The Mechanism of Microcin C Resistance Provided by the MccF Peptidase*

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The heptapeptide-nucleotide microcin (McC) is a potent inhibitor of enteric bacteria growth. Inside a sensitive cell, McC is processed by aminopeptidases, which release a nonhydrolyzable aspartyl-adenylate, a strong inhibitor of aspartyl-tRNA synthetase. The mccABCDE operon is sufficient for McC production and resistance of the producing cell to McC. An additional gene, mccF, which is adjacent to but not part of the mccABCDE operon, also provides resistance to exogenous McC. MccF is similar to Escherichia coli LdeA, an L-D-carboxypeptidase whose substrate is monomeric murotetrapeptide L-Ala-D-Glu-meso-A2pm-D-Ala or its UDP-activated murein precursor. The mechanism by which MccF provides McC resistance remained unknown. Here, we show that MccF detoxifies both intact and processed Mcc by cleaving an amide bond between the C-terminal aspartate and the nucleotide moiety. MccF also cleaves the same bond in nonhydrolyzable aminoacyl sulfamoyl adenosines containing aspartyl, glutamyl, and, to a lesser extent, seryl aminoacyl moieties but is ineffective against other aminoacyl adenylates.

Microcins are a class of small (less than 10 kDa) antibacterial peptides produced by Escherichia coli and its close relatives (1, 2). Microcins are produced from ribosome-synthesized, gene-encoded precursors. Precursors of post-translationally modified microcins are heavily modified by dedicated maturation enzymes (3, 4). One post-translationally modified microcin, microcin C (McC), is a heptapeptide with covalently attached C-terminal modified adenosine monophosphate (Fig. 1A) (5, 6). McC is produced by E. coli cells harboring plasmids carrying the mccABCDE operon (Fig. 1B), which encodes the seven-amino acid McC precursor (the product of the mccA gene), the enzymes necessary for McC synthesis (MccB, MccD, and N-terminal domain of MccE), and proteins that make the producer cell resistant to McC (MccC and C-terminal domain of MccE). Mature McC (molecular mass of 1178 Da; Fig. 1A) consists of modified Mcca heptapeptide with the C-terminal aspartate instead of asparagine encoded by the mccA gene, an AMP residue attached to the α-carboxyl group of the aspartate through an N-acyl phosphoramidate linkage, and a propylamine group attached to the phosphate. The N-terminal methionine residue of McC, encoded by the first codon of mccA, is formylated.

Once inside a sensitive cell, McC is processed, and the product of processing (Fig. 1A), a nonhydrolyzable analog of aspartyl-adenylate (Fig. 1A) that is an intermediate of reaction catalyzed by aspartyl-tRNA synthetase (AspRS), inhibits translation by preventing the synthesis of Asp-tRNAAsp (7). McC processing involves deformylation of the N-terminal Met residue by peptidase deformylase, followed by degradation of the peptide moiety by any one of the three broad specificity aminopeptidases, peptidases A, B, and N (8). Whereas unprocessed McC has no effect on the aminoaetylation reaction, processed McC has no effect on the growth of sensitive cells at concentrations at which intact McC efficiently inhibits growth (7). Thus, McC is a Trojan-horse inhibitor (7, 9); the peptide moiety is required for the entry of unprocessed Mcc into sensitive cells where it must be processed by peptidases to release the aminoacyl-nucleotide part of the drug, which is required for the inhibition. Other known Trojan horse inhibitors targeting aminoacyl-tRNA synthetases include albomycin, a nonhydrolyzable seryl pyrimidyl attached to ferritin transport moiety (10), and agrocin 84, a nonhydrolyzable leucyl adenylate modified by an opine necessary for transport inside agrobacterial cells (11–13). Albomycin targets SerRS, whereas agrocin 84 targets LeuRS.

Although some Mcc processing should occur inside the cytoplasm of the producing cells, no poisoning of cells in the producing culture is observed, and cells remain viable. Resistance to internally produced Mcc is provided by the action of the MccC pump and through acetyltransferase activity of the
C-terminal domain of the MccE protein. MccECTD acetylates the amino group of processed McC, thus preventing its interaction with (and inhibition of) AspRS (14).

The natural plasmid responsible for McC production, pMcc7, is a large low copy number plasmid (15). The mccABCDE operon was subcloned from pMcc7 during early studies aimed at elucidating the minimal determinants sufficient for McC production and immunity. However, it was observed that both the growth rate and levels of McC resistance of cells harboring pMcc7 were higher than the corresponding rates and levels in cells harboring multicopy plasmids carrying the mccABCDEF operon (16). It was subsequently shown that a fragment of pMcc7 containing an additional gene, located immediately downstream of the mccABCDEF operon and transcribed in an opposite direction, leads to McC resistance when placed on a multicopy plasmid (16). This gene was named mccF (Fig. 1B). The mechanism by which MccF contributes to McC resistance was not defined, however. Here, we show that MccF is a peptidase that is able to specifically cleave both intact and processed McC at the carboxamide bond connecting, respectively, the peptidyl or aminoacyl moieties and the nucleotide moiety. The results thus describe a novel mechanism of detoxification of antibacterial compounds based on aminoacyl adenylates. Given the abundance of MccF homologs, the mechanism may be widespread in the bacterial world.

EXPERIMENTAL PROCEDURES

Molecular Cloning and Recombinant Protein Purification—The mccF gene was amplified by PCR with primers containing engineered XhoI and BplI restriction sites using pBM43 plasmid as a template (18). PCR product was digested with XhoI and BplI, and the mccF fragment was cloned into appropriately treated pET19 expression vector. The S118A substitution was introduced into cloned mccF by site-directed mutagenesis using appropriate oligonucleotides. Recombinant MccF and MccF S118A were overproduced in E. coli BL21(DE3) cells. The cells were grown at 37 °C in 400 ml of LB supplemented with ampicillin (100 µg/ml) until A 600 of culture reached 0.6, and expression of plasmid-borne mccF was induced by the addition of 0.2 mM isopropyl β-D-thiogalactopyranoside. After induction, the cells were grown at 30 °C until A 600 reached 1. The cells were harvested by centrifugation and resuspended in 6 ml of buffer A (20 mM Tris-HCl, pH 8.0, 50 mM NaCl). After a 1-h incubation on ice, the cells were disrupted by sonication, and cell debris was removed by centrifugation. The supernatant was loaded on a 1-ml chelating HiTrap column (GE Healthcare) charged with Ni²⁺ according to the manufacturer’s instructions. The column was washed with buffer A containing 50 mM imidazole, and the bound protein was eluted with the same buffer containing 300 mM imidazole. The resulting MccF proteins were 95% pure as judged by Coomassie staining of SDS gels. Glycerol was added to a final concentration 50%, and the samples were stored at −20 °C until further use.

In Vivo Sensitivity Test—E. coli BL21(DE3) cells carrying pET19, pET19mccF, and pET19mccF S118A plasmids were grown in 5 ml of LB supplemented with ampicillin (100 µg/ml) at 37 °C until A 600 reached ~1. Cells carrying pet28mccE (14) and pet11aspRS (7) plasmid were grown in 5 ml of LB supplemented with kanamycin (50 µg/ml) and isopropyl β-D-thiogalactopyranoside (0.1 mM) at 30 °C overnight (~18 h). 100–200 µl of culture was mixed with 5 ml of melted top (0.75%) LB agar, and the mixture was poured on a surface of LB agar plates supplemented with kanamycin (in the case of pet28mccE and pet11aspRS plasmids) or LB agar plates supplemented with ampicillin (in the case of pET19-based plasmids). The sensitivity of cells to different compounds was measured by placing 2-µl drops of McC (10 µM), XDSA (10 µM),5 XESA (35 µM), XLSA (35 µM), albomycin (10 µM), DSA (10 µM), or LSA (10 µM) on the surface of solidified soft agar. The plates were incubated at 37 °C overnight, and diameters of growth inhibition zones were determined. Each experiment was repeated at least three times, and the mean values as well as standard deviations are reported in the figures.

MccF Peptidase Assay—Incubation of recombinant MccF proteins with Mcc was carried out in the following conditions. 50 µl of reaction mixture contained 20 mM Tris-HCl, pH 8.0, 50 mM NaCl, 1 µM recombinant MccF or MccF S118A. The reactions were preincubated at 30 °C for 1 h, following the addition of McC to the final concentration 40 nM and an additional 1-h incubation at 37 °C. The reactions containing DSA/ESA contained, in 200 µl, 20 mM of Tris-HCl, pH 8.0, 50 mM NaCl, and 40 nM MccF or MccF S118A. The reaction mixtures were preincubated at 37 °C for 30 min, followed by the addition of 100 µM substrates and an additional 2-h incubation at the same temperature. The reactions without added MccF served as controls. Effect of serine-peptidase inhibitor PMSF on MccF activity was studied by including 4 mM PMSF in the starting reaction mixture. The reactions were terminated by flash-freezing in liquid nitrogen and lyophilization. Dried residue was dissolved in 5 µl of ultrapure water. Aliquots of dissolved reaction products were placed on the surface of solidified soft agar. The plates were incubated at room temperature overnight, and the sizes of growth inhibition zones were determined as described above.

Preparation of S30 Extracts—E. coli cells were grown in 50 ml of LB medium containing 50 µg/ml ampicillin until mid log phase. After centrifuging at 3,000 × g for 10 min, the supernatant was discarded, and the cell pellet was resuspended in 40 ml of buffer containing 20 mM Tris-HCl or HEPES-KOH, pH 8.0, 10 mM MgCl₂, 100 mM KCl. The cell suspension was centrifuged again as above. This procedure was repeated two times. The pellet was resuspended in 1 ml of the same buffer containing 1 mM DTT and kept at 0 °C. The cells were disrupted by several successive rounds of sonication. The lysate was centrifuged at 15,000 × g for 30 min at 4 °C. The supernatant was aliquoted and stored at −80 °C until further use.

The tRNA Aminoacylation Reaction—The tRNA aminoacylation reactions were performed as described in Ref. 7 with minor modifications. To 1 µl of solution containing an inhibitor tested (McC or AaSAs), 3 µl of E. coli S30 extract, and,
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A

Intact McC

Processed McC

Asp-AMP

DSA

B

mccA  mccB  mccC  mccD  mccE  mccF

C

MccF

AgnC5

LdcA

MccF

AgnC5

LdcA

MccF

AgnC5

LdcA

MccF

AgnC5

LdcA

MccF

AgnC5

LdcA

MccF

AgnC5

LdcA

MccF

AgnC5

LdcA

MccF

AgnC5

LdcA

MccF

AgnC5

LdcA

MccF

AgnC5

LdcA
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HPLC Analysis—The reverse phase C18 column was equilibrated with buffer A (25 mM triethylamine acetate buffer, pH 7.5, in H₂O). Probes were diluted in 1 ml of buffer A and injected to the column. Then a column was ramped to buffer B (25 mM TEAB in CH₃CN). The flow rate was 1 ml/min throughout. The retention times are relative to the flow start.

RESULTS

Overproduction of MccF Renders Cells Resistant to McC—If MccF alone were sufficient for McC resistance, then overexpression of mccf should make cells McC-resistant. To test this, a pET-based mccf expression plasmid was created and introduced into McC-sensitive BL21(DE3) E. coli cells. Cells transformed with pET vector or pET-based plasmid expressing mcee, an acetyltransferase that inactivates processed McC (14), were used as, respectively, negative and positive controls. McC sensitivity was determined by depositing 2-µl drops of 10 µM McC solution on freshly prepared lawns of cells tested. Upon overnight growth, the presence and the size of growth inhibition zones around McC spots were recorded. As can be seen from Fig. 2A, overexpression of MccF made BL21(DE3) cells resistant to McC. As expected, overexpression of MccE (positive control) had a similar effect, whereas cells harboring the negative control (pET vector) were sensitive.

We also tested three synthetic McC analogs: XDSA, XESA, and XLSA (17), for their ability to inhibit the growth of cells overproducing MccF. The synthetic compounds contain a hexapeptide corresponding to the first six amino acids of McC attached to, respectively, aspartyl sulfamoyl adenosine DSA (nonhydrolyzable analog of aspartyl adenylate, inhibits AspRS), glutamyl sulfamoyl adenosine ESA (inhibits GluRS), and leucyl sulfamoyl adenosine LSA (inhibits LeuRS). As shown elsewhere (17), synthetic compounds retain the Trojan horse mechanism of McC and target AspRS (XDSA), GluRS (XESA), and LeuRS (XLSA). Cells overproducing MccF were resistant to XDSA and XESA but were as sensitive to XLSA as control cells harboring pET vector (Fig. 2A). In contrast, cells overproducing MccE were resistant to all three synthetic McC analogs, as expected (Fig. 2A). MccF-overproducing cells were fully sensitive to albomycin (Fig. 2A), whereas cells overproducing MccE were resistant, in agreement with earlier data (14). The effect of MccF overproduction on sensitivity to DSA and LSA was also investigated (ESA was excluded from the analysis because it does not permeate E. coli cells). The results showed that cells overproducing MccF and MccE were resistant to DSA, whereas only MccE-overproducing cells were resistant to LSA (Fig. 2B).

Based on these results, we conclude that when overexpressed, MccF alone can provide resistance to McC and its synthetic derivatives targeting AspRS or GluRS, as well as to processed...
McC analog DSA. MccF is clearly more specific than MccE, because the latter but not the former protein overproduction renders cells resistant to XLSA, LSA, and albomycin.

**MccF Rescues the tRNAAsp Aminoacylation Reaction from McC Inhibition in Vitro**—Recombinant MccF was purified to homogeneity, and its ability to act on McC was determined in vitro. E. coli S30 extracts were prepared and supplemented with McC or DSA with or without MccF addition, and tRNAAsp aminoacylation reactions were carried out (Fig. 3A). Because McC requires processing to release the inhibitory aspartyl adenylate, it was preincubated with cell extract for 15 min prior to the addition of MccF (the time of MccF addition was considered as a zero time point; see Fig. 3A). Because DSA does not require processing for AspRS inhibition, MccF was added to extracts together with DSA, at which point the first measurement of tRNAAsp aminoacylation (a zero time point) was performed. As can be seen, McC or DSA inhibited tRNAAsp aminoacylation in control extracts, as expected. In contrast, the inhibition in extracts containing MccF was poor at the time of the first measurement (80% of activity observed in the absence of added inhibitor). Upon further incubation, MccF-containing extracts fully recovered from inhibition. MccF alone had no effect on the reaction (data not shown). Additional experiments demonstrated that the addition of MccF had no effect on aminoacylation reactions inhibited by several other aminoacyl adenylates (isoleucyl-sulfamoyl-adenosine, lysyl-sulfamoyl-adenosine, phenylalanyl-sulfamoyl-adenosine, glycyl-sulfamoyl-adenosine, glutaminyl-sulfamoyl-adenosine, ornithinyl-sulfamoyl-adenosine, and prolyl-sulfamoyl-adenosine).

**FIGURE 2.** Overproduced MccF makes cells resistant to McC, XDSA, XESA, and DSA. **A**, the sizes of growth inhibition zones around 2-μl drops containing McC and XDSA (10 μM solutions), XESA and XLSA (35 μM solutions), and albomycin (10 μM solution) deposited onto cell lawns formed by E. coli BL21(DE3) cells carrying indicated expression plasmids are shown. The error bars show standard deviations of measurements obtained in at least three independent experiments. **B**, the sizes of growth inhibition zones around 2-μl drops of 10 mM solutions of DSA and LSA deposited on cell lawns formed by E. coli BL21(DE3) cells carrying the indicated expression plasmids are shown.

**FIGURE 3.** Recombinant MccF is sufficient to overcome tRNA aminoacylation inhibition caused by the addition of McC and DSA. **A**, AspRS-catalyzed aminoacylation of tRNAAsp in S30 extracts prepared from E. coli cells with or without the addition of recombinant MccF. Where indicated, the extracts were supplied with intact McC or DSA. Reactins were indicated for the times indicated, and tRNAAsp aminoacylation reactions were carried out. A representative result of three independent experiments is shown. **B**, LeuRS-catalyzed aminoacylation of tRNALeu in S30 extracts prepared from E. coli cells with or without the addition of recombinant MccF. Where indicated, the extracts were supplied with LSA, and tRNALeu aminoacylation reactions were carried out at the time points indicated. A representative result of three independent experiments is shown.
enzyme removes the C-terminal l-Ala residue from its substrates. The amino acid sequence alignment of MccF, LdcA, and a homologous protein encoded by the biosynthetic cluster of agrocyn 84, a Trojan horse antibiotic targeting LeuRS (9), is shown in Fig. 1C. We hypothesized that MccF is also a peptidase and that it contributes to Mcc resistance through its peptidase activity. Two experiments support this view. First, a mutation substituting an evolutionary conserved serine residue Ser118 that likely forms the catalytic triad of the MccF peptidase catalytic center for alanine was created (Fig. 1C). An expression plasmid carrying the mutant gene overproduced MccF at high level, and all overproduced protein was soluble (data not shown), yet the cells overproducing the mutant protein were fully sensitive to Mcc, indicating that the point substitution annihilated MccF protective function (Fig. 4A). The mutant protein was purified and found to be unable to detoxify Mcc in vitro (Fig. 4B). Second, the in vitro test described above was repeated in the presence of PMSF, an inhibitor of serine proteases. As can be seen (Fig. 4B), the addition of 4 mM PMSF to MccF prior to the addition of Mcc prevented MccF function, and growth inhibition zones of the same size as in controls (no MccF added) were observed. Together, these experiments support a view that MccF is a serine peptidase and that its peptidase activity is required for Mcc detoxification.

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**FIGURE 4.** Incubation with pure MccF abolishes antibacterial activity of Mcc. A, the sizes of growth inhibition zones around 2-$\mu$l drops of Mcc (10 $\mu$M solution) and DSA (10 $\mu$M solution) deposited on cell lawns formed by cells expressing wild-type MccF, MccF mutant carrying a S118A substitution, as well as cells harboring the pET vector control are shown. The error bars show standard deviations of measurements obtained in at least three independent experiments. B, Mcc was incubated with purified MccF in the presence or in the absence of PMSF, or with MccF S118A mutant as described under “Experimental Procedures.” The reactions were terminated by lyophilization, reaction products were dissolved in water, and aliquots were deposited onto cell lawns formed by Mcc-sensitive E. coli cells. The sizes of growth inhibition zones observed after overnight incubation are shown. The error bars show standard deviations of measurements obtained in at least three independent experiments.

In another series of experiments, purified MccF was incubated with Mcc, and the reactions were then applied onto lawns of sensitive E. coli, and aliquots were deposited onto cell lawns formed by Mcc-sensitive E. coli cells. The sizes of growth inhibition zones observed after overnight incubation are shown. The error bars show standard deviations of measurements obtained in at least three independent experiments.
and nucleotide fragment produced upon MccF cleavage of McC without the propylamine modification are the same; Fig. 1A). Based on the results of MS analysis and mutagenesis data (above), we conclude that MccF is a highly unusual serine peptidase that cleaves McC, its maturation derivatives, and synthetic analogs at the amide bond connecting the peptidyl part with the nucleotide part of these compounds.

**In Vitro MccF Treatment of DSA and ESA Leads to Production of Antibacterially Active Sulfamoyl Adenosine**—Mass spectrometric identification of the products of incubation of pure MccF with DSA, ESA, and LSA was also performed. A mass peak corresponding to LSA remained unaltered upon MccF treatment, and no new products were observed, in agreement with *in vivo* and *in vitro* data presented above (Fig. 5D). In contrast, treatment of DSA and ESA with MccF led to a complete disappearance of mass peaks corresponding to the original compounds. Instead, a mass peak corresponding to sulfamoyl adenosine (SA) generated by the cleavage of carboxamide bond between the aminoacyl and nucleotidyl parts of initial aminoacyladenylates was observed in both cases (Fig. 5, C and E). The complementing aminoacyl moieties were not detected in the MALDI experiment because of interference from matrix peaks. Both DSA and LSA remained intact upon incubation with MccF mutant described above (data not shown). Several additional aminoacyl sulfamoyl adenylates (listed above) were also tested. Of these, only seryl-sulfamoyl-adenosine was found to be a substrate for MccF-catalyzed cleavage (Fig. 5F).

An unexpected result was obtained when MccF-treated ESA or DSA reactions were applied onto lawns of McC-sensitive *E. coli* cells. In control reactions (incubation of ESA or DSA without MccF), no growth inhibition was observed in the case of ESA, whereas DSA produced growth inhibition, as expected. Surprisingly, upon incubation with MccF, reactions containing either compound led to the appearance of exceptionally large growth inhibition zones (Fig. 6A). The zones of identical size were observed when MccF-treated DSA was tested on lawns of cells overproducing MccF, MccE, or AspRS from expression plasmids (Fig. 6B). Cells overproducing either of these proteins were resistant to DSA and Mcc, as expected.

Because in our MccF-treated DSA or ESA samples the initial compounds were completely converted to SA (and a corresponding amino acid) as judged by MALDI-MS analysis, we hypothesized that the modified nucleotide was by itself responsible for the strong antibacterial action observed. Indeed, published literature contains reports documenting potent antibacterial activity of SA (22). SA was prepared by chemical synthesis and was found to be identical to SA generated by MccF treatment of DSA by both MALDI MS and HPLC retention time (Fig. 6, compare C and E). Synthetic SA indeed strongly inhibited *E. coli* growth, including the growth of *E. coli* overproducing MccF, MccE, or AspRS (Fig. 6B). In contrast and as expected, aspartic or glutamic acid solutions had no effect on cell growth at these concentrations (data not shown). We therefore conclude that the appearance of antibacterial activity of ESA, and increased activity of DSA observed upon MccF treatment is due to the accumulation of SA that results from MccF-mediated cleavage.

**MccF Homologs Encoded by the *E. coli* Genome and Agrocin 84 Biosynthetic Operon Are Unable to Detoxify McC and Processed McC Analogs**—As mentioned above, the *E. coli* genome encodes a homolog of MccF, the product of *ldcA* gene. To determine whether sequence similarity between the two proteins leads to functional similarity, an *E. coli* strain lacking *ldcA* and a strain harboring an *ldcA* expression plasmid were
obtained, respectively, from the Kejo and ASKA collections, and the sensitivity of these strains to McC was determined. No difference from control levels (an \( ldcA \) strain alone or harboring a vector plasmid) was observed. Moreover, the addition of purified recombinant LdcA protein had no effect on tRNA\(^{Asp} \) aminoacylation reactions inhibited by McC at conditions when MccF efficiently relieved the inhibition (data not shown). We therefore conclude that the LdcA protein plays no role in McC resistance.

A plasmid expressing the MccF homolog encoded by the agrocin 84 biosynthetic cluster was also constructed. Cells expressing this protein were as sensitive to McC or DSA as control cells carrying the pET vector alone. Although agrocin 84 does not affect the growth of \( E. coli \), its addition leads to inhibition of tRNA\(^{Asp} \) aminoacylation reaction in \( E. coli \) S30 extracts. The addition of either MccF or its agrobacterial homolog did not relieve the inhibition (data not shown), suggesting that \( E. coli \) MccF does not recognize and cleave agrocin 84 (an expected result because MccF also does not cleave LSA) and that the agrobacterial MccF homolog does not detoxify agrocin 84 or McC, at least in \( E. coli \) extracts.

**DISCUSSION**

In this work, we uncovered the molecular mechanism of McC resistance provided by the MccF protein. MccF is a serine protease that cleaves the carboxamide bond connecting the McC peptide part with its nucleotide part. DSA, an analog of processed McC, is also cleaved by MccF, indicating that MccF does not require the peptide part of McC for cleavage. With regards to aminoacyl specificity, MccF appears to recognize aspartate and glutamate but not leucine, indicating that it is more specific than MccE acetyltransferase, which acetylates...
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(and detoxifies) every aminoacyl sulfamoyl adenylate tested with the exception of PSA (14). MccF is also unable to cleave processed albomycin, an inhibitor of SerRS that contains a seryl residue attached to thioxylfuranosyl pyrimidine. This compound is acetylated by MccE (14). Elucidation of the molecular determinants of MccF substrate specificity will have to await structural information on MccF and its complexes with substrates.

The mechanism of MccF function to provide resistance to McC and other aminoacyl adenylates is formally similar to the mechanism of Hint protein action. Hint is a cellular purine nucleoside phosphorylase that cleaves, among other substrates, certain aminoacyl adenylates (23). It remains to be seen whether E. coli hinT contributes to basal levels of resistance to McC and processed McC analogs.

It is interesting to speculate why the system of McC production requires three different proteins (the MccC export pump, the MccF acetyltransferase, and the MccF protease) for immunity. One idea is that MccF is a backup immunity system that is expressed continuously throughout the growth of mcc gene cluster carrying cell culture. The basal level of immunity afforded by MccF may guarantee that cells other before other cells in the culture) do not gain undue advantage. Indeed, preliminary data indicate that although the mccABCDE operon transcription is activated in the stationary phase, mccF transcription is detected in the logarithmic phase of growth.

In the course of this work, we made a serendipitous observation that MccF treatment strongly increases the antibacterial potency of DSA and ESA. This effect was shown to be due to the accumulation of SA, which was earlier shown to be a broad spectrum antibacterial agent. The mechanism of SA antibacterial action is unknown. Our observation that MccF overproducing cells are resistant to DSA suggests, rather unexpectedly, that internally generated SA does not affect cell growth. If so, external SA should affect the cell surface.

An MccF homolog is encoded in the agrocin 84 biosynthesis cluster. The role of this gene in agrocin 84 synthesis and/or immunity of the producing cell is unknown. In the agrocin 84 system of agrocin 84 maturation using purified proteins shall provide LdcA clones from Keio and ASKA collection.

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