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Letter to the Editor

Cysteine induces resistance of lactobacilli to erythromycin and azithromycin

Sir,

The Laboratory of Microbial Genetics of the Federal University of Pernambuco (Recife, Brazil) is dedicated to the study of the biology of *Lactobacillus vini* strains isolated from ethanol fermentation industries and their tolerance to different forms of environmental stress. We have started the enterprise of producing recombinant strains for gene functional characterisation in the Laboratory of Lactic Acid Bacteria and Prebiotics of IATA-CSIC (Paterna, Spain). The amino acid cysteine was believed to be added to the medium to increase the aerobic growth rate and to increase the efficiency of transformation. Thus, MRS (De Man, Rogosa and Sharpe) agar medium containing 40 mM cysteine was sterilised by autoclavation, was cooled to 50 °C for antibiotic addition and was spread on sterile Petri dishes. In the course of these genetic modifications, an unexpected cell tolerance to 5 μg/mL erythromycin was observed when non-transformed cells were spread on MRS plates supplemented with cysteine (data not shown). The unexpected resistance led us to investigate the possible interference of this amino acid with the action of agents that inhibit protein synthesis, such as erythromycin. Minimum inhibitory concentrations (MICs) were determined by serial microdilution tests as recommended by the Clinical and Laboratory Standards Institute (CLSI) and previously used in our work with *L. vini* [1]. We observed that the response to the addition of cysteine regarding antibiotic resistance varied among *Lactobacillus* spp. and the type of antibiotics (Table 1). The MIC of erythromycin increased for all bacterial strains tested to different levels, including both strains of *L. vini*, and higher MICs were obtained for all strains when testing azithromycin. In addition, a similar effect of cysteine was observed for other protein synthesis-blocking agents (tetracycline and chloramphenicol), although not so expressive as observed for macrolides (Table 1). Afterwards, cells were pre-cultivated in MRS containing cysteine and were tested for induction of antibiotic resistance in MRS without cysteine. The results showed that only *Lactobacillus rhamnosus* CECT 278^T and *Lactobacillus acidophilus* CECT 903^T showed induced resistance to erythromycin (>10 μg/mL). There are some reports on the modulation of antibiotic effectiveness by *N*-acetylcysteine (NAC) and its precursor amino acid cysteine. NAC is produced from cysteine and is converted to glutathione, and the thiol group (–SH) of these compounds is effective in protecting cells against oxidative stress. In the present study, supplementation with 50 mM of reduced glutathione also increased the MIC of erythromycin, but not for all of the species for which resistance was induced by cysteine (data not shown). NAC was effective in reducing the antibacterial activity of antibiotics of different classes such as aminoglycosides, fluoroquinolones and also erythromycin [2]. On the other hand, it appears to potentiate the efficacy of β-lactams [2]. NAC at a concentration of 50 mM was also responsible for increasing the MICs of carbapenems and amikacin against 40 bacterial pathogens [3]. In addition, 10 mM NAC was enough to increase the MIC of erythromycin and ciprofloxacin against several bovine mastitis pathogens, although it decreased the MICs of penicillin and ampicillin against these bacteria [4]. The specific mechanisms of the modulation of antibiotic activity are still unclear, however

Table 1
Minimum inhibitory concentrations (MICs) of antibiotics in the absence or presence of cysteine (Cys) for different *Lactobacillus* spp. isolates determined by the serial microdilution method according to Clinical and Laboratory Standards Institute (CLSI) recommendations[a]

Lactobacillus sp.	MIC (μg/mL)							
	Erythromycin		Azithromycin		Tetracycline		Chloramphenicol	
	−Cys	+Cys	−Cys	+Cys	−Cys	+Cys	−Cys	+Cys
L. acidophilus CECT 903^T	≤1	>25	3	>10	3	>10	7.5	>10
L. acidophilus CECT 362	≤1	10	1	>10	7.5	>10	5	7.5
L. alimentarius CECT 570	≤1	3	2	>10	>10	>10	>10	7.5
L. brevis CECT 216	≤1	3	2	>10	>10	>10	7.5	7.5
L. casei BL23	≤1	20	4	>10	4	>10	7.5	>10
L. casei ATCC 334	≤1	15	3	>10	4	7.5	7.5	>10
L. fermentum CECT 4007^T	≤1	20	1	>10	>10	>10	7.5	>10
L. halotolerans CECT 573^T	2	>25	>10	>10	>10	>10	>10	>10
L. plantarum CECT 748^T	2	>25	>10	>10	>10	>10	>10	>10
L. rhamnosus CECT 278^T	≤1	10	5	>10	2	4	7.5	7.5
L. sakei CECT 906^T	≤1	3	5	>10	>10	>10	>10	>10
L. vini JP789	≤1	20	3	>10	4	10	3	7.5
L. vini MONT4	≤1	20	2	>10	2	>10	7.5	>10

[a] MICs are the result of triplicate tests of two biological replicates. Numbers represent the means of four replicates.

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there are indications that it works through the thiol group [2]. This is indicated by the early report of cysteine-induced bacterial resistance of *Clostridium difficile* [5]. To test this hypothesis, we replaced cysteine (cys-SH) by its oxidised form cystine (cys-S-S-cys). The results showed that the resistance profile was similar to the condition without cysteine and supported the hypothesis of the protective action of the –SH group. Given the fact that cysteine is a common component for several cultivation media for bacterial growth and is commonly used as a food supplement for humans and animals, it is paramount to further evaluate how it could affect antibiotic therapy against pathogenic bacteria.

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Competing interests

None declared.

Ethical approval

Not required.

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