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Effect of antibiotic ceftriaxone on elimination of ABI and GV3101 strains of *Agrobacterium tumefaciens*

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Aim. To find out, at which concentration the antibiotic ceftriaxone of β–lactam group causes the elimination of ABI and GV3101 strains of *Agrobacterium tumefaciens*. **Methods.** The disc diffusion method. **Results.** Antibiotic ceftriaxone was used for the cell elimination of the *Agrobacterium tumefaciens* ABI strain for the first time**.** The same zones of inhibition were observed when using the 400 mg/l ceftriaxone and 500 mg/l cefotaxime solutions for both *Agrobacterium* strains (ABI and GV3101) studied. **Conclusions.** Ceftriaxone inhibits the *Agrobacterium* growth more effectively than cefotaxime. The ceftriaxone concentration for elimination of ABI and GV3101 *Agrobacterium tumefaciens* strains is 400 mg/l**.**

K e y w o r d s: ceftriaxone, *Agrobacterium tumefaciens,* elimination

Introduction

Various *Agrobacterium tumefaciens* strains mediate the genetic transformation of plants. The antibiotics of β-lactam group are used for the bacterial cell elimination during *Agrobacterium*-mediated transformation [1, 2]. They kill bacteria by specific interfering with biosynthesis of the peptidoglycan component of the bacterial cell wall via binding to Penicillin-Binding Proteins (PBPs) whereas there is only little or no detrimental effect on the eukaryotic plant cells [3–5]. The antibiotics cefotaxime and carbenicillin are commonly used for the *Agrobacterium* cell elimination. Nowadays the usage of carbenicillin becomes undesirable because of its toxicity. Cefotaxime is mostly used in concentrations of 500 mg/l and higher [6]. However, the numerous studies prove the negative effect of its high concentrations on the organogenesis, embryogenesis and shoot regeneration of most plant species [4]. Now, timentin and ceftriaxone (β-lactam group) are frequently used instead of cefotaxime [7, 8]. These antibiotics eliminate *A. tumefaciens* efficiently during the genetic transformation *in vitro* and do not affect the regeneration frequency in most cases. Thus, ceftriaxone may be considered as an alternative to cefotaxime.

Ceftriaxone, a third-generation cephalosporin, characterized by a prolonged half-value period, has the broad-spectrum activity. The successful elimination of various *Agrobacterium tumefaciens* strains (EHA105, LBA4404, AGL_1) by means of ceftriaxone has been earlier reported [9–13]. However, it has not been yet used for the elimination of ABI *Agrobacterium* strain.

The aim of our study was to find out the concentration of ceftriaxone, the antibiotic of β–lactam group, causing the eliminating effect on the ABI and GV3101 strains of *Agrobacterium tumefaciens.*

Materials and Methods

The ceftriaxone effect on the ABI and GV3101 *Agrobacterium tumefaciens* strains was evaluated by

disk diffusion method [13]. Cefotaxime (500 mg/l) was used as a control. We also compared the eliminating capability of ceftriaxone and timentin. *Agrobacterium* night culture was sown on Himedia M001 (LB analog) agar medium [14]. The earlier sterilized paper disks (6 mm diameter) were moistened by antibiotic solutions (ceftriaxone: 300 mg/l, 350 mg/l, 400 mg/l, 450 mg/l, 500 mg/l; timentin: 100 mg/l, 150 mg/l, 200 mg/l, 250 mg/l, 300 mg/l, 350 mg/l, 400 mg/l) and put on the medium surface. Bacterial cultures were (OD_{600} = 0.8) cultivated in thermostat at 27 °C during 48 hours. Linear measurement of the inhibition zone diameter was carried out during 48-hour period. The experiment was repeated three times.

Results and Discussion

The inhibiting effects of ceftriaxone, cefotaxime and timentin on the cells of ABI and GV3101 *Agrobacterium tumefaciens* strains were compared in our study.

The ceftriaxone concentrations used by Chinese investigators for elimination of various *Agrobacterium* strains were as follows: 200 mg/l or 500 mg/l for LBA4404 [4]; 200 mg/l or 500 mg/l for EHA105 [12]; 300 mg/l for AGL_1 [11]; 300, 400 or 500 mg/l for GV3101 [9] in case of genetic transformation of various plant species. Ceftriaxone in all the studied concentrations was proved to eliminate the bacterium growth. Timentin was also used for bacteria elimination: 250 mg/l [13] or 400 mg/l [15] for EHA105 strain, 250 mg/l for C58 strain [7], 150 mg/l [16] or 400 mg/l [17] for LBA4404 strain, 50 mg/l for KYRT1strain [18]; 400 mg/l for GV3101 strain [12]. Cefotaxime (500 mg/l) is commonly used for elimination of most mentioned strains.

The suppression zone of 12.5 mm diameter was shown for ABI strain in case of using the solutions: 400 mg/l ceftriaxone, 500 mg/l cefotaxime and 350 mg/l timentin (Fig. 1). The inhibiting zone was 10.5 mm in case of using the mentioned concentrations of antibiotics for GV3101 strain. This way, ceftriaxone was proved to inhibit the *Agrobacterium* growth more effectively than cefotaxime and less effectively than timentin. We consider that ceftriaxone should be used in the concentration of 400 mg/l for elimination of the GV3101 *Agrobacterium* strain.

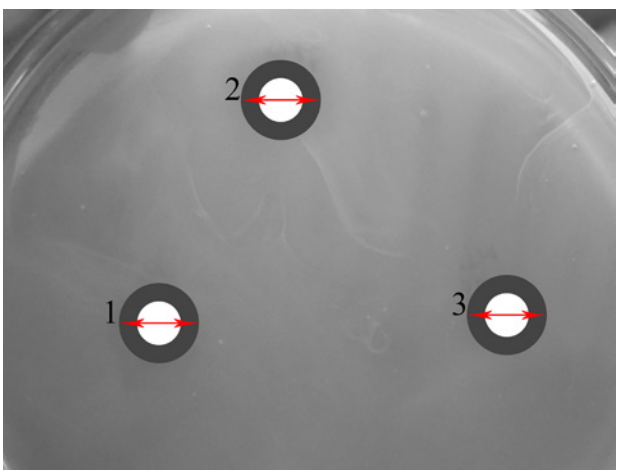

Fig. 1. Zones of ABI *Agrobacterium tumefaciens* strain inhibition by the studied antibiotics: 1. 400 mg/l ceftriaxone; 2. 500 mg/l cefotaxime; 3. 350 mg/l timentin.

Conclusions

Ceftriaxone has been shown to inhibit the *Agrobacterium* growth more effectively comparing to the cefotaxime effect: the zones of inhibition were proved to be of the same size in cases of using the 400 mg/l ceftriaxone and 500 mg/l cefotaxime solutions for both studied *Agrobacterium* strains (ABI and GV3101).

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Ефект антибіотика цефтриаксона на елімінацію *Agrobacterium tumefaciens* штаммів ABI та GV3101

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Мета. З'ясувати за якої концентрації антибіотик цефтриаксон з групи β-лактамів викликає елімінацію штамів ABI та GV3101 *Agrobacterium tumefaciens*. **Методи.** Метод дифузії з дисків. **Результати.** Для елімінації клітин *Agrobacterium tumefaciens* штаму ABI антибіотик цефтриаксон використано вперше. Для обох досліджуваних штамів агробактерії (ABI та GV3101) спостерігалися однакові зони ігібування за використання розчину 400 мг/л цефтриаксону та 500 мг/л цефотаксиму. **Висновки.** Цефтриаксон пригнічує ріст агробактерій більш ефективно, ніж цефотаксим. Концентрація цефтриаксону для еліміннації штамів ABI та GV3101 *Agrobacterium tumefaciens* – 400 мг/л.

Ключові слова: цефтриаксон, *Agrobacterium tumefaciens,* елімінація

Эффект антибиотика цефтриаксона на элиминацию *Agrobacterium tumefaciens* штаммов ABI и GV3101

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Цель. Выяснить при какой концентрации антибиотик цефтриаксон из группы β-лактамов вызывает элиминацию штаммов ABI и GV3101 *Agrobacterium tumefaciens*. **Методы.** Метод диффузии из дисков. **Результаты.** Для элиминации клеток *Agrobacterium tumefaciens* штамма ABI антибиотик цефтриаксон использован впервые. Для обоих изучаемых штаммов агробактерии (ABI и GV3101) наблюдались одинаковые зоны ингибирования при использовании раствора 400 мг/л цефтриаксона и 500 мг/л цефотаксима. **Выводы.** Цефтриаксон ингибирует рост агробактерий более эффективно, чем цефотаксим. Концентрация цефтриаксона для элиминации штамов ABI и GV3101 *Agrobacterium tumefaciens* – 400 мг/л.

Ключевые слова: цефтриаксон, *Agrobacterium tumefaciens,* элиминация

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