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Production and Properties of Bacteriocin-Like Inhibitory Substances from the Swine Pathogen *Streptococcus suis* Serotype 2

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Streptococcus suis **serotype 2 is a major pathogen found in the upper respiratory tract of swine. In this study, isolates of this bacterial species were tested for the production of bacteriocin-like inhibitory substances (BLIS). Of the 38 strains tested, four inhibited the growth of other *S. suis* isolates according to a deferred-antagonism plate assay. Interestingly, three of the strains were originally isolated from healthy carrier pigs and were considered nonvirulent. Three isolates (94-623, 90-1330, and AAH4) that produced BLIS in liquid broth were selected for further characterization. None of the inhibitory activities was related to the production of either organic acids or hydrogen peroxide. The BLIS produced by these strains were heat stable and proteinase K, pronase, and elastase sensitive but were trypsin and chymotrypsin resistant. They were stable at pH 2 and 12 and had molecular masses in the range of 14 to 30 kDa. Maximum production was observed during the mid-log phase. Following a curing procedure with novobiocin, only 90-1330 lost the ability to produce BLIS, suggesting that the BLIS might be plasmid encoded. Analysis of the inhibitory spectra revealed that the BLIS-producing strains also inhibited the growth of *Actinobacillus minor*, *Actinobacillus porcinus*, *Enterococcus durans*, *Micrococcus luteus*, *Streptococcus agalactiae*, *Streptococcus dysgalactiae subsp. dysgalactiae*, *Streptococcus equi* subsp. *zooepidemicus*, and *S. dysgalactiae subsp. equisimilis*. This study reports for the first time the ability of the swine pathogen *S. suis* serotype 2 to produce BLIS with the characteristics of classic bacteriocins. Further studies are required to investigate the possibility of using bacteriocin-producing strains to prevent swine infections caused by virulent strains of *S. suis* serotype 2.**

A number of chemical substances, including bacteriocins, are secreted by cells during the various stages of bacterial growth. Bacteriocins have the ability to inhibit closely related and sometimes more distantly related strains of bacteria and thus play a major role in the natural defense systems of several bacterial species (12). Bacteriocins produced by indigenous bacteria may be critical for the maintenance of normal microflora and host health by preventing invasion by exogenous pathogens (3). Since a number of bacterial species, including *Streptococcus suis*, *Haemophilus parasuis*, *Rothia nasimurium*, *Staphylococcus aureus*, *Arcanobacterium pyogenes*, and *Actinomyces hyovaginalis*, are found in the upper respiratory tract (URT) of pigs (2), it is possible that the URT may be a favorable environment for the secretion of competition factors like bacteriocins.

The predominant streptococcal species in the URT of swine, more particularly the tonsils and nasal cavities, is *S. suis* (2). There are presently 35 serotypes of *S. suis* (serotypes 1 to 34 and serotype 1/2) recognized on the basis of capsular antigens, although serotype 2 is the strain most frequently associated with infections in pigs (8, 24), especially meningitis (7, 8, 24). Other pathologies, such as arthritis, pneumonia, endocarditis, and septicemia leading to sudden death, have also been reported (7, 8, 24). *S. suis* infections are largely controlled by using prophylactic and therapeutic antibiotics, including penicillin (8). However, a recent increase in antibiotic-resistant *S. suis* isolates combined with the growing public concern over the use of prophylactic antimicrobials in farming indicates that alternative strategies for controlling *S. suis* infections are required (1, 4, 13, 15, 26). Until now, most vaccines developed to protect swine against *S. suis* infections have used inactivated bacteria and results have been inconsistent (8). In this study, we propose that bacterial interference or bacteriocins may be a promising new avenue for the prevention and treatment of *S. suis* infections. Since there are no scientific data on antagonistic interactions involving *S. suis*, we investigated the production of bacteriocin-like inhibitory substances (BLIS) by *S. suis* serotype 2 isolates.

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MATERIALS AND METHODS

Bacterial strains and culture conditions. The bacterial isolates used in this study are listed in Table 1 and were kindly provided by M. Gottschalk from the Groupe de Recherche sur les Maladies Infectieuses du Porc at Université de Montréal (Montreal, Quebec, Canada). *Micrococcus luteus* ATCC 272 was obtained from M. Lavoie (Université Laval, Quebec City, Quebec, Canada). Bacteria were routinely grown aerobically at 37°C in Todd-Hewitt broth (THB; Becton Dickinson, Cockeysville, Md.) or on THB agar (THA) plates. LA broth medium was used to investigate the kinetics of BLIS production and to obtain BLIS-containing supernatants. LA broth contains 1% glucose, 2% peptone (Proteose Peptone no. 3; Difco, Detroit, Mich.), 0.3% K_2HPO_4, 0.2% KH_2PO_4, 0.01% $MgSO_4 \cdot 7H_2O$, 0.002% $MnSO_4 \cdot 6H_2O$, and 0.5% NaCl (9). BLIS production in THB, Trypticase soy broth (Becton Dickinson) supplemented with 3% yeast extract, and M17 broth (Difco) supplemented with 0.5% glucose was also evaluated.

Deferred-antagonism plate assay. The bacterial isolates listed in Table 1 were tested for antagonistic interactions on THA plates. The producing bacteria were inoculated along a straight line through the center of THA plates by using sterile cotton-tipped swabs dipped in overnight broth cultures. After a 24-h incubation at 37°C to allow bacterial growth and BLIS production, the indicator strains were inoculated perpendicular to the producing bacteria with sterile cotton-tipped

TABLE 1. Bacterial strains used in this study

Strain	Origin	Geographic origin
A. minor AMX 2B	Healthy carrier pig	Quebec, Canada
A. porcinus 96-0088-3F	Healthy carrier pig	Quebec, Canada
E. durans DM	NA[c]	NA
E. faecalis ATCC 19433	NA	NA
E. faecalis ATCC 29212	Urine	NA
E. hirae ATCC 8043	NA	NA
H. parasuis 99-9048-B	Diseased pig	Quebec, Canada
M. luteus ATCC 272	NA	NA
P. multocida 01-14-97-2	Diseased pig	Quebec, Canada
S. hyicus ATCC 11249	Pig with epidermitis	NA
S. agalactiae ATCC 13813	NA	NA
S. dysgalactiae subsp. *dysgalactiae* ATCC 27957	Infected bovine	NA
S. dysgalactiae subsp. *dysgalactiae* ATCC 9926	NA	NA
S. equi subsp. *zooepidemicus* ATCC 6580	Horse with strangles	United States
S. equi subsp. *zooepidemicus* ATCC 43079	Bovine mastitis	England
S. dysgalactiae subsp. *equisimilis* ATCC 9542	NA	NA
S. bovis ATCC 9809	NA	NA
S. bovis B457C	Diseased pig, kidney	NA
S. suis S428 serotype 1	Diseased pig	Europe
S. suis 2651 serotype 1/2	Diseased pig	Europe
S. suis 31533 serotype 2	Diseased pig	France
S. suis 89-999 serotype 2	Diseased pig	Quebec, Canada
S. suis 94-623 serotype 2	Healthy carrier pig	France
S. suis 90-1330 serotype 2	Healthy carrier pig	Canada
S. suis 94-3037 serotype 2	Human; meningitis	Quebec, Canada
S. suis S735 serotype 2[a]	Diseased pig	The Netherlands
S. suis mutant 2A serotype 2[b]	S-735	Quebec, Canada
S. suis Reims serotype 2	Human	France
S. suis 166 serotype 2	Diseased pig	France
S. suis 24 serotype 2	Diseased pig	France
S. suis 65 serotype 2	Healthy carrier pig	Europe
S. suis B268a serotype 2	Healthy carrier pig	Quebec, Canada
S. suis T15 serotype 2	Healthy carrier pig	Europe
S. suis TD10 serotype 2	Healthy carrier pig	United Kingdom
S. suis AAH4 serotype 2	Diseased pig	United States
S. suis SS 93 serotype 2	Diseased pig	France
S. suis D 282 serotype 2	Diseased pig	The Netherlands
S. suis JL 590 serotype 2	Diseased pig	Mexico
S. suis 17 serotype 2	NA	The Netherlands
S. suis 89-5046 serotype 2	Diseased pig	Quebec, Canada
S. suis 4/3 HI serotype 2	Healthy carrier pig	Quebec, Canada
S. suis LM 90-559 serotype 2	Diseased pig	Canada
S. suis EA 0891/90 serotype 2	Diseased pig	Canada
S. suis 95-8242 serotype 2	Diseased pig	Quebec, Canada
S. suis 89-4223 serotype 2	Healthy carrier pig	Quebec, Canada
S. suis 98-3473-5 serotype 2	Pig; clinical strain	Quebec, Canada
S. suis 98-B575 serotype 2	Pig; clinical strain	Quebec, Canada
S. suis 90-2741-7 serotype 2	Diseased pig	Quebec, Canada
S. suis 770 353 serotype 2	Human; meningitis	The Netherlands
S. suis 770 297 serotype 2	Human; meningitis	The Netherlands
S. suis 96-52466 serotype 2	Human; arthritis	France
S. suis 98-3634 serotype 2	Human; endocarditis	Quebec, Canada
S. suis 4961 serotype 3	Diseased pig	Europe
S. suis Amy12C serotype 5	Pig; clinical strain	Quebec, Canada
S. suis 4B serotype 5	Pig; clinical strain	Quebec, Canada

[a] ATCC 43765 *S. suis* serotype 2 reference strain.
[b] *S. suis* S735 mutant that does not express capsular polysaccharide.
[c] NA, not available.

swabs dipped in overnight broth cultures. The plate contents were incubated for a further 24 h at 37°C before they were examined for the presence of inhibition zones at the intersections of the streaks.

Kinetics of BLIS production. The kinetics of BLIS production by *S. suis* 94-623 was determined in LA broth. Tubes of LA broth were inoculated with a 10% (vol/vol) inoculum of an 18-h culture and were incubated at 37°C. Samples were taken every 20 min (15 min during the exponential growth phase). The optical densities at 660 nm (OD_{660}) were monitored, and the BLIS titers of the culture supernatants were determined by using a plate diffusion assay. The culture supernatants were adjusted to pH 7.0, filter sterilized, and serially diluted in LA broth containing 1% (vol/vol) Tween 20. Fifty-microliter samples of the dilutions were placed in glass penicylinders (8 by 8 mm; Bellco Glass Inc., Vineland, N.J.) on THA plates that had been inoculated with a standardized quantity of indicator strain *S. suis* 24 (100 μl of a bacterial suspension at an OD_{660} of 0.4). After a 24-h incubation at 37°C, the BLIS titer was expressed in arbitrary units (AU) defined as the reciprocal of the highest dilution for which the growth of the indicator strain was inhibited.

Mode of action. The mode of action of the BLIS was investigated as follows: the BLIS-producing strains (*S. suis* 94-623, 90-1330, and AAH4) were grown in LA broth for 48 h. The culture supernatants were collected by centrifugation (10,000 × *g* for 15 min), adjusted to pH 7.0, and filter sterilized. One milliliter of each culture supernatant was added to 10 ml of an early-log-phase culture of *S. suis* S735 in THB. The OD_{660} was then measured at various times for up to 24 h. One milliliter of culture supernatant from the non-BLIS-producing *S. suis* S735 strain was used as a negative control.

Plasmid curing. Plasmid curing of the four BLIS-producing *S. suis* isolates was attempted by using the procedure described by Shehane and Sizemore (23). Tubes of THB medium containing 1.0, 4.0, 8.0, 10.0, or 20.0 μg of novobiocin/ml (Sigma Chemical Co., St. Louis, Mo.) were inoculated with overnight *S. suis* cultures and were incubated for 48 h at 37°C prior to spreading serial dilutions on THA plates. After an overnight incubation, 100 bacterial colonies from each isolate were selected and tested for BLIS production as follows: bacterial colonies grown on THA plates were covered with 7 ml of soft THA (0.75% [wt/vol] agar) containing 0.75 ml of an overnight culture of the indicator strain *S. suis* 24 (adjusted to an OD_{660} of 0.2). The plate contents were incubated for a further 24 h and were then examined for inhibition zones.

Characterization of BLIS. The susceptibility of the BLIS produced by strains 94-623, 90-1330, and AAH4 to enzymatic, pH, and temperature treatments was determined by using the plate diffusion assay described above. The proteolytic enzymes used were trypsin (type I; Sigma) α-chymotrypsin (type II; Sigma), elastase (type II-A; Sigma), pronase (Boehringer Mannheim, Laval, Quebec, Canada), and proteinase K (Boehringer Mannheim), each at a final concentration of 0.5 mg/ml. The effect of catalase (Sigma) at a final concentration of 1,000 U/ml was also investigated. Aliquots of culture supernatants from BLIS-producing *S. suis* strains grown in LA broth medium were treated for 2 h at 37°C with each enzyme individually. They were then boiled for 5 min to inactivate the enzymes. The pHs of the culture supernatants were adjusted to 2 or 12 by using 1 N HCl or 1 N NaOH, respectively, to evaluate the sensitivity of the BLIS to extreme pHs. The supernatants were incubated at room temperature for 2 h, the pH was then readjusted to 7.0, and the BLIS titers were determined. To evaluate temperature stability, the supernatants were incubated at 100°C for 15 min or 121°C for 20 min and were rapidly chilled on ice and the BLIS titers were determined. Lastly, the culture supernatants of BLIS-producing *S. suis* strains grown in LA broth were dialyzed by using tubing with a 12- to 14-kDa molecular mass cutoff. Following dialysis for 18 h at 4°C, samples were taken and tested for the presence of BLIS with the plate diffusion assay. BLIS-containing supernatants were also subjected to ultrafiltration by using membranes with 10- and 30-kDa molecular mass cutoffs. The ultrafiltrates were tested for the presence of BLIS with the plate diffusion assay. To exclude the possibility that growth inhibition could be related to a low pH resulting from acid production, the effect on the inhibition zones of adding 1% $CaCO_3$ to THA plates was tested. The indicator strain used throughout the characterization process was *S. suis* 24.

RESULTS

Thirty-eight strains of *S. suis* serotype 2 were tested for BLIS production with a deferred-antagonism plate assay. Four strains (94-623, 90-1330, AAH4, and 65) produced inhibitory activity against other *S. suis* strains (serotypes 1, 1/2, 2, 3, and 5) (Table 2). Strains 94-623, 90-1330, and AAH4 produced BLIS that were active against all *S. suis* isolates except themselves, while strain 65 inhibited the growth of all *S. suis* isolates except itself. Figure 1 shows the growth-inhibitory zones of the indicator strain *S. suis* 24 caused by the four BLIS-producing isolates. Strains 94-623, 90-1330, and 65 were originally isolated from healthy carrier pigs (from Canada and Europe), while AAH4 was isolated from a diseased pig (from the United

TABLE 2. Susceptibility of *S. suis* strains to the four BLIS-producing *S. suis* strains as determined by a deferred-antagonism plate assay

Indicator strain	Susceptibility to BLIS-producing strain:			
	94-623	90-1330	AAH4	65
94-623	−[a]	−	−	+
90-1330	−	−	−	+
AAH4	−	−	−	+
65	+[b]	+	+	−
T15	+	+	+	+
TD10	+	+	+	+
S428	+	+	+	+
2651	+	+	+	+
31533	+	+	+	+
89-999	+	+	+	+
94-3037	+	+	+	+
S735	+	+	+	+
Mutant 2A	+	+	+	+
Reims	+	+	+	+
166	+	+	+	+
24	+	+	+	+
B268a	+	+	+	+
SS 93	+	+	+	+
D 282	+	+	+	+
JL 590	+	+	+	+
17	+	+	+	+
89-5046	+	+	+	+
4/3 HI	+	+	+	+
LM 90-559	+	+	+	+
EA 0891/90	+	+	+	+
95-8242	+	+	+	+
89-4223	+	+	+	+
98-3473-5	+	+	+	+
98-B575	+	+	+	+
90-2741-7	+	+	+	+
770 353	+	+	+	+
770 297	+	+	+	+
96-52466	+	+	+	+
98-3634	+	+	+	+
4961	+	+	+	+
Amy12C	+	+	+	+
4B	+	+	+	+

[a] −, not inhibited.
[b] +, inhibited.

States). BLIS production in LA broth by these four isolates was tested by using a plate diffusion assay. The supernatants of three of the isolates (94-623, 90-1330, and AAH4) contained BLIS activity, which was further characterized.

The kinetics of BLIS production in LA broth by *S. suis* 94-623 is presented in Fig. 2. Anti-*S. suis* 24 BLIS activity reached a maximum (640 AU/ml) in the culture supernatant after a 5.5-h incubation period, which corresponded to the mid-log growth phase. The activity appeared relatively stable, since the titer did not decrease when the culture was incubated for up to 24 h. On the other hand, the BLIS titer in THB, supplemented with 3% yeast extract and M17 supplemented with 0.5% glucose reached only a maximum of 20 AU/ml, despite comparable cell densities in all four media.

The modes of action of the BLIS produced by 94-623, 90-1330, and AAH4 are presented in Fig. 3. The growth of indicator strain S735 stopped immediately after the addition of the BLIS-containing supernatants. The BLIS produced by AAH4 appeared to be the most efficient in inhibiting bacterial growth. However, after a 24-h incubation period, the OD_{660} of all three test cultures was comparable to that of the control culture, suggesting that the BLIS were bacteriostatic.

Following a curing procedure with novobiocin, 100 colonies of each BLIS-producing strain were tested to determine whether they retained their ability to produce BLIS. For 90-1330, all colonies lost their ability to produce BLIS when 10 μg of novobiocin/ml was added to the media, whereas only 73% of the colonies lost their BLIS activity when 4 μg of novobiocin/ml was used. All the colonies from 94-623, AAH4, and 65 retained their ability to produce BLIS in the presence of 10 μg of novobiocin/ml.

The four BLIS-producing strains were tested for their capacity to inhibit the growth of a number of gram-positive and gram-negative bacteria, most of which were isolated from swine (Table 3). *Actinobacillus minor* AMX 2B, *Actinobacillus porcinus* 96-0088-3F, *Micrococcus luteus* ATCC 272, *Streptococcus equi* subsp. *zooepidemicus* ATCC 6580, *Streptococcus dysgalactiae* subsp. *dysgalactiae* ATCC 27957, *Streptococcus dysga-*

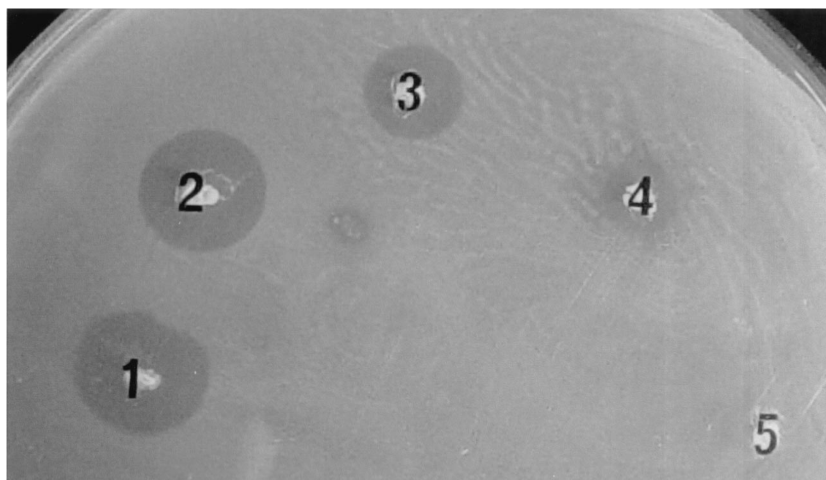

FIG. 1. BLIS production by *S. suis* 94-623 (1), *S. suis* 90-1330 (2), *S. suis* AAH4 (3), *S. suis* 65 (4), and *S. suis* 31-533 (5) (negative control). Fresh overnight cultures were spotted on THA plates and were incubated overnight. Plates were then overlaid with THA soft agar that had been inoculated with the indicator strain used (*S. suis* 24), and their contents were further incubated overnight.

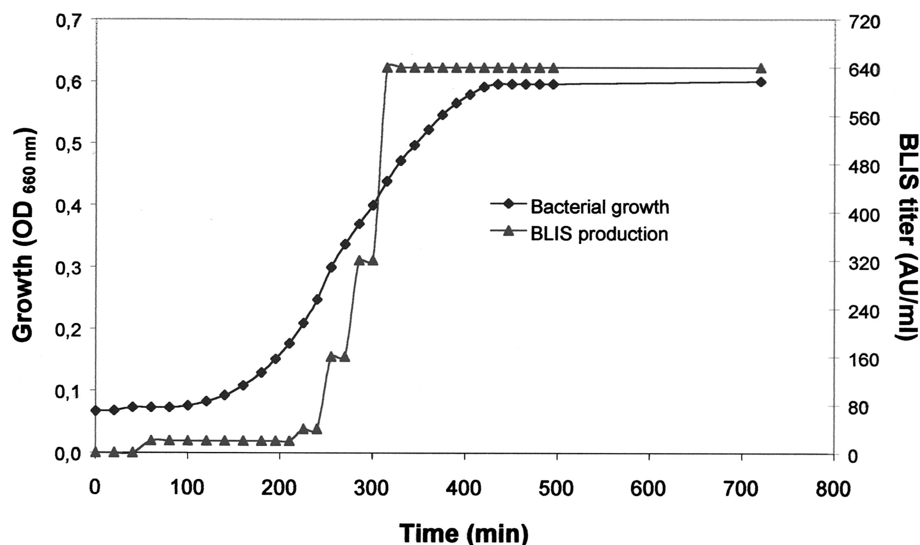

FIG. 2. Kinetics of BLIS production during growth of *S. suis* 94-623. The growth of *S. suis* 94-623 was evaluated by measuring the OD_{660}, and the BLIS titer against *S. suis* 24 was expressed as AU per milliliter as described in Materials and Methods.

lactiae subsp. equisimilis ATCC 9542, *Streptococcus agalactiae* ATCC 13813, and *Enterococcus durans* DM were inhibited by all four BLIS-producing strains, while the growth of *Streptococcus bovis* ATCC 9809, *Pasteurella multocida* 01-14-97-2, *Haemophilus parasuis* 99-9048-B, *S. equi* subsp. *zooepidemicus* ATCC 43079, *Enterococcus faecalis* ATCC 19433, *E. faecalis* ATCC 29212, *Enterococcus hirae* ATCC 8043, and *Staphylococcus hyicus* ATCC 11249 was unaffected. In addition, strain 65, but not strains 94-623, 90-1330, and AAH4, inhibited *E. hirae* ATCC 8043, *S. dysgalactiae* subsp. *dysgalactiae* ATCC 9926, and *S. bovis* B457C.

Table 3 lists the effects of various treatments on anti-*S. suis* 24 activity. BLIS from 94-623, 90-1330, and AAH4 retained their activity after heating at 100°C for 15 min but were inactivated following treatment at 121°C for 20 min. All three BLIS were resistant to pH 2 and 12 and trypsin and chymotrypsin treatments. However, they were completely inactivated by pronase, proteinase K, and elastase. The BLIS did not pass through a dialysis membrane with a 12- to 14-kDa molecular mass cutoff but were detected in the ultrafiltrate following ultrafiltration through a membrane with a 30-kDa molecular mass cutoff. The above properties could not be determined for the BLIS produced by *S. suis* 65, since it could not be recovered from the culture supernatant.

DISCUSSION

Positive and negative interactions are known to modulate microbial ecosystems. Because of the ecological diversity and the high bacterial density of the URT in pigs, bacteria must compete for the same ecological niches. Bacteriocins or BLIS may play an important role in this competitive process. The aim of our study was to highlight the presence of antagonistic interactions between various *S. suis* serotype 2 isolates and to determine the properties of the BLIS that they produced. Only 4 of 38 *S. suis* serotype 2 isolates produced BLIS that were active against other *S. suis* isolates and other swine pathogens. Since the BLIS produced by strains 94-623, 90-1330, AAH4, and 65 inhibited only one of two *S. dysgalactiae* and *S. equi* subsp. *zooepidemicus* strains, it is possible that the BLIS produced by these two strains and by *S. suis* are closely related. Indeed, *S. dysgalactiae* ATCC 9926 and *S. equi* subsp. *zooepidemicus* ATCC 43079 produced BLIS that were active against several *S. suis* serotype 2 strains (data not shown). The fact that different BLIS can have similar immunity proteins could explain these results. For instance, the immunity proteins of curvacin A and acidocin A share 50% similarity, even though the bacteriocins share only 34% sequence homology (10).

S. suis 65 consistently produced BLIS on THA plates but never in broth media. We tried to extract the BLIS from THA plates, but we obtained inconsistent results. Similar observations have been reported for bacteriocins produced by other streptococci (11, 21). The hydrophobic nature of some BLIS and bacteriocins, which can attach to the bacterial cell surface, may explain their absence in the culture supernatant. BLIS produced by 94-623, 90-1330 and AAH4 were heat stable, were unaffected by extreme pHs, and did not pass through a membrane with a 12- to 14-kDa cutoff. However, the BLIS could pass through an ultrafilter with a 30-kDa cutoff, indicating that they had molecular masses between 12 to 14 and 30 kDa. Although bacteriocins with molecular masses in the 30- to 50-kDa range have been previously reported (25, 27), it is possible that the BLIS produced by *S. suis* are low- molecular-weight molecules but form protein aggregates because they are highly hydrophobic. The fact that the BLIS were inactivated by proteinase K, pronase, and elastase indicated that they are proteins. Considering their properties, the BLIS produced by *S. suis* 94-623, 90-1330, and AAH4 may be classic bacteriocins, although studies on purified BLIS would be required to confirm their true nature. They had similar inhibitory spectra and did not interfere with the growth of the other producing strains, suggesting that they may be similar molecules. Bacteriocins produced by lactic acid bacteria are known to possess a specific mechanism that provides self-protection against the toxicity of their own bacteriocins (5). Each bacteriocin is asso-

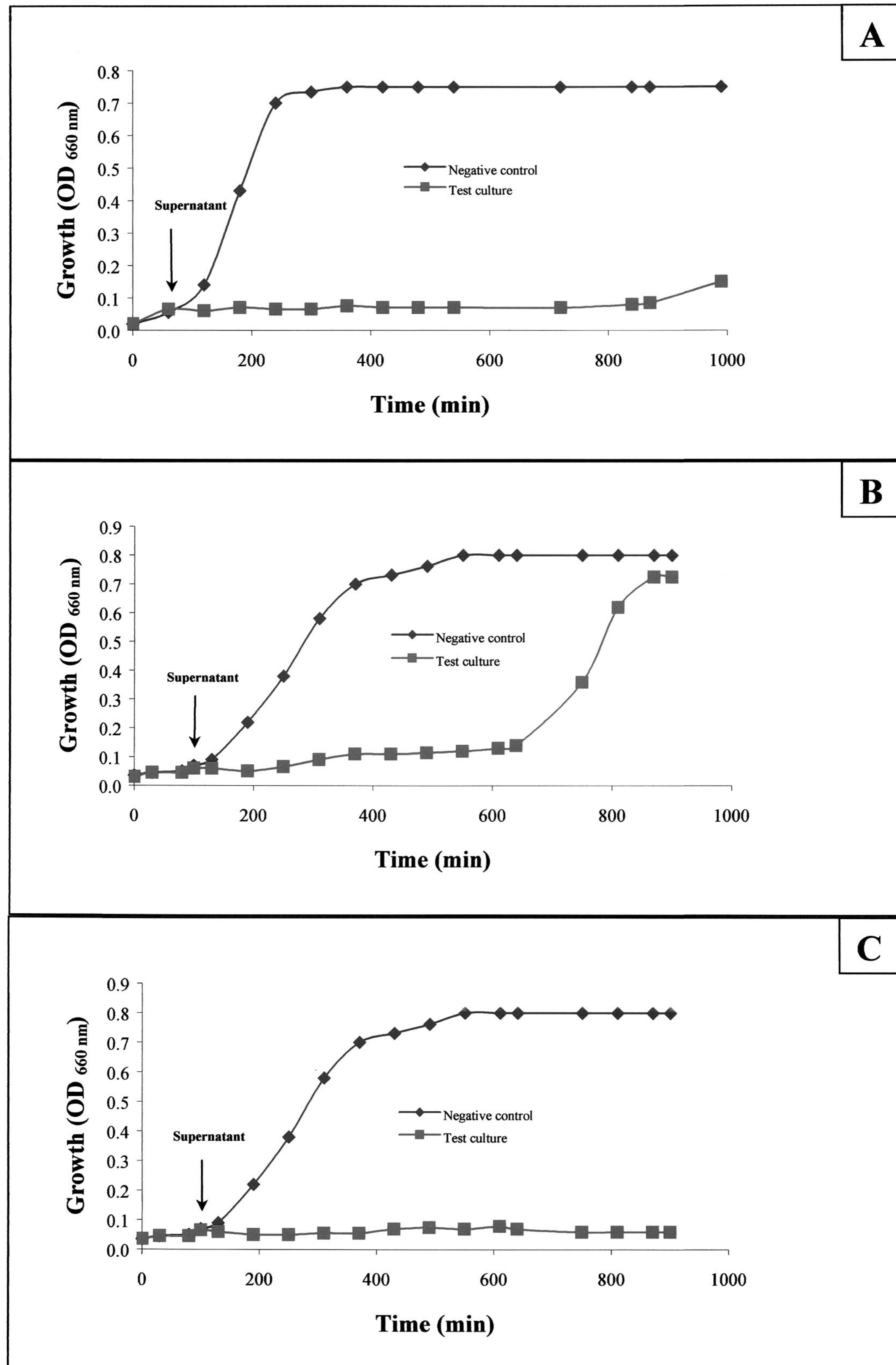

FIG. 3. Mode of action of the BLIS produced by *S. suis* 90-1330 (A), 94-623 (B), and AAH4 (C) on the growth of *S. suis* S735. The BLIS-containing supernatant (test culture) or the supernatant from a nonproducing strain (negative control) was added in the early exponential growth phase.

TABLE 3. Properties of the BLIS produced by *S. suis* serotype 2

Property or strain tested	Inhibitory activity or resistance shown by producing strain:			
	94-623	90-1330	AAH4	65
Production in				
Todd-Hewitt agar plates	+[a]	+	+	+
Todd-Hewitt agar plates + $CaCO_3$	+	+	+	+
Todd-Hewitt broth	+	+	+	−[b]
LA broth	+	+	+	−
Sensitivity to				
100°C, 15 min	R[c]	R	R	ND[e]
121°C, 20 min	S[d]	S	S	ND
pH 2.0	R	R	R	ND
pH 12.0	R	R	R	ND
Trypsin	R	R	R	ND
Chymotrypsin	R	R	R	ND
Elastase	S	S	S	ND
Proteinase K	S	S	S	ND
Pronase	S	S	S	ND
Dialysis (12–14 kDa)[f]	+	+	+	ND
Ultrafiltration (30 kDa)[g]	+	+	+	ND
Activity spectrum for bacteria				
Gram-positive				
E. durans DM	+	+	+	+
E. faecalis ATCC 19433	−	−	−	−
E. faecalis ATCC 29212	−	−	−	−
E. hirae ATCC 8043	−	−	−	−
M. luteus ATCC 272	+	+	+	+
S. hyicus ATCC 11249	−	−	−	−
S. bovis ATCC 9809	−	−	−	−
S. bovis B457C	−	−	−	+
S. agalactiae ATCC 13813	+	+	+	+
S. dysgalactiae subsp. *dysgalactiae* ATCC 27957	+	+	+	+
S. dysgalactiae subsp. *dysgalactiae* ATCC 9926	−	−	−	+
S. equi subsp. *zooepidemicus* ATCC 6580	+	+	+	+
S. equi subsp. *zooepidemicus* ATCC 43079	−	−	−	−
S. dysgalactiae subsp. *equisimilis* ATCC 9542	+	+	+	+
Gram-negative				
A. minor AMX 2B	+	+	+	+
A. porcinus 96-0088-3F	+	+	+	+
H. parasuis 99-9048-B	−	−	−	−
P. multocida 01-14-97-2	−	−	−	−

[a] +, inhibitory activity.
[b] −, no inhibitory activity.
[c] R, resistant.
[d] S, sensitive.
[e] ND, not determined.
[f] BLIS activity in dialysis tubing.
[g] BLIS activity in ultrafiltrates.

ciated with an immunity protein, and they are generally produced concomitantly. These immunity proteins may provide cross-immunity against bacteria that produce related bacteriocins (12). Amino acid sequencing of the BLIS produced by 94-623, 90-1330, and AAH4 is required to determine whether they are different molecules.

The extended lag times observed following the addition of the BLIS-containing supernatants to the S735 cultures and the fact that the OD_{660} after the 24-h incubation periods was similar to that of the control culture indicated that the BLIS produced by *S. suis* 94-623, 90-1330, and AAH4 were bacteriostatic. *S. suis* 94-623 produced BLIS constitutively in broth media, and maximum activity was reached at the mid-log phase. This is in agreement with previous reports on bacteriocin production by various gram-positive bacteria (6, 9, 17, 18, 20). Bacteriocin production was influenced by the culture medium used and was most pronounced in LA broth medium, which had the highest concentration of glucose. Previous studies with *Lactobacillus plantarum* and *Pediococcus damnosus* also showed that bacteriocin production is dependent on glucose concentration and a decrease in pH (14, 19).

Following a curing procedure, only 90-1330 lost the capacity to produce BLIS, suggesting that this BLIS may be encoded by a plasmid. Conversely, the fact that BLIS production by 94-623, AAH4, and 65 was not affected by this curing procedure suggested that these BLIS were not encoded by a plasmid. Analysis of the plasmid profiles of 90-1330 and the cured strains by agarose gel electrophoresis revealed no differences in plasmids under 10 kb (data not shown). Pulsed-field gel electrophoresis analysis, which allows large plasmids to be detected, is currently in progress to identify the nature of the plasmid coding for the BLIS produced by 90-1330. Bacteriocins encoded by large plasmids have been previously reported (16, 18).

As *S. suis* 94-623, 90-1330, and 65 were isolated from healthy carrier pigs and produce BLIS that were against many virulent *S. suis* serotype 2 strains as well as a number of other swine pathogens, they may be of great interest for use as probiotic strains. The possibility of preventing *S. suis* serotype 2 infections by a precocious colonization of piglets with bacteriocin-producing nonvirulent *S. suis* strains deserves serious consideration. Bacteriocin treatments have already been proposed to control infectious diseases because they appear to be inexpensive, effective, and nontoxic to animals and humans (3, 22).

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REFERENCES

1. **Aarestrup, F. M., S. R. Rasmussen, K. Artursson, and N. E. Jensen.** 1998. Trends in the resistance to antimicrobial agents of *Streptococcus suis* isolates from Denmark and Sweden. Vet. Microbiol. **63:**71–80.
2. **Baele, M., K. Chiers, L. A. Devriese, H. E. Smith, H. J. Wisselink, M. Vaneechoutte, and F. Haesebrouck.** 2001. The gram-positive tonsillar and nasal flora of piglets before and after weaning. J. Appl. Microbiol. **91:**997–1003.
3. **Brook, I.** 1999. Bacterial interference. Crit. Rev. Microbiol. **25:**155–172.
4. **Cantin, M., J. Harel, R. Higgins, and M. Gottschalk.** 1992. Antimicrobial resistance patterns and plasmid profiles of *Streptococcus suis* isolates. J. Vet. Diagn. Investig. **4:**170–174.
5. **Cintas, L. M., M. P. Casaus, C. Herranz, I. F. Nes, and P. E. Hernandez.** 2001. Review: bacteriocins of lactic acid bacteria. Food Sci. Tech. Int. **7:**281–305.
6. **Crupper, S. S., and J. J. Iandolo.** 1996. Purification and partial characterization of a novel antibacterial agent (Bac1829) produced by *Staphylococcus aureus* KSI1829. Appl. Environ. Microbiol. **62:**3171–3175.
7. **Gottschalk, M., and M. Segura.** 2000. The pathogenesis of the meningitis caused by *Streptococcus suis*: the unresolved questions. Vet. Microbiol. **76:**259–272.
8. **Higgins, R., and M. Gottschalk.** 1999. Streptococcal diseases, p. 563–578. *In* B. E. Straw, S. D'Allaire, W. L. Mengelin, and D. J. Taylor (ed.), Diseases of swine, 8th ed. Iowa State University Press, Ames.
9. **Ikeda, T., T. Iwanami, M. Hirasawa, C. Watanabe, J. R. McGhee, and T. Shiota.** 1982. Purification and certain properties of a bacteriocin from *Streptococcus mutans.* Infect. Immun. **35:**861–868.
10. **Ingolf, F. N., and H. Helge.** 2000. Class II antimicrobial peptides from lactic acid bacteria. Biopolymers **55:**50–61.

11. **Iverson, W. G., and N. F. Millis.** 1976. Bacteriocins of *Streptococcus bovis.* Can. J. Microbiol. **22:**1040–1047.
12. **Jack, R. W., J. R. Tagg, and B. Ray.** 1995. Bacteriocins of gram-positive bacteria. Microbiol. Rev. **59:**171–200.
13. **Kataoka, Y., T. Yoshida, and T. Sawada.** 2000. A 10-year survey of antimicrobial susceptibility of *Streptococcus suis* isolates from swine in Japan. J. Vet. Med. Sci. **62:**1053–1057.
14. **Leal-Sánchez, M. V., R. Jiménez-Diaz, A. Maldonado-Barragán, A. Garrido-Fernández, and J. L. Ruiz-Barba.** 2002. Optimization of bacteriocin production by batch fermentation of *Lactobacillus plantarum* LPC010. Appl. Environ. Microbiol. **68:**4465–4471.
15. **Martel, A., M. Baele, L. A. Devriese, H. Goossens, H. J. Wisselink, A. Decostere, and F. Haesebrouck.** 2001. Prevalence and mechanism of resistance against macrolides and lincosamides in *Streptococcus suis* isolates. Vet. Microbiol. **83:**287–297.
16. **Martinez-Cuesta, M. C., G. Buist, J. Kok, H. H. Hauge, J. Nissen-Meyer, C. Pelaez, and T. Requena.** 2000. Biological and molecular characterization of a two-peptide lantibiotic produced by *Lactobacillus lactis* IFPL 105. J. Appl. Microbiol. **89:**249–260.
17. **Nakamura, T., N. Yamazaki, H. Taniguchi, and S. Fujimura.** 1983. Production, purification and properties of a bacteriocin from *Staphylococcus aureus* isolated from saliva. Infect. Immun. **39:**609–614.
18. **Navaratna, M. A., H. G. Sahl, and J. R. Tagg.** 1998. Two-component anti-*Staphylococcus aureus* lantibiotic activity produced by *Staphylococcus aureus* C55. Appl. Environ. Microbiol. **64:**4803–4808.
19. **Nel, H. A., R. Bauer, E. J. Vandamme, and L. M. Dicks.** 2001. Growth optimization of *Pediococcus damnosus* NCFB 1832 and the influence of pH and nutrients on the production of pediocin PFD-1. J. Appl. Microbiol. **91:**1131–1138.
20. **Ocaña, V. S., A. A. Pesce de Ruiz Holgado, and M. E. Nader-Macías.** 1999. Characterization of a bacteriocin-like substance produced by a vaginal *Lactobacillus salivarius* strain. Appl. Environ. Microbiol. **65:**5631–5635.
21. **Parrot, M., M. Charest, and M. C. Lavoie.** 1989. Production of mutacin-like substances by *Streptococcus mutans.* Can. J. Microbiol. **35:**366–372.
22. **Reid, G., J. Howard, and B. S. Gan.** 2001. Can bacterial interference prevent infection? Trends Microbiol. **9:**424–428.
23. **Shehane, S. D., and R. K. Sizemore.** 2002. Isolation and preliminary characterization of bacteriocin produced by *Vibrio vulnificus.* J. Appl. Microbiol. **92:**322–328.
24. **Staats, J. J., I. Feder, O. Okwumabua, and M. M. Chengappa.** 1997. *Streptococcus suis*: past and present. Vet. Res. Commun. **21:**381–407.
25. **Thompson, J. K., M. A. Collins, and W. D. Mercer.** 1996. Characterization of a proteinaceous antimicrobial produced by *Lactobacillus helveticus* CNRZ450. J. Appl. Microbiol. **80:**338–348.
26. **Turgeon, P. L., R. Higgins, M. Gottschalk, and M. Beaudoin.** 1994. Antimicrobial susceptibility of *Streptococcus suis* isolates. Br. Vet. J. **50:**263–269.
27. **Van de Guchte, M., S. D. Ehrlich, and E. Maguin.** 2001. Production of growth-inhibiting factors by *Lactobacillus delbrueckii.* J. Appl. Microbiol. **91:**147–153.