Surface-Active Properties of Antifungal Lipopeptides Produced by *Bacillus subtilis*

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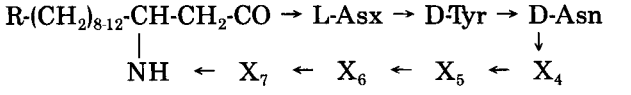
The interfacial behavior of antifungal lipopeptides of the iturinic group was studied in comparison with that of surfactin, an anionic lipopeptide. All these lipopeptides were isolated from various strains of *Bacillus subtilis*; each strain produced surfactin and one antifungal compound. The iturinic compounds differ from surfactin by their lower surfactant properties. The critical micelle concentration (CMC) values were dependent on the nature of the peptide moiety in the iturinic compounds. The highest values were observed for anionic antibiotics. The arrangement of lipopeptides at the air-water interface was largely dependent on the size of the lipid moiety; surfactin, which has a C_{14} or C_{15} β-hydroxy fatty acid, iturins A, C, and bacillomycins D, L, which have a C_{14} or C_{15} β-amino fatty acid, occupied a smaller area than mycosubtilin and bacillomycin F, which have a C_{16} or C_{17} β-amino fatty acid. These data can be related to bioactivity of these lipopeptides.

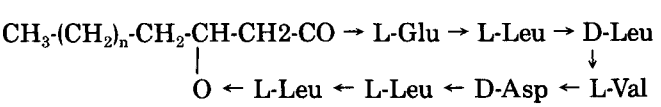
KEY WORDS: Antifungal lipopeptides, *Bacillus subtilis*, surfactant property.

Several antifungal lipopeptides have been isolated from various strains of *Bacillus subtilis*. They possess related structures consisting of a heptapeptide sequence, closed in a ring, with a lipophilic β-amino acid. Iturin A was the first compound of this family (1,2); afterwards, other compounds, such as iturin C, mycosubtilin, bacillomycins D, L, F, were studied and their structures were determined (3-6). They are of the following type:



R is a CH_3, CH_3-CH(-CH_3)- or CH_3-CH_2-CH(-CH_3)- group in accordance with *n* C_{14}, *iso* C_{15}, *anteiso* C_{15}, *n* C_{16}, *iso* C_{14}, *iso* C_{17}, *anteiso* C_{17} β-amino acids. The nature of L-Asx (X_1) and X_4 to X_7 residues is summarized in Table 1.

The name iturinic lipopeptides is used to designate compounds belonging to this group. Recently, we found that surfactin (7), a well-known lipopeptide with surfactant properties, was coproducted with iturinic lipopeptides (8). The structure of surfactin differs from those of iturinic lipopeptides essentially by the presence of a β-hydroxy fatty acid instead of a β-amino fatty acid (9):



n=8-10

The presence in the culture medium of two lipopeptides having essentially the surfactant properties of surfactin and antifungal properties of iturinic lipopeptides prompted us to examine the surfactant activity of iturinic lipopeptides to establish a possible correlation between the surfactant or antifungal properties and the structure of lipopeptides.

TABLE 1

Nature of X_1 to X_7 Residues of the Uncommon Part of the Iturin Group of Antibiotics

Antibiotics	(X_1) L-Asx	X_4	X_5	X_6	X_7
Iturin A	L-Asn	L-Gln	L-Pro	D-Asn	L-Ser
Iturin C	L-Asp	L-Gln	L-Pro	D-Asn	L-Ser
Mycosubtilin	L-Asn	L-Gln	L-Pro	D-Ser	L-Asn
Bacillomycin L	L-Asp	L-Ser	L-Gln	D-Ser	L-Thr
Bacillomycin D	L-Asn	L-Pro	L-Glu	D-Ser	L-Thr
Bacillomycin F	L-Asn	L-Gln	L-Pro	D-Asn	L-Thr

EXPERIMENTAL PROCEDURES

Iturinic antibiotics and surfactin were prepared as previously described (1-7). Surface tension of the lipopeptide solution was measured with a Krüss tensiometer by the procedure of Du Noüy with a platinum ring, as a function of concentration. The lipopeptides were dissolved in 0.1 M $NaHCO_3$ solution. The surface tension-concentration plots were used to determine critical micelle concentration (CMC), which is the point of intersection of two linear segments, and the surface tension close to the CMC (γ_{CMC}).

The minimum inhibitory concentration (MIC) against *Saccharomyces cerevisiae* was determined by the dilution method in liquid medium (glucose 40 g, peptone (bio-Mérieux) 10 g, yeast extract 2 g in 1 liter). Serial dilutions of the antifungal agent were inoculated with the organism and incubated 24 hr at 28°C.

RESULTS AND DISCUSSION

Iturinic antibiotics and surfactin displayed good solubility in 0.1 M $NaHCO_3$. Figure 1 shows the variation of

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surface tension *versus* log concentration in 0.1 M $NaHCO_3$ solution at 25°C for mycosubtilin, a neutral antibiotic; bacillomycin D, an anionic antibiotic; and surfactin, an anionic surfactant. Similar plots were obtained with other iturinic antibiotics. These curves give CMC values at 25°C. Other physical parameters were calculated by using the Gibbs adsorption equation and they are reported in Table 2. The antifungal activity against *Saccharomyces cerevisiae* is also indicated as minimal inhibitory concentration (MIC) *i.e.* the smallest concentration which inhibits the growth of the yeast.

All the iturinic antibiotics have close γ_{CMC} values, which are significantly higher than that of surfactin, but the CMC values are quite variable, from 9 μM for surfactin to 170 μM for bacillomycin D. Among iturinic compounds, the highest CMC values are observed for iturin C, bacillomycin D and bacillomycin L. These three compounds have a carboxyl group from an aspartyl or a glutamyl residue of the peptide moiety, while other compounds of this family do not. The arrangement of lipopeptides at the interface water-air is expressed by Γmax and A values. Two sets of A values are observed corresponding to two groups of antibiotics: for mycosubtilin and bacillomycin F, A = 0.97 ± 0.07 nm^2/mol; for iturins A, C and bacillomycins D, L, A = 0.72 ± 0.08 nm^2/mol. These differences could be related to the tridimensional structure of these molecules. Previous studies have reported different conformations for iturin A and mycosubtilin, as determined by nuclear magnetic resonance (NMR) spectrometry (10,11). Moreover, the lipid moiety could be involved in the orientation at the interface as the compounds of the first group, mycosubtilin and bacillomycin F, have a C_{16} or C_{17} β-amino acid, whereas the compounds of the second group, iturins A, C and bacillomycins D, L, have a C_{14} or C_{15} β-amino acid. The length of the lipidic chain could be a parameter that determines the mode of orientation of the lipopeptides at the water-air interface. We have also observed that the MIC against *Saccharomyces cerevisiae* varied in the same way. The lowest MIC is reported for mycosubtilin and bacillomycin F. The possibility of a reciprocal influence of a powerful surfactant, surfactin, and of an antibiotic, iturin A, was examined. The presence of iturin A did not modify the surfactant properties of surfactin, but the antifungal activity of iturin A was enhanced by 30% by the presence of surfactin. Thus the interfacial properties of these lipopeptides are related to their bioactivity and these data are interesting for possible applications.

TABLE 2

Interfacial Properties and MIC on *Saccharomyces cerevisiae* of Surfactin and Iturinic Antibiotics

	γ_{CMC} mN/m	CMC (25°C) μM	Γmax mol/nm^2	A nm^2/mol	MIC μM
Surfactin	31	9	2.2	0.45	no activity
Bacillomycin F	50.5	27	0.96	1.04	10
Mycosubtilin	55	37	1.1	0.91	8
Iturin A	54.5	43	1.54	0.65	20
Iturin C	49.5	80	1.48	0.67	no activity
Bacillomycin L	46	160	1.26	0.8	60
Bacillomycin D	53	170	1.28	0.78	250

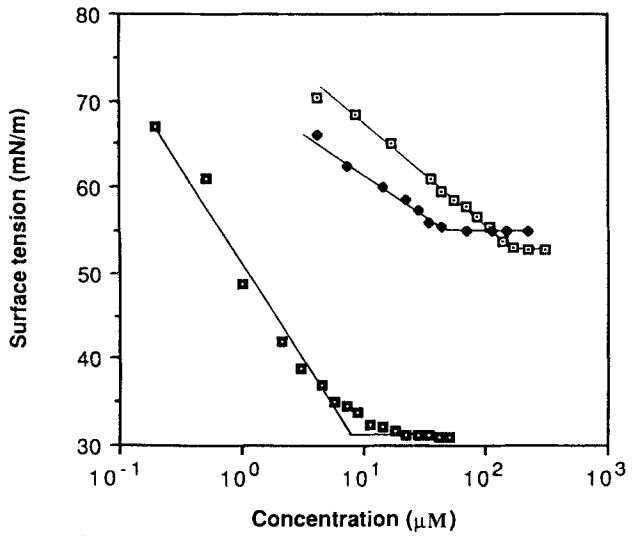

FIG. 1. Surface tension *vs* concentration of surfactin (=), mycosubtilin (=) and bacillomycin D (=).

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