RESEARCH LETTER

Tyrosine 9 is the key amino acid in microcin J25 superoxide overproduction

Miriam C. Chalon, Augusto Bellomio, José O. Solbiati, Roberto D. Morero, Ricardo N. Farias & Paula A. Vincent

Departamento de Bioquímica de la Nutrición, Instituto Superior de Investigaciones Biológicas (Consejo Nacional de Investigaciones Científicas y Técnicas-Universidad Nacional de Tucumán) and Instituto de Química Biológica 'Dr. Bernabé Bloj,' San Miguel de Tucumán, Tucumán, Argentina

Correspondence: Paula A. Vincent, Departamento de Bioquímica de la Nutrición, Instituto Superior de Investigaciones Biológicas (Consejo Nacional de Investigaciones Científicas y Técnicas-Universidad Nacional de Tucumán) and Instituto de Química Biológica 'Dr. Bernabé Bloj,' Chacabuco 461, San Miguel de Tucumán 4000, Tucumán, Argentina. Tel./fax: +54 381 424 8921; e-mail: pvincent@fbqf.unt.edu.ar

Present address: José O. Solbiati, Department of Microbiology, University of Illinois at Urbana-Champaign, Urbana, IL 61801, USA.

Received 28 April 2009; accepted 19 August 2009.
Final version published online 15 September 2009.

DOI:10.1111/j.1574-6968.2009.01770.x

Editor: Robert Gunsalus

Keywords
microcin; oxygen consumption; antibiotic; antimicrobial peptide; respiratory chain; superoxide.

Abstract

Escherichia coli microcin J25 (MccJ25) is a lasso-peptide antibiotic comprising 21 L-amino acid residues (G^1-G-A-G-H^5-V-P-E-Y-F^{10}-V-G-I-G-T^{15}-P-I-S-F-Y^{20}-G). MccJ25 has two independent substrates: RNA-polymerase (RNAP) and the membrane respiratory chain. The latter is mediated by oxygen consumption inhibition together with an increase of superoxide production. In the present paper, the antibiotic MccJ25 was engineered by substituting Tyr^9 or Tyr^{20} with phenylalanine. Both mutants were well transported into the cells and remained active on RNAP. Only the Y9F mutant lost the ability to overproduce superoxide and inhibit oxygen consumption. The last results confirm that the Tyr^9, and not Tyr^{20}, is involved in the MccJ25 action on the respiratory chain target.

Introduction

Escherichia coli microcin J25 (MccJ25) is a plasmid-encoded antibiotic peptide comprising 21 amino acid residues (G^1-G-A-G-H^5-V-P-E-Y-F^{10}-V-G-I-G-T^{15}-P-I-S-F-Y^{20}-G) (Salomon & Farias, 1992; Blond *et al*., 1999). MccJ25 is a lasso-peptide (Bayro *et al*., 2003; Rosengren *et al*., 2003; Wilson *et al*., 2003) that contains a lactam linkage between the α-amino group of Gly^1 and the γ-carboxyl of Glu^8, forming an eight-residue ring (Gly^1–Glu^8), which is termed a lariat ring. The 'tail' (Tyr^9-Gly^{21}) passes through the ring, with Phe^{19} and Tyr^{20} straddling each side of the tail, sterically trapping the tail within the ring. MccJ25 amino acids F^{10}-P^{16} form a β-hairpin structure comprising two β-strands (F^{10}-V^{11} and T^{15}-P^{16}) and a β-turn (V^{11}-G^{14}).

Four genes (*mcjA*, *mcjB*, *mcjC*, and *mcjD*) are required for MccJ25 synthesis, export, and immunity (Solbiati *et al*., 1996, 1999). The *mcjA* gene encodes the primary structure of MccJ25 as a 58-amino acid precursor, from which a 37-amino acid N-terminal leader is removed. The 21-residue mature peptide has a compact, extraordinary structure described above. The *mcjB* and *mcjC* gene products are involved in this assembly process (Duquesne *et al*., 2007). The *mcjD* product has a dual role. It works as a dedicated

© 2009 Federation of European Microbiological Societies
Published by Blackwell Publishing Ltd. All rights reserved

Table 1. Strains and plasmids

	Description	References
Strain		
AB1133	F⁻ *thr-1ara-14 leuB6 lacY1* Δ*(gtp-proA)62 supE44 galK2*λ*- rac his G4 rfbD1 rpsL31* (Sm^r) *kggK51 xyl-5 mtl-1 argE3 thi-1*	CGSC
PA232	AB1133 *rpoC* T931I	Bellomio *et al*. (2007)
Plasmid		
pGC01	pBR322 *fhu*A$^+$, Ap^r	Coulton *et al*. (1983)
pTUC203-6	pACYC184 with 5.2-kb HindIII–SalI fragment encompassing *mcjABCD* genes with a Tn*5* insertion in *mcjA*, Cm^r Km^r	Solbiati *et al*. (1996)
pTUC202	pACYC184 with 6-kb BamHI–SalI fragment encompassing *mcjABCD* genes, Ap^r	Solbiati *et al*. (1996)
pCR 2.1-Topo	Cloning vector for PCR products, Km^r Ap^r	Invitrogen
pTopoY9F	pCR 2.1-Topo *mcjA* (Tyr^{9}xPhe), Ap^r	This work
pTopoY20F	pCR 2.1-Topo *mcjA* (Tyr^{20}xPhe), Ap^r	This work

Cm^r, chloramphenicol resistant; Ap^r, ampicillin resistant, Km^r, kanamycin resistant; CGSC, *Escherichia coli* Genetic Stock Center.

ATP-binding cassette exporter of MccJ25 and, at the same time, provides immunity against both endogenous MccJ25 synthesized in producer cells and exogenous microcin that gains entry (Delgado *et al*., 1999; Solbiati *et al*., 1999).

Microcin J25 is active against Gram-negative bacteria related to the producer strain, many of which are human pathogens (Salomon & Farias, 1992; Sable *et al*., 2000). MccJ25 intake is mediated by the outer membrane receptor FhuA and the inner membrane proteins TonB, ExbB, ExbD, and SbmA (Salomon & Farias, 1993, 1995).We reported that RNA-polymerase (RNAP) is the target of MccJ25 activity in *E. coli* (Delgado *et al*., 2001). MccJ25 inhibits RNAP activity by obstructing the secondary channel, thus preventing access of the nucleotide substrates to RNAP's active site (Delgado *et al*., 2001; Yuzenkova *et al*., 2002; Adelman *et al*., 2004; Mukhopadhyay *et al*., 2004). Recently, we showed that MccJ25 also targets the bacterial membrane respiratory enzyme chain, consequently inhibiting cell oxygen consumption (Rintoul *et al*., 2001; Vincent *et al*., 2004). This effect is mediated by an increase in superoxide production by the membrane respiratory processes (Bellomio *et al*., 2007). Therefore, one or more of the MccJ25 amino acids may participate in the superoxide-production redox reaction.

We observed that MccJ25 antibiotic activity was significantly reduced when its tyrosine residues were chemically modified with 4-fluoro-7-nitrobenz-2-oxa-1,3-diazole (unpublished data). The MccJ25 has two tyrosines (Tyr^9 and Tyr^{20}). The peptide structure implies that Tyr^9 and Tyr^{20} have different solvent accessibility: whereas Tyr^{20} forms hydrophobic patches with the Val^6 side chain and the methylene groups of the Glu^8 side chain (Rosengren *et al*., 2003), Tyr^9 is sufficiently exposed to the solvent to allow it to participate in any chemical reaction. Based on these functional and structural considerations, Tyr^9 and Tyr^{20} were mutated to examine the activity of this amino acid in superoxide generation. We reasoned that at least one of these amino acids could be essential during antibiotic action on the membrane.

The results of the present study showing evidence regarding the nature and position of the amino acid responsible for the respiratory chain target action contribute to a better understanding of the structure–function relationship of MccJ25.

Materials and methods

Bacterial culture media and growth conditions

The bacteria and plasmids used are listed in Table 1. Luria–Bertani (LB) broth and M9 minimal salts were purchased from Sigma Chemical Co. (St. Louis, MO). Minimal medium was supplemented with 0.2% glucose, 1 mM $MgSO_4$, and 1 μg mL^{-1} vitamin B_1. Solid media were prepared by adding agar to a final concentration of 1.5%. When required, 50 μg mL^{-1} ampicillin, 30 μg mL^{-1} kanamycin, or 30 μg mL^{-1} chloramphenicol was added. Liquid cultures were grown under agitation in 100-mL flasks. For anaerobic growth, liquid cultures were grown without agitation in filled tubes with the medium overlaid with mineral oil. Growth was monitored by measuring the $OD_{600\,nm}$ for 24 h. Thereafter, the culture was followed under aerobic conditions in 100-mL flasks with vigorous shaking. All cultures were incubated at 37 °C. Aliquots were obtained to determine CFU.

Mutagenesis, isolation, and MS of MccJ25 analogues

Plasmid pTUC202, carrying the *mcjABCD* genes under their own promoters, was used to produce the MccJ25 mutants. Plasmids were constructed by PCR with the appropriate primers to obtain the substitutions (Chalon *et al*., 2007). After amplification, PCR fragments were linked to pCR

© 2009 Federation of European Microbiological Societies
Published by Blackwell Publishing Ltd. All rights reserved

2.1-Topo, yielding pTopoY9F and pTopoY20F plasmids to produce the Y9F and Y20F substitutions, respectively. Mutations were confirmed by DNA sequencing of the plasmids. To produce and purify the microcin variants, the pTopoY9F or pTopoY20F plasmid was transformed into a strain carrying the compatible plasmid pTUC203-6, containing the *mcjABCD* genes with a Tn*5* insertion in *mcjA* (Solbiati *et al.*, 1996). Native MccJ25 and MccJ25 analogues were purified by HPLC, as described previously (Bellomio *et al.*, 2003).

Molecular mass determinations (MS and MS–MS) were carried out by electrospray ionization and matrix-assisted laser-desorption time-of-flight using VG Quattro and VG TofSpec mass spectrometers.

Microcin activity sensitivity test

Sensitivity to microcin was tested by a spot-on-lawn assay as follows: to avoid aggregation (Blond *et al.*, 1999), concentrated methanolic solutions of the peptides (2 mM) were prepared for the antibiotic activity assay, and serial doubling dilutions were performed in double-distilled water containing 0.1% Tween 80 in Eppendorf tubes. Samples (10 μL) of each dilution were spotted onto M9 medium plates supplemented with 0.2% tryptone. After the drops had dried, the plates were overlaid with 4 mL soft agar (0.6%) containing 30 μL of an overnight LB culture of the strain to be assayed. The plates were incubated for 12 h at 37 °C and examined for different degrees of inhibition. The concentration (μM) of the last dilution yielding a clear or turbid spot was defined as the minimal inhibitory concentration of MccJ25 or its analogues.

Oxygen consumption

The strain PA232 (pGC01) was grown to the exponential phase ($OD_{600\,nm}$ between 0.4 and 0.5) in LB. Samples were diluted in M9, supplemented with 10 mM glucose to an $OD_{600\,nm}$ of 0.15, and incubated at 37 °C for 30 min without MccJ25 (control) and with 20 μM of the different peptides. The average cell respiration rate over the subsequent 5 min was polarographically measured using a Clark-type electrode oxygraph and normalized to the $OD_{600\,nm}$.

In vivo and *in vitro* transcription assay

In vitro RNAP activity was determined as described by Gross *et al.* (1976) with a few modifications. The assay mixtures (100 μL) contained the NTP [^{3}H]UTP instead of [^{33}P]UTP, purified plasmid pBR322 (2 μg) as a template, and 4 μM of wild-type or mutant microcins. The reaction was started by adding 1.5 U RNAP holoenzyme purified from *E. coli* (Pharmacia, Piscataway, NJ) and was allowed to proceed at 37 °C for 15 min before precipitation with 1 mL of cold 10% trichloroacetic acid (TCA). After 1 h of incubation on ice, the precipitates were collected on glass fiber filters (Millipore type APFC), washed with 10 mL cold 10% TCA, dried, and the radioactivity retained on the filters was estimated in a Beckman LS-1801 liquid scintillation counter (Beckman-Coulter, Pasadena, CA).

The *in vivo* transcription assay was performed as described by Delgado *et al.* (2001). Strain AB1133 was grown in M9 medium to the early exponential phase. The culture was divided into four parts. The first three received one of the antibiotic peptides, native MccJ25, MccJ25(Y9F), and MccJ25(Y20F), at a final concentration of 4 μM, and the last (without peptide) served as a control. After that, [^{3}H]uridine (0.5 $\mu Ci\,mL^{-1}$) was added to the cell suspension (10 mL) and then 0.5-mL aliquots were extracted at 0, 15, and 30 min. The extracted samples were mixed with 1.5 mL cold 10% TCA, incubated on ice, filtered through Millipore HAWP02500 filters (Millipore, Bedford, MA), and washed with cold 10% TCA. The retained radioactivity was estimated as described before. RNAP activity was defined as the difference between the radioactivity level at 15 and 30 min.

Superoxide production rate

Superoxide formation was measured as superoxide dismutase-sensitive cytochrome *c* reduction (Imlay & Fridovich, 1991). Bacterial membrane from *E. coli* strain PA232 (final concentration, 1 mg protein mL^{-1}) was assayed in 0.6-mL reaction volumes of 50 mM phosphate buffer (pH 7.8) containing 40 μM cytochrome *c* in the absence or presence of 40 μM peptides. Reduction of cytochrome *c* was initiated by the addition of 10 mM succinate (final concentration) and monitored in a Beckman DU 7500 spectrophotometer at 550 nm. Duplicate reactions were performed by adding 30 U *E. coli* Mn-superoxide dismutase (Sigma Chemical Co.). The extent of reduced cytochrome *c* was calculated using an absorption coefficient (ε) of 0.21 $mM^{-1}\,cm^{-1}$. Membranes from the PA232 strain were obtained as described previously by Evans (1969) and stored frozen in 20 mM Tris/HCl (pH 7.4) containing 1 mM $MgCl_2$.

Results

Generation of MccJ25 Tyr mutants and their antibiotic activity

Phenylalanine substitutions of Tyr^{9} or Tyr^{20} were generated following the protocol described in Materials and methods and they were confirmed by sequencing the recombinant plasmids (data not shown). Cells harboring plasmids with mutant *mcjA* and wild-type *mcjBCD* produced mature processed MccJ25 mutants, based on the appearance of characteristic peaks during reverse-phase HPLC (data not shown) and MS. No contamination with native MccJ25 was

© 2009 Federation of European Microbiological Societies
Published by Blackwell Publishing Ltd. All rights reserved

Table 2. Comparison of the sensitivities of several *Escherichia coli* strains to MccJ25 and MccJ25 Tyr mutants

	Sensitivity by spot-on-lawn test [microcin MIC (μM)]		
Strain	MccJ25	MccJ25(Y20F)	MccJ25(Y9F)
PA232	2.0	7.8	> 500
PA232 (pGC01)	0.5	0.5	> 500
AB1133	0.25	0.25	7.8

MIC, minimal inhibitory concentration.

detected. The MS^2 spectra showed ions with an *m*/*z* of 687 corresponding to the eight-residue ring (Gly^1–Glu^8) for both mutants, confirming the integrity of the lariat ring (Wilson *et al.*, 2003).

The sensitivity of the *E. coli* strain AB1133 containing wild-type RNAP and strain PA232, harboring a mutated-MccJ25-resistant RNAP (*rpoC* T931I), to the native MccJ25 and Tyr variants was compared and is reported in Table 2. It was previously found that the respiratory chain target is also present in laboratory *E. coli* strains. The effect on the oxygen consumption inhibition becomes noticeable when the bacteria overexpress the external membrane transport protein of MccJ25 (FhuA) (Bellomio *et al.*, 2007). For this reason, the sensitivity of the strain PA232 transformed with pGC01 (carrying the *fhuA* gene) was also tested (Table 2). The mutant MccJ25(Y20F) showed antibiotic activity similar to that of the wild-type MccJ25 on the three strains, whereas the antibiotic activity of MccJ25(Y9F) was significantly lower. The two-dilution difference between MccJ25(Y20F) and the wild-type peptide in the PA232 strain was not considered significant taking into account the considerable difference observed between the Y9F mutant and the native

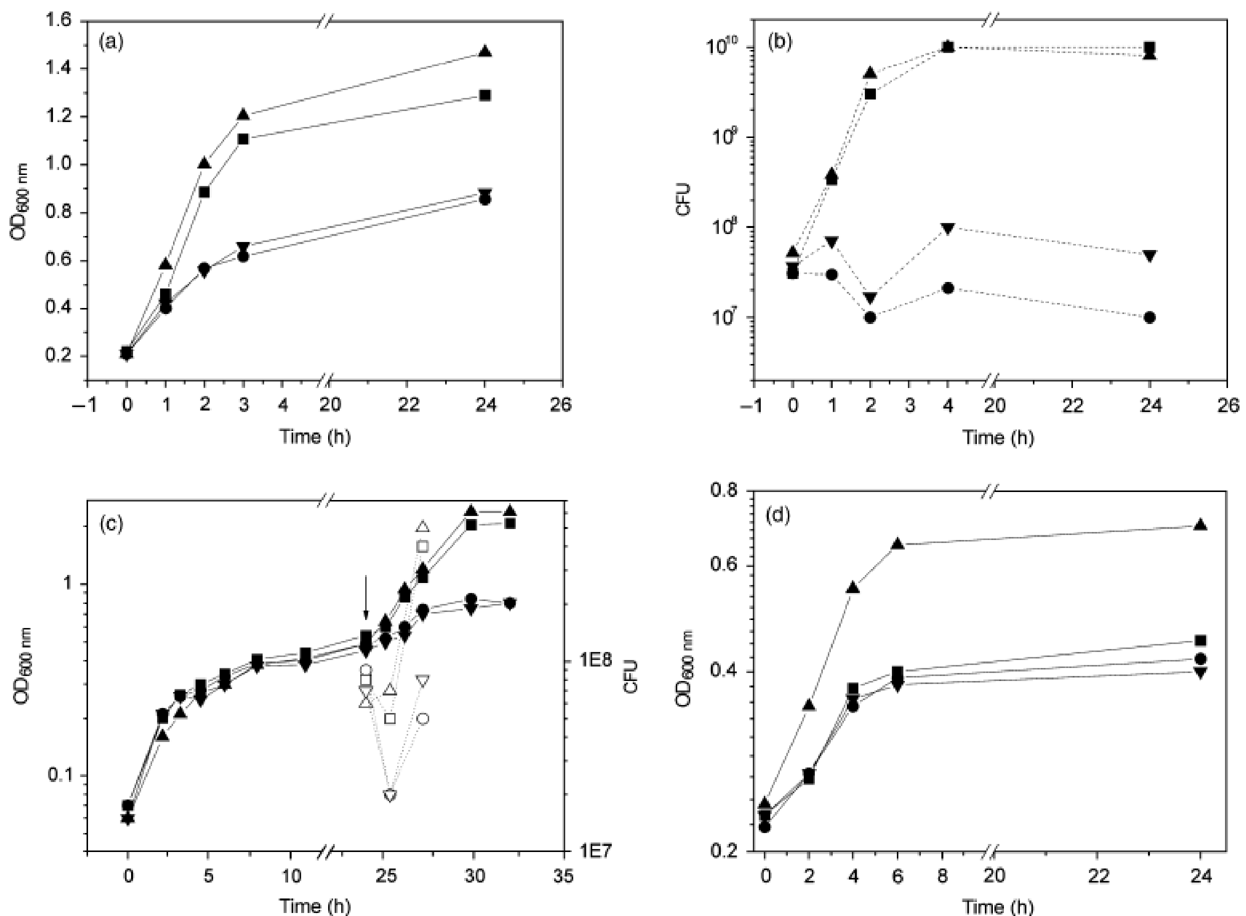

Fig. 1. Peptide effect on the growth of several *Escherichia coli* strains under aerobic and anaerobic conditions. Culture growth and cell viability of PA232 (pGC01) and AB1133 strains in the absence (triangle) or presence of 20 μM MccJ25 (circles), MccJ25(Y9F) (squares), and MccJ25(Y20F) (inverted triangles). (a) Culture growth of PA232 (pGC01) strain under aerobic conditions. (b) Cell viability of PA232 (pGC01) strain under aerobic conditions. (c) PA232 (pGC01), the strain was grown without agitation in filled tubes with liquid cultures lacking oxygen and the medium was overlaid with mineral oil. The culture was then changed to aerobic conditions (see the arrow) in 100-mL flasks with vigorous shaking. (d) Anaerobic culture growth of strain AB1133. All cultures were incubated at 37 °C. The experiments were performed three times, with similar results. The values shown are from one representative experiment.

© 2009 Federation of European Microbiological Societies
Published by Blackwell Publishing Ltd. All rights reserved

peptide; in fact, the strains PA232 and PA232 (pGC01) were totally resistant to MccJ25(Y9F). Note that the susceptibility conferred by PA232 and PA232 (pGC01) strains to MccJ25 and MccJ25(Y20F) could only be attributed to the antibiotic action on the respiratory chain target, while in the AB1133 strain the two targets are present.

Effect of Tyr mutants on aerobic and anaerobic cell growth

Previously, we demonstrated that growth inhibition of the PA232 (pGC01) strain by MccJ25 occurred only under aerobic conditions or when the anaerobic culture was changed to an oxygen atmosphere (Bellomio *et al.*, 2007). Figure 1a and b show that the variant MccJ25(Y20F) displayed antibiotic activity similar to that of the native peptide. In contrast, MccJ25(Y9F) did not inhibit the PA232 (pGC01) strain in either the aerobic liquid culture (Fig. 1a and b) or the anaerobic culture when changed to aerobic conditions (Fig. 1c). To reach the cell cytoplasm, MccJ25 must be transported through the outer and inner membrane by the FhuA and SbmA proteins, respectively (Salomon & Farias, 1993, 1995). The fact that both mutant peptides had *in vivo* inhibitory capacity similar to that of wild-type microcin on the AB1133 strain growing under anaerobic conditions (Fig. 1d) excludes the possibility that the mutants could not enter the cell.

Effect of the MccJ25 Tyr mutants on transcription

To elucidate the influence of the MccJ25 Tyr^9 and Tyr^{20} residues on transcription, the effect of MccJ25 Tyr mutants on RNA synthesis *in vivo* and *in vitro* was studied using AB1133 cells and purified RNAP, respectively. The two variants inhibited both *in vivo* and *in vitro* RNA synthesis to the same extent as the wild-type MccJ25 (Fig. 2). Further, both purified Tyr mutants similarly inhibited anaerobic growth of the AB1133 strain, as noted above. This result, in addition to *in vivo* transcription inhibition observed by Tyr mutants, allowed us to conclude that both peptides can enter the cell.

Effect of MccJ25(Y9F) mutants on the respiratory chain target

The above findings suggest that MccJ25(Y9F) does not exhibit the alternative mechanism of action on the respiratory chain target that was previously described for the PA232 (pGC01) strain (Bellomio *et al.*, 2007). Consistent with this finding, MccJ25(Y9F) did not inhibit cell respiration in the PA232 (pGC01) strain (Fig. 3) and did not increase membrane superoxide generation *in vitro* (Fig. 4), whereas the MccJ25(Y20F) variant behaved the same as the native peptide (Figs 3 and 4).

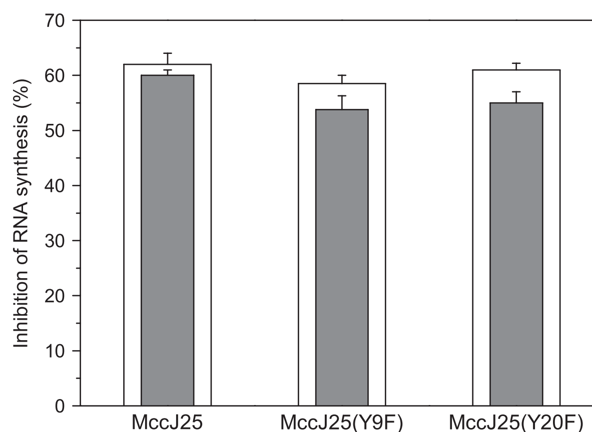

Fig. 2. Effect of MccJ25 Tyr mutants on RNA synthesis. Effect of 4 μM of MccJ25, MccJ25(Y9F), or MccJ25(Y20F) on *in vivo* RNAP activity (white bars) and *in vitro* RNAP activity (gray bars). The transcription activity was plotted as a percent of activity observed in the absence of MccJ25. Error bars represent SDs from five experiments.

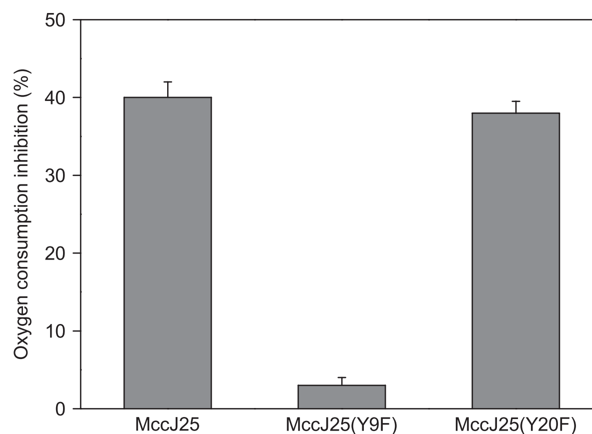

Fig. 3. Effect of MccJ25 Tyr mutants on oxygen consumption. Respiratory rate was determined in the presence of 20 μM of MccJ25, MccJ25(Y9F), or MccJ25(Y20F) as described in Materials and methods, and the results are expressed as percent respiratory inhibition relative to the controls in the absence of MccJ25. Error bars represent SDs from five experiments.

Discussion

The findings of the present report indicate that a single Y9F mutation in the tail region of the MccJ25 molecule abolishes the antibiotic action on superoxide overproduction. Any possible role of this amino acid in peptide cell uptake and its action on RNAP (intracellular target) has been ruled out. Together, these findings strongly suggest that Tyr^9 is involved in the microcin J25 action on the respiratory chain of

© 2009 Federation of European Microbiological Societies
Published by Blackwell Publishing Ltd. All rights reserved

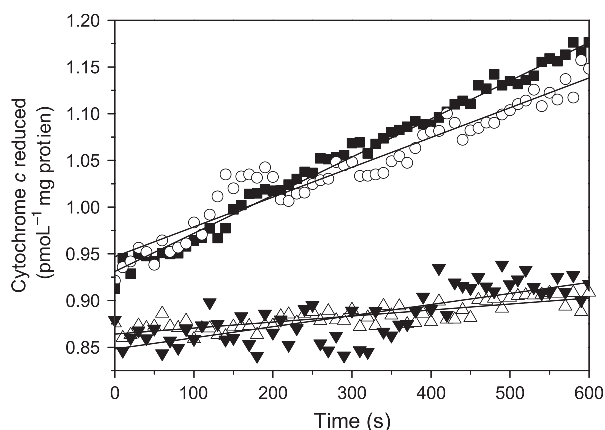

Fig. 4. MccJ25 Tyr mutant effects on the time course of superoxide production by *Escherichia coli* membrane. A solution containing membranes (1 mg protein mL^{-1}) and 40 μM cytochrome *c* in 50 mM phosphate buffer, pH 7.8 (final volume, 0.6 mL), was incubated at 37 °C in the absence (open triangle) or presence of 40 μM of MccJ25 (filled square), MccJ25(Y9F) (filled inverted triangle), or MccJ25(Y20F) (open circle). The time course reaction was initiated with 10 mM succinate (final concentration). The cytochrome *c* reduction was followed spectrophotometrically at 550 nm, as described in Materials and methods. The experiment was performed three times, with similar results. The values shown are from one representative experiment.

E. coli. In a recent paper, Pavlova *et al.* (2008) asserted that the residue immediately following the MccJ25 cycle (Tyr9) is the sole residue for which no non-wild-type side chain is tolerated in the inhibition of RNAP. This result, which seems to contradict our findings, was obtained by studying RNAP inhibition with crude supernatants containing MccJ25 derivatives. Inhibition was not observed, however, in our *in vivo* or *in vitro* assays of RNAP transcription induced by the culture supernatant of strains producing MccJ25 or MccJ25(Y9F). We are currently studying the possible reasons for this discrepancy.

The antibiotic may be capable of producing radicals directly or indirectly by an intermediary in the respiratory chain that is able to generate superoxide radicals. The exact mechanism of superoxide generation by MccJ25 is actually under investigation by our group. The present results define the nature and position (Tyr^9) of the amino acid in MccJ25 necessary for its action on the respiratory chain target. This observation, together with findings from our previous studies, leads us to propose four important conclusions about the regions of the MccJ25 molecule involved in the physiologic actions of the molecule (see Supporting Information, Fig. S1): (1) Tyr^9 is the determinant for respiration inhibition by superoxide overproduction; (2) the β-hairpin region is important for MccJ25 uptake through FhuA transport (Bellomio *et al.*, 2004; de Cristobal *et al.*, 2006); (3) His^5 and the lariat ring are involved in SbmA transport (de Cristobal *et al.*, 2006); and (4) the C-terminal region is an important determinant for RNAP inhibition (Bellomio *et al.*, 2003; Vincent *et al.*, 2005).

These findings provide a starting point for the development of more potent inhibitors based on the respiratory chain target by strategic replacement of amino acids in the MccJ25 molecule or the development of an MccJ25 mutant with selective RNAP inhibition, free of the respiratory chain target action. The last possibility is important for possible clinical antibiotic applications because the pathogenic actions of MccJ25 on mammalian mitochondria under aerobic conditions would not be eliminated (Niklison Chirou *et al.*, 2004, 2008).

Acknowledgements

This work was funded by Grants PICT 2107, PAE 22642, and PICTO 843 from the Agencia Nacional de Promoción Científica y Tecnológica (ANPCyT). M.C.C. was the recipient of a fellowship from CONICET. R.D.M, R.N.F., A.B., and P.A.V. are Career Investigators from CONICET. A preliminary communication of this work was presented at the 43rd Annual Meeting of the Sociedad Argentina de Investigación Bioquímica y Biología Molecular (Chalon *et al.*, 2007).

References

Adelman K, Yuzenkova J, La Porta A *et al.* (2004) Molecular mechanism of transcription inhibition by peptide antibiotic microcin J25. *Mol Cell* **14**: 753–762.

Bayro MJ, Mukhopadhyay J, Swapna GV *et al.* (2003) Structure of antibacterial peptide microcin J25: a 21-residue lariat protoknot. *J Am Chem Soc* **125**: 12382–12383.

Bellomio A, Rintoul MR & Morero RD (2003) Chemical modification of microcin J25 with diethylpyrocarbonate and carbodiimide: evidence for essential histidyl and carboxyl residues. *Biochem Bioph Res Co* **303**: 458–462.

Bellomio A, Vincent PA, de Arcuri BF, Salomon RA, Morero RD & Farias RN (2004) The microcin J25 beta-hairpin region is important for antibiotic uptake but not for RNA polymerase and respiration inhibition. *Biochem Bioph Res Co* **325**: 1454–1458.

Bellomio A, Vincent PA, de Arcuri BF, Farias RN & Morero RD (2007) Microcin J25 has dual and independent mechanisms of action in *Escherichia coli*: RNA polymerase inhibition and increased superoxide production. *J Bacteriol* **189**: 4180–4186.

Blond A, Peduzzi J, Goulard C *et al.* (1999) The cyclic structure of microcin J25, a 21-residue peptide antibiotic from *Escherichia coli*. *Eur J Biochem* **259**: 747–755.

Chalon MC, Bellomio A, Morero RD, Farias RN & Vincent PA (2007) Tyr9 of the microcin J25 molecule is involved in the overproduction of superoxide. *Biocell* **31** (suppl): 67.

Coulton JW, Mason P & DuBow MS (1983) Molecular cloning of the ferrichrome-iron receptor of *Escherichia coli* K-12. *J Bacteriol* **156**: 1315–1321.

© 2009 Federation of European Microbiological Societies
Published by Blackwell Publishing Ltd. All rights reserved

de Cristobal RE, Solbiati JO, Zenoff AM *et al.* (2006) Microcin J25 uptake: His5 of the MccJ25 lariat ring is involved in interaction with the inner membrane MccJ25 transporter protein SbmA. *J Bacteriol* **188**: 3324–3328.

Delgado MA, Solbiati JO, Chiuchiolo MJ, Farias RN & Salomon RA (1999) *Escherichia coli* outer membrane protein TolC is involved in production of the peptide antibiotic microcin J25. *J Bacteriol* **181**: 1968–1970.

Delgado MA, Rintoul MR, Farias RN & Salomon RA (2001) *Escherichia coli* RNA polymerase is the target of the cyclopeptide antibiotic microcin J25. *J Bacteriol* **183**: 4543–4550.

Duquesne S, Destoumieux-Garzon D, Zirah S, Goulard C, Peduzzi J & Rebuffat S (2007) Two enzymes catalyze the maturation of a lasso peptide in *Escherichia coli*. *Chem Biol* **14**: 793–803.

Evans DJ Jr (1969) Membrane adenosine triphosphatase of *Escherichia coli*: activation by calcium ion and inhibition by monovalent cations. *J Bacteriol* **100**: 914–922.

Gross C, Engbaek F, Flammang T & Burgess R (1976) Rapid micromethod for the purification of *Escherichia coli* ribonucleic acid polymerase and the preparation of bacterial extracts active in ribonucleic acid synthesis. *J Bacteriol* **128**: 382–389.

Imlay JA & Fridovich I (1991) Assay of metabolic superoxide production in *Escherichia coli*. *J Biol Chem* **266**: 6957–6965.

Mukhopadhyay J, Sineva E, Knight J, Levy RM & Ebright RH (2004) Antibacterial peptide microcin J25 inhibits transcription by binding within and obstructing the RNA polymerase secondary channel. *Mol Cell* **14**: 739–751.

Niklison Chirou M, Bellomio A, Dupuy F, Arcuri B, Minahk C & Morero R (2008) Microcin J25 induces the opening of the mitochondrial transition pore and cytochrome *c* release through superoxide generation. *FEBS J* **275**: 4088–4096.

Niklison Chirou MV, Minahk CJ & Morero RD (2004) Antimitochondrial activity displayed by the antimicrobial peptide microcin J25. *Biochem Bioph Res Co* **317**: 882–886.

Pavlova O, Mukhopadhyay J, Sineva E, Ebright RH & Severinov K (2008) Systematic structure–activity analysis of microcin J25. *J Biol Chem* **283**: 25589–25595.

Rintoul MR, de Arcuri BF, Salomon RA, Farias RN & Morero RD (2001) The antibacterial action of microcin J25: evidence for disruption of cytoplasmic membrane energization in *Salmonella newport*. *FEMS Microbiol Lett* **204**: 265–270.

Rosengren KJ, Clark RJ, Daly NL, Goransson U, Jones A & Craik DJ (2003) Microcin J25 has a threaded sidechain-to-backbone ring structure and not a head-to-tail cyclized backbone. *J Am Chem Soc* **125**: 12464–12474.

Sable S, Pons AM, Gendron-Gaillard S & Cottenceau G (2000) Antibacterial activity evaluation of microcin J25 against diarrheagenic *Escherichia coli*. *Appl Environ Microb* **66**: 4595–4597.

Salomon RA & Farias RN (1992) Microcin 25, a novel antimicrobial peptide produced by *Escherichia coli*. *J Bacteriol* **174**: 7428–7435.

Salomon RA & Farias RN (1993) The FhuA protein is involved in microcin 25 uptake. *J Bacteriol* **175**: 7741–7742.

Salomon RA & Farias RN (1995) The peptide antibiotic microcin 25 is imported through the TonB pathway and the SbmA protein. *J Bacteriol* **177**: 3323–3325.

Solbiati JO, Ciaccio M, Farias RN & Salomon RA (1996) Genetic analysis of plasmid determinants for microcin J25 production and immunity. *J Bacteriol* **178**: 3661–3663.

Solbiati JO, Ciaccio M, Farias RN, Gonzalez-Pastor JE, Moreno F & Salomon RA (1999) Sequence analysis of the four plasmid genes required to produce the circular peptide antibiotic microcin J25. *J Bacteriol* **181**: 2659–2662.

Vincent PA, Delgado MA, Farias RN & Salomon RA (2004) Inhibition of *Salmonella enterica* serovars by microcin J25. *FEMS Microbiol Lett* **236**: 103–107.

Vincent PA, Bellomio A, de Arcuri BF, Farias RN & Morero RD (2005) MccJ25 C-terminal is involved in RNA-polymerase inhibition but not in respiration inhibition. *Biochem Bioph Res Co* **331**: 549–551.

Wilson KA, Kalkum M, Ottesen J *et al.* (2003) Structure of microcin J25, a peptide inhibitor of bacterial RNA polymerase, is a lassoed tail. *J Am Chem Soc* **125**: 12475–12483.

Yuzenkova J, Delgado M, Nechaev S *et al.* (2002) Mutations of bacterial RNA polymerase leading to resistance to microcin J25. *J Biol Chem* **277**: 50867–50875.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Fig. S1. Schematic representation of different regions of the MccJ25 molecule involved in its physiologic actions and the mechanism of action in *Escherichia coli*.

Please note: Wiley-Blackwell is not responsible for the content or functionality of any supporting materials supplied by the authors. Any queries (other than missing material) should be directed to the corresponding author for the article.

© 2009 Federation of European Microbiological Societies
Published by Blackwell Publishing Ltd. All rights reserved