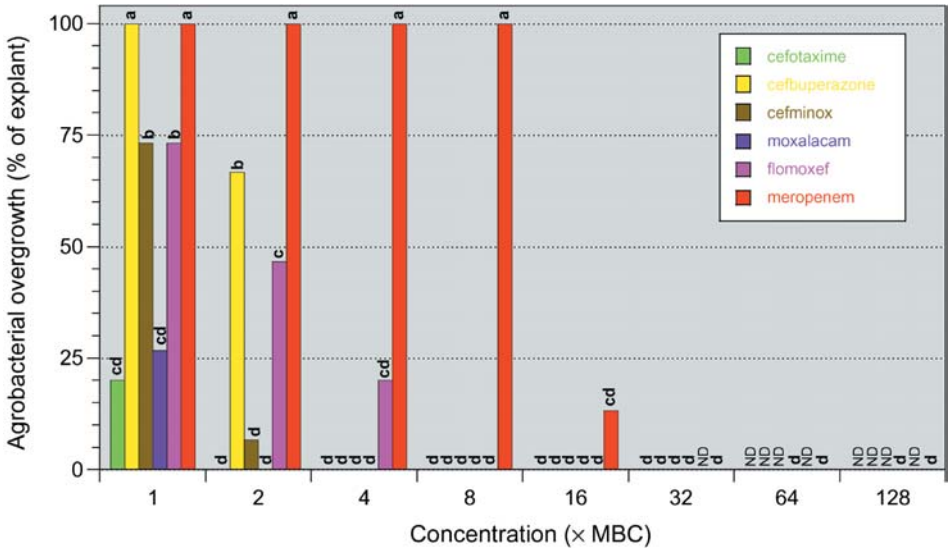

Fig. 1 Frequencies of explants showing agrobacterial overgrowth. Treatments with the same letter are not significantly different according to Fisher's PLSD. Data for the other six β-lactams are not shown because overgrowth was not observed on explants treated with these antibiotics. *ND* Not determined

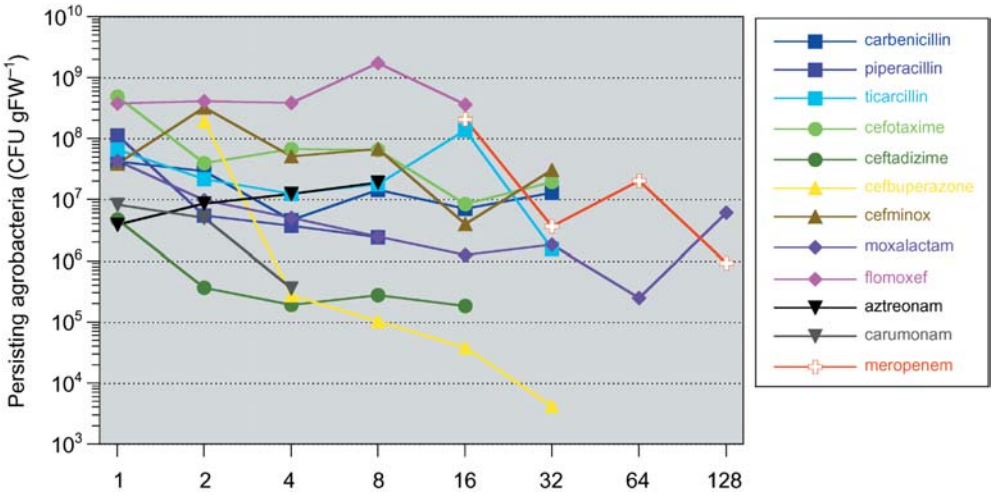

Fig. 2 In planta bactericidal activities of β-lactams against *Agrobacterium tumefaciens* EHA101. Data represent the number of surviving agrobacteria. Significant differences were detected by ANOVA (P=4.47×10^{-18}). SD and significance of each data point are not shown to minimize complexity of the figure (average SD ±6.38×10^{7} cfu gFW^{-1})

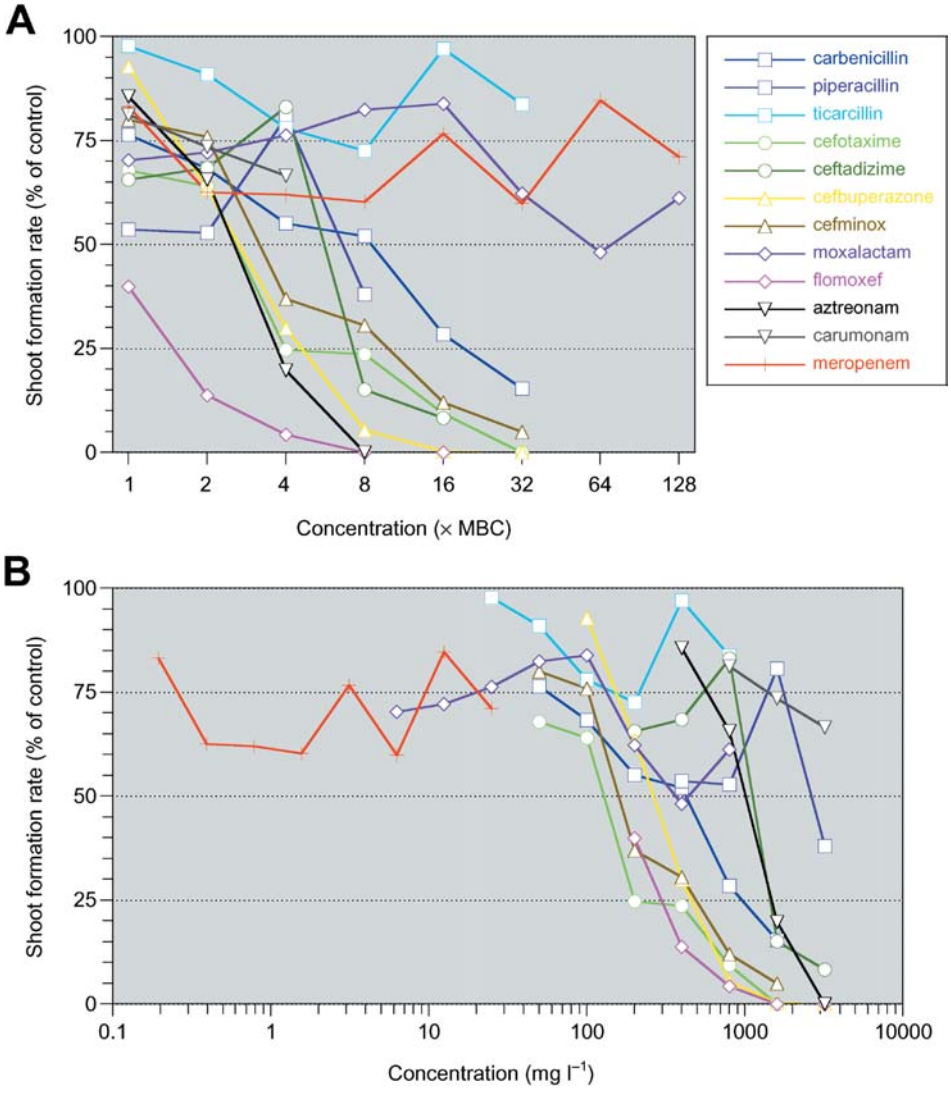

Fig. 3a,b Effect of β-lactams on shoot regeneration from tobacco leaf explants without inoculation with *A. tumefaciens* EHA101. The data are arranged by **a** relative concentration (×MBC), and **b** absolute concentration (mg l^{-1}) of β-lactams. Significant differences were detected by ANOVA (P=3.36×10^{-21}). SD and significance of each data point are not shown to minimize complexity of the figure (average SD ±18.6%)

tumefaciens strains as well as clinical pathogens (Craig and Ebert 1991) are known to exhibit "concentration-independent killing" or "time-dependent killing" (Ogawa and Mii 2004). Therefore, to avoid the risk of releasing genetically engineered *Agrobacterium* into the environment, β-lactams should be continuously supplied to the culture medium until the elimination of agrobacteria persisting in the plant tissue has been confirmed.

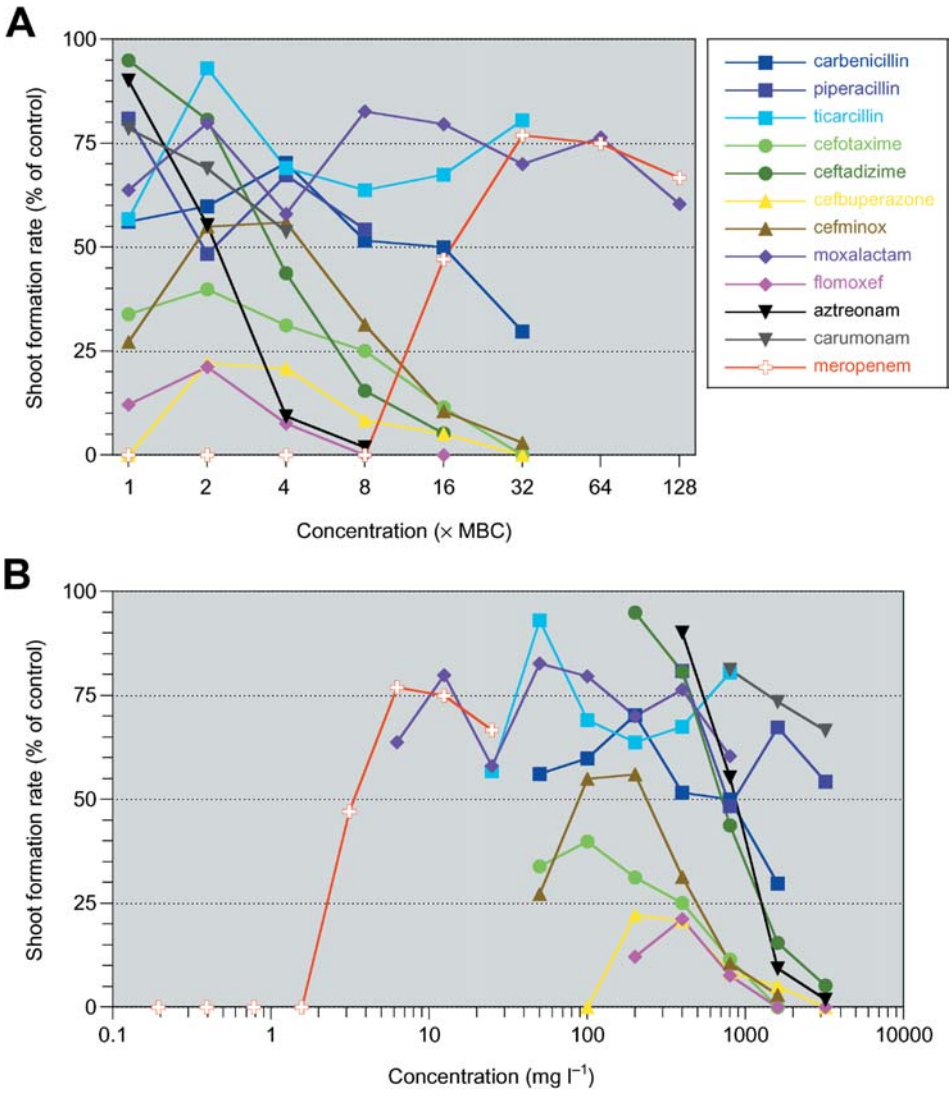

Fig. 4a,b Effect of β-lactams on shoot regeneration from tobacco leaf explants inoculated with *A. tumefaciens* EHA101. The data are arranged by **a** relative concentration (×MBC) and **b** absolute concentration (mg l^{-1}) of β-lactams. Significant differences were detected by ANOVA (P=3.36×10^{-21}). SD and significance of each data point are not shown to minimize complexity of the figure (average SD ±15.8%)

Side effects of β-lactams on plant tissues at concentrations bactericidal to EHA101

At concentrations bactericidal against EHA101, the 12 β-lactams were generally toxic to tobacco tissue and inhibited its organogenesis (Fig. 3), and an inhibitory effect of all β-lactams on shoot regeneration was observed at the concentration that suppressed agrobacterial overgrowth, irrespective of inoculation with EHA101 (Fig. 4). According to the concentration dependency of the inhibitory effects on shoot regeneration, all but two β-lactams could be classified as either: (1) highly phytotoxic drugs (carbenicillin, cefotaxime, ceftazidime, cefbuperazone, cefminox, flomoxef, and aztreonam), or (2) moderately phytotoxic drugs (ticarcillin, moxalactam, and meropenem).

Most of the concentration-dependent phytotoxic β-lactams reduced the shoot regeneration rate to below 50% at 4× MBC, and at 8× or more MBC, all these drugs severely inhibited shoot regeneration, leading to shoot formation rates of less than 10% (Figs. 3, 4). The inhibitory effect generally appeared at concentrations over 100 mg l^{-1} (Figs. 3, 4). Leaf explants that were treated with these β-lactams at inhibitory concentrations showed chlorosis within 2 weeks and finally showed necrosis, although leaf explants treated with carbenicillin formed calli without showing chlorosis.

In the concentration-independent, moderately phytotoxic, group, both moxalactam and meropenem maintained a relatively high shoot formation rate (approximately 60–80%) over a wide range of concentration, i.e., 1–128× MBC (6.25–800 mg l^{-1} moxalactam and 0.20–25 mg l^{-1} meropenem). Ticarcillin-treated explants also showed high regeneration capacity at 1–32× MBC (25–800 mg l^{-1}) and had higher shoot formation rates (73–98%) than moxalactam- and meropenem-treated explants.

The remaining two antibiotics, piperacillin and carumonam, maintained a shoot formation rate of 38–81% at the high concentrations of antibiotics tested: 400–3,200 mg l^{-1} for piperacillin and 800–3,200 mg l^{-1} for carumonam, respectively. However, it was not possible to classify carumonam and piperacillin into these two groups

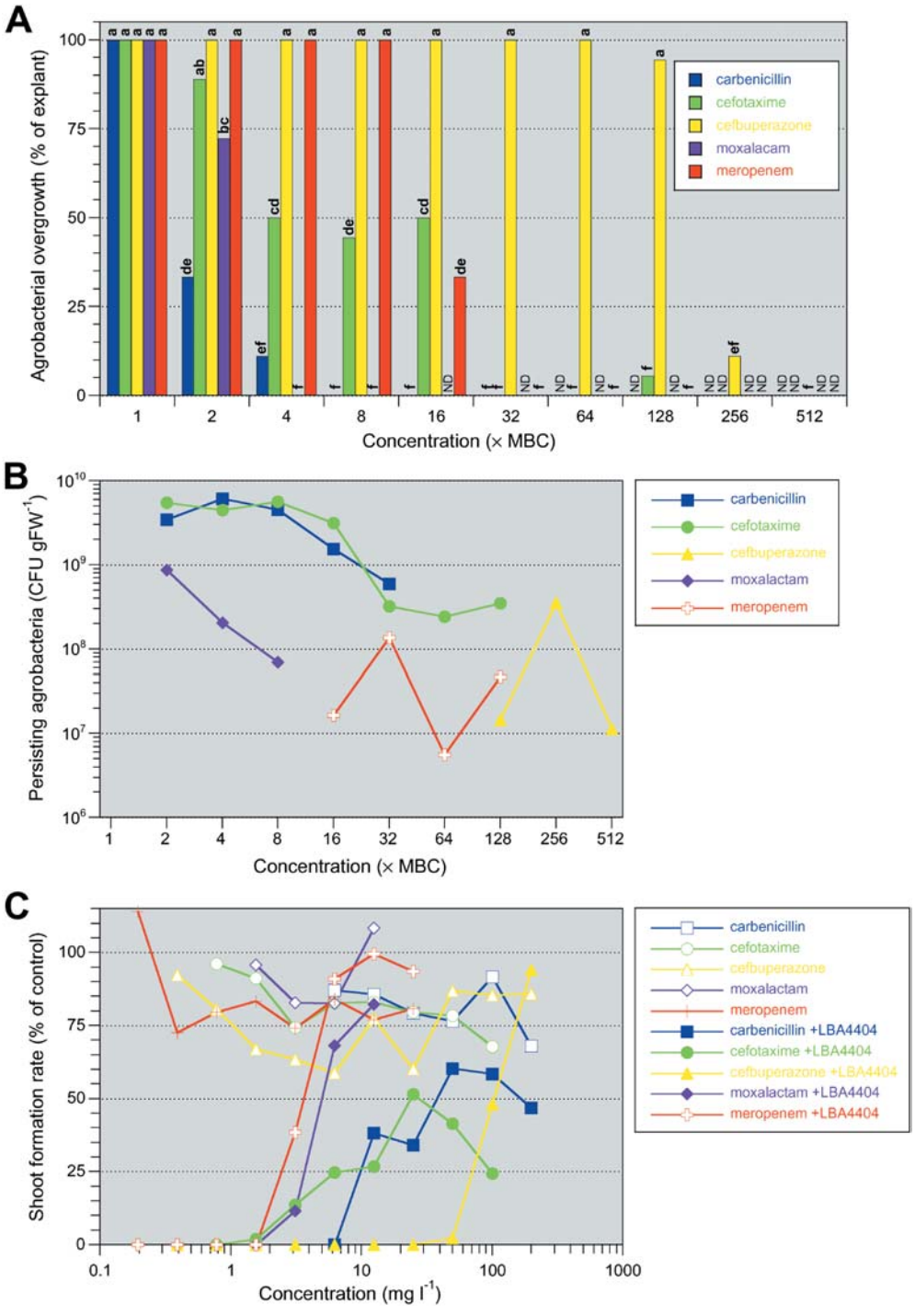

Fig. 5a–c In planta antibacterial activities of four β-lactams against *A. tumefaciens* LBA4404 and phytotoxicities against tobacco leaf explants. **a** Effect of β-lactams on overgrowth of agrobacteria. Columns followed by the same letter are not significantly different according to Fisher's PLSD. **b** Effect of β-lactams on survival of agrobacteria. Significant differences were detected by ANOVA (P=4.39×10^{-3}). SD and significance of each data point are not shown to minimize complexity of the figure (average SD ±1.93×10^{9} cfu gFW^{-1}). **c** Effect of β-lactams on shoot regeneration from tobacco leaf explants inoculated with or without LBA4404. Significant differences were detected by ANOVA (P=3.41×10^{-34}). SD and significance of each data point are not shown to minimize complexity of the figure (average SD ±14.6%)

because concentrations greater than 3,200 mg l^{-1} were not examined.

It has been reported that β-lactams have both stimulatory and inhibitory effects on plant cell growth and organogenesis (Nauerby et al. 1997). The β-lactams tested in this study showed no apparent stimulatory effect, although callus formation was observed with carbenicillin and three of the concentration-independent, moderately phytotoxic, antibiotics (ticarcillin, moxalactam and meropenem) without any marked delay or decline in shoot regeneration and growth.

In planta antibacterial activities and phytotoxicities of β-lactams in LBA4404

After evaluation of 12 β-lactams on EHA101, similar experiments were performed on LBA4404 using five β-lactams (carbenicillin, cefotaxime, cefbuperazone, mox-

alactam, and meropenem) that were highly active against this bacterial strain in vitro (Table 1; Ogawa and Mii 2004). Although all five β-lactams controlled overgrowth of LBA4404 on explants inoculated at 1× MBC for the first 2 weeks, they required 4× or higher MBC to suppress overgrowth during 6 weeks of culture (Fig. 5a). An extremely high concentration of cefbuperazone (256× MBC) was required to inhibit agrobacterial overgrowth. While cefotaxime usually suppressed overgrowth of LBA4404 at 32× MBC, it occasionally failed to suppress overgrowth even at 128× MBC. In planta bactericidal activities of these five β-lactams against LBA4404 were low and concentration-independent, as observed against EHA101. Numbers of persisting LBA4404 cells after β-lactam-treatment were 10-fold to 1,000-fold higher (10^8–10^{10} cfu gFW^{-1}) than with EHA101 (Fig. 5b). Amongst five β-lactams tested, only meropenem showed similar high in planta bacterial activity against both LBA4404 and EHA101.

In the absence of LBA4404, shoot formation rate was 60% or higher (Fig. 5c). As shown in prior experiments, the same high shoot formation rate was also obtained when β-lactams were applied at low phytotoxic concentration ranges (Fig. 3). It is interesting to note that shoot formation rate was reduced by inoculation with LBA4404 (Fig. 5c) but not with EHA101 (Figs. 3, 4) when the explants were exposed to either carbenicillin, cefotaxime or moxalactam at concentrations that completely suppressed agrobacterial overgrowth. This phenomenon might be related to the difference in number of persisting agrobacteria in explants after inoculation with these two bacterial strains as described above. Occasional overgrowth of LBA4404 on explants treated with 128× MBC cefotaxime may be explained by the large number of persisting agrobacteria as well as by the concentration-independent bactericidal activity of this β-lactam.

Conclusions

One of the most important requirements for *Agrobacterium*-mediated transformation is the selection of suitable antibiotics that do not inhibit growth, organogenesis or embryogenesis of plant tissues at concentrations effective for control of agrobacteria. Based on the results presented here, we conclude that meropenem and moxalactam are the most effective antibiotics for *Agrobacterium*-mediated plant genetic transformation of tobacco leaf explants. Meropenem exhibited the highest antibacterial activities against EHA101 and LBA4404 while also allowing a high level of shoot formation rate at rates of $\geq$6.25 mg l^{-1}. Therefore, it could be utilized for *Agrobacterium*-mediated transformation studies with strains such as LBA4404, EHA101, and probably other C58 derivatives. Moxalactam also appears to be suitable for *Agrobacterium*-mediated transformation, since it showed effects similar to those of meropenem on both agrobacterial strains and plant tissues. Appropriate concentrations of moxalactam are 25 mg l^{-1} for EHA101 and 6.25 mg l^{-1} for LBA4404. Carbenicillin and cefotaxime, which are currently utilized in many laboratories, appear to be less optimal for *Agrobacterium*-mediated transformation of tobacco leaf explants. However, optimization of the concentration of these β-lactams could improve transformation efficiencies. Based on these results, the optimal concentration of carbenicillin is at least 50 mg l^{-1} for LBA4404 or EHA101 and, for cefotaxime, 25–200 mg l^{-1} for LBA4404 and 100–200 mg l^{-1} for EHA101. Since side effects of antibiotics may vary depending on plant species and explant sources (Nauerby et al. 1997), preliminary experiments would be required for other antibiotic-plant combinations. The MBCs established in our previous study (Ogawa and Mii 2004) as well as the results presented here could be utilized as a baseline.

Incorporating β-lactams such as meropenem and moxalactam into *Agrobacterium*-mediated transformation procedures appears to be a simple and effective strategy for achieving high transformation efficiencies while minimizing persistent *Agrobacterium* growth. Although the novel, highly active, drugs described in this study are more expensive than conventionally used β-lactams, such as penicillin derivatives, lower concentration requirements will help minimize additional expense.

Acknowledgement We thank Dr. R. Aida (National Institute of Floricultural Science, Japan) for the generous gift of ticarcillin and for helpful suggestions.

References

Aida R, Hirose Y, Kishimoto S, Shibata M (1999) *Agrobacterium tumefaciens*-mediated transformation of *Cyclamen persicum* Mill. Plant Sci 148:1–7

Asbel LE, Levison ME (2000) Cephalosporins, carbapenems, and monobactams. Infect Dis Clin North Am 14:435–447

Barrett C, Cassells AC (1994) An evaluation of antibiotics for the elimination of *Xanthomonas campestris* pv. *pelargonii* (Brown) from *Pelargonium* × *domesticum* cv. 'Grand Slam' explant in vitro. Plant Cell Tissue Organ Cult 36:169–175

Barrett C, Cobb E, McNicol R, Lyon G (1997) A risk assessment study of plant genetic transformation using *Agrobacterium* and implications for analysis of transgenic plants. Plant Cell Tissue Organ Cult 47:135–144

Cooke DL, Waites WM, Leifert C (1992) Effect of *Agrobacterium tumefaciens*, *Erwinia caratovora*, *Pseudomonas syringae* and *Xanthomonas campestris* on plant tissue cultures of *Aster*, *Cheiranthus*, *Delphinium*, *Iris*, and *Rosa*; disease development in vivo as a result of latent infection in vitro. Plant Dis Prot 99:469–481

Craig WA, Ebert SC (1991) Killing and regrowth of bacteria in in vitro: a review. Pediatr Infect Dis J S74:63–70

Demain AL, Elander RP (1999) The β-lactam antibiotics: past, present, and future. Antonie Van Leeuwenhoek 75:5–19

Enomoto S, Itoh H, Ohshima M, Ohashi Y (1990) Induced expression of a chimeric gene construct in transgenic lettuce plants using tobacco pathogenesis-related protein gene promoter region. Plant Cell Rep 9:6–9

Falkiner FR (1988) Strategy of the selection of antibiotics for use against common bacterial pathogens and endophytes of plants. Acta Hortic 225:53–56

Falkiner FR (1990) The criteria for choosing an antibiotic for control of bacteria in plant tissue culture. Int Assoc Plant Tissue Cult Newsl 60:13–23

Frary A, Earle ED (1996) An examination of factors affecting the efficiency of *Agrobacterium*-mediated transformation of tomato. Plant Cell Rep 16:235–240

Fukasawa M, Sumita Y, Tada E, Okuda T (1992) In vitro antibacterial activity of meropenem. Chemotherapy 40(S-1):74–89

Ghuysen J-M (1997) Penicillin-binding proteins. Wall peptidoglycan assembly and resistance to penicillin: facts, doubts and hopes. Int J Antimicrob Agents 8:45–60

Hammerschlag FA, Zimmerman RH, Yadava UL, Hunsucker S, Gercheva P (1997) Effect of antibiotics and exposure to an acidified medium on the elimination of *Agrobacterium tumefaciens* from apple leaf explants and on shoot regeneration. J Am Soc Hortic Sci 122:758–763

Hayward AC (1974) Latent infections by bacteria. Annu Rev Phytopathol 12:87–97

Hoekema A, Hirsch PR, Hooykaas PJJ, Schilperoort RA (1983) A binary vector strategy based on separation of *vir*- and T-region of the *Agrobacterium tumefaciens* Ti-plasmid. Nature 303:179–180

Hood EE, Helmer GC, Fraley RT, Chilton M-D (1986) The hypervirulence of *Agrobacterium tumefaciens* A281 is encoded in a region of pTiBo542 outside the T-DNA. J Bacteriol 168:1291–1301

Matzk A, Mantell S, Schiemann J (1996) Localization of persisting agrobacteria in transgenic plants. Mol Plant Microbe Interact 9:373–381

Murashige T, Skoog F (1962) A revised medium for rapid growth and bioassays with tobacco tissue culture. Physiol Plant 15:473–497

Nauerby B, Billing K, Wyndaele R (1997) Influence of the antibiotic timentin on plant regeneration compared to carbenicillin and cefotaxime in concentrations suitable for elimination of *Agrobacterium tumefaciens*. Plant Sci 123:169–177

Ogawa Y, Mii M (2004) Screening for highly active β-lactam antibiotics against *Agrobacterium tumefaciens*. Arch Microbiol 181:331–336

Okkels FT, Pedersen MG (1988) The toxicity to plant tissue and to *Agrobacterium tumefaciens* of some antibiotics. Acta Hortic 225:199–207

Pollock K, Barfield DG, Shields R (1983) The toxicity of antibiotics to plant cell culture. Plant Cell Rep 2:36–39

Schroeder HE, Schotz AH, Wardley-Ricardson T, Spencer D, Higgins TJV (1993) Transformation and regeneration of pea (*Pisum sativum* L.). Plant Physiol 101:751–757

Shackelford NJ, Chlan CA (1996) Identification of antibiotics that are effective in eliminating *Agrobacterium tumefaciens*. Plant Mol Biol Rep 14:50–57

van der Hoeven C, Dietz A, Landsmann J (1991) Agrobacteria shown to reside in transgenic plants. Nachrichtenbl Dtsch Pflanzenschutzdienstes 43:249–251

Vergauwe A, Van Geldre E, Inzé D, Van Montagu M, Van den Eeckhout E (1996) The use of amoxicillin and ticarcillin in combination with a β-lactamase inhibitor as decontaminating agents in the *Agrobacterium tumefaciens*-mediated transformation of *Artemisia annua* L. J Biotechnol 52:89–95

Young PM, Hutchins AS, Canfield ML (1984) Use of antibiotics to control bacteria in shoot cultures of woody plants. Plant Sci Lett 34:203–209

Zhang F-L, Takahata Y, Watanabe M, Xu J-B (2000) *Agrobacterium*-mediated transformation of cotyledonary explants of Chinese cabbage (*Brassica campestris* L. ssp. *pekinensis*). Plant Cell Rep 19:569–575