Recently, we reported the purification and characterization of three distinct lantibiotics (named suicin 90-1330, suicin 3908, and suicin 65) produced by \textit{Streptococcus suis}. In this study, we investigated the distribution of the three suicin lantibiotic gene clusters among serotype 2 \textit{S. suis} strains belonging to sequence type (ST) 25 and ST28, the two dominant STs identified in North America. The genomes of 102 strains were interrogated for the presence of suicin gene clusters encoding suicins 90-1330, 3908, and 65. The gene cluster encoding suicin 65 was the most prevalent and mainly found among ST25 strains. In contrast, none of the genes related to suicin 90-1330 production were identified in 51 ST25 strains nor in 35/51 ST28 strains. However, the complete suicin 90-1330 gene cluster was found in ten ST28 strains, although some genes in the cluster were truncated in three of these isolates. The vast majority (101/102) of \textit{S. suis} strains did not possess any of the genes encoding suicin 3908. In conclusion, this study indicates heterogeneous distribution of suicin genes in \textit{S. suis}.

1. Introduction

Bacteriocins are cationic bactericidal peptides that primarily act by disrupting the cell membrane integrity of the target bacteria [1]. More specifically, lantibiotics are an important family of heat stable low molecular weight bacteriocins that possess unusual posttranslationally modified amino acids, such as lanthionine, methyllanthionine, dehydroalanine, and dehydrobutyrine with thioether linkages [2]. Nisin A, produced by \textit{Lactococcus lactis} subsp. \textit{lactis} and so far the most studied lantibiotic, was approved as GRAS (Generally Recognized As Safe) by the US Food and Drug Administration (FDA) in 1988 and is currently used commercially in more than 50 countries as a food preservative, especially for dairy products [3]. The lantibiotic biosynthesis machinery involves many enzymes which are encoded by genes organized in gene clusters. Typically, lantibiotic gene clusters include a structural gene encoding a prelantibiotic peptide, as well as genes required for the modification of amino acids, maturation, export, regulation, and immunity [2]. More specifically, the structural gene encodes a prepeptide containing a leader sequence at the N-terminus, which is ultimately cleaved, and a propeptide at the C-terminus in which many or all of the serine and threonine residues are modified [2].

\textit{Streptococcus suis} is a major swine pathogen responsible for a wide array of infections including life-threatening meningitis, septicemia, and pneumonia [4]. In addition, the organism is an emerging agent of zoonotic disease, particularly in some Asian countries where the general population is at risk [5]. Of the 35 serotypes (1 to 34 and 1/2) that
have been originally described based on the composition and structure of the capsular polysaccharides, serotype 2 is the most frequently associated with disease [4, 6]. In the last decade, other serotypes with particular geographical distributions have also been identified as the source of many infections [6]. For instance, in North America, serotypes 2 and 3 show a prevalence of 24.3% and 21%, respectively, followed by serotypes 1/2, 8, and 7 [6]. S. suis serotype 2 can be genotyped using multilocus sequence typing (MLST) [7]. In North America, three genotypes have been described among S. suis serotype 2 strains; the relatively frequent sequence type (ST) 25 and ST28 and the less frequent ST1 [8]. Animal infection models have shown that strains belonging to ST1 are highly virulent whereas ST25 and ST28 isolates have an intermediate and low virulence, respectively [8].

Recently, we purified and characterized three different lantibiotics, named suicins, produced by three distinct strains of S. suis serotype 2 [9–11]. The mature peptides corresponding to each suiciun showed poor identity (≤24%) between each other, thus indicating that they are unrelated. Suicin 90-1330 (SslA) is a type A (linear) lantibiotic secreted by a nonvirulent (in both mouse and pig infection models) isolate of S. suis (strain 90-1330) belonging to ST28 and exhibits high homology (90.9%) with nisin U produced by Streptococcus uberis [9]. The production of suiciun 90-1330 involves a gene cluster comprising eleven genes [9]. Suicin 3908 (SuiA) is a type B (N-terminal linear and C-terminal globular moieties) lantibiotic produced by a nonvirulent (newborn germ-free pig infection model) isolate of S. suis (strain 3908; unknown ST) isolated from a healthy carrier pig, and shows some identity with bovicin H50 (Streptococcus bovis; 49.2% identity) [10]. The suiciun 3908 gene cluster contains nine genes [10]. Lastly, a second type B lantibiotic (suiciun 65; SssA) showing a high identity with lantibiotics produced by Streptococcus pyogenes (streptococcin FF22, 84.6% identity) and L. lactis subsp. lactis (lactacin 481, 74.1% identity) was identified and characterized in S. suis 65, an ST28 avirulent (newborn germ-free pig infection model) strain isolated from a healthy carrier pig [11]. The gene cluster involved in the production of suiciun 65 was found to contain ten genes, including a duplication of the structural gene. Since all three suiciuns were found to be bactericidal for highly virulent ST1 strains of S. suis [9–11], the use of the purified lantibiotics or the lantibiotic-producing strains may represent a valuable strategy to control S. suis infections and for reducing antibiotic use in the swine industry and consequently the spread of antibiotic resistance. However, the prevalence of the different suiciun gene clusters among the circulating population of S. suis strains is not known. In this study, we investigated the distribution and genetic diversity of suiciun gene clusters in S. suis serotype 2 belonging to ST25 and ST28, the two dominant STs found in North America.

2. Materials and Methods

2.1. Bacterial Strains. One hundred and two strains of S. suis serotype 2 belonging to either ST25 (n = 51) or ST28 (n = 51) were included in this study (Table 1). These strains were isolated from diseased pigs in Canada, the United States, Japan, and Thailand and have been described previously [12, 13].

2.2. Genome Analysis. The genomes of the 102 strains were sequenced as paired-end reads with either a HiSeq 2500 or a MiSeq instrument (Illumina, San Diego, CA, USA) as described in previous reports [12, 13]. Sequences with accession numbers SRR056866 and SRR058193, for ST25 and ST28 strains, respectively, were retrieved from the NCBI sequence read archive (SRA). The A5 pipeline was used for de novo assembly of Illumina sequenced strains [14]. Contigs were ordered relative to the reference genome NSUI01060 (accession number: CP012911) or NSUI01002 (accession number: CP011419), for ST25 and ST28, respectively, using Progressive Mauve [15]. Pseudochromosomes were next created by concatenating the ordered contigs using the sequence NNNSNCACTTCATTCAATTAAATTAAATGAAATGATGNNNN, which introduces start and stop codons in all 6 reading frames, as a separator. Pseudochromosomes and the genome sequences of reference strains NSUI0102 and NSUI01060 were converted into queryable BLASTN databases using BLAST+. Gene clusters encoding suiciuns 90-1330 (accession number: KU867866), 3908 (accession number: KU867867), and 65 (accession number: KU867868) have been described previously and are illustrated in Figure 1 [9–11]. The sequences of individual genes, as well as the full regions for each suiciun cluster, were blasted against each genome and pseudochromosome database to test for the presence of genes encoding suiciuns 90-1330, 3908, and 65. The presence of putative promoter sequences upstream the genes coding for immunity proteins was analyzed with the bacterial promoter recognition program BProm (http://linux1.softberry.com) [16].

2.3. Plate Diffusion Assay for Detecting Suiciun Susceptibility. Overnight cultures of the suiciun-producing S. suis strains (90-1330, 3908, and 65) were spotted (2 μL) on Todd–Hewitt broth (THB; BD-Canada, Mississauga, ON, Canada) agar plates. After a 24 h incubation at 37°C to allow growth, the plates were overlaid with soft THB agar (0.75% agar, w/v) that had been inoculated (700 μL of culture/7 mL of agar) with a 24 h culture of S. suis NSUI018 (possessing immunity gene for suiciun 90-1330), NSUI036 (possessing immunity gene for suiciun 3908), and NSUI060 (possessing immunity gene for suiciun 65). The plates were further incubated for 24 h at 37°C, and the presence of an inhibitory zone was observed.

3. Results and Discussion

Suiciun 90-1330 was originally identified in S. suis strain 90-1330, which belongs to ST28 [9]. Interestingly, 35 out of 51 ST28 strains analyzed did not possess any of the genes involved in the production of this suiciun (Figure 2). On the other hand, all genes expected in a complete suiciun 90-1330 gene cluster were identified in ten ST28 strains, while the locus was partially present in six ST28 strains. However, it is worth mentioning that a full, uninterrupted suiciun 90-1330 gene cluster was identified in only seven of these ten ST28 strains (i.e., all genes were identified as a continuum of
Table 1: Presence and absence of suicin regions and genes among S. suis serotype 2 strains of ST25 and ST28.

| Strain | Sequence type | Suicin 65 Present or absent | Number of contigs | Suicin 90-1330 Present or absent | Number of contigs | Suicin 3908 Present or absent | Number of contigs |
|--------|---------------|-----------------------------|-------------------|----------------------------------|-------------------|-------------------------------|-------------------|
| NSUI001 | 25            | Mostly present*             | 1                 | Absent                           | N/A               | Absent                        | N/A               |
| NSUI006 | 25            | Mostly present              | 1                 | Absent                           | N/A               | Absent                        | N/A               |
| NSUI012 | 25            | Mostly present              | 1                 | Absent                           | N/A               | Absent                        | N/A               |
| NSUI033 | 25            | Present*                    | 1                 | Absent                           | N/A               | Absent                        | N/A               |
| NSUI034 | 25            | Present                     | 1                 | Absent                           | N/A               | Absent                        | N/A               |
| NSUI035 | 25            | Present                     | 2                 | Absent                           | N/A               | Absent                        | N/A               |
| NSUI037 | 25            | Present                     | 1                 | Absent                           | N/A               | Absent                        | N/A               |
| NSUI038 | 25            | Present                     | 1                 | Absent                           | N/A               | Absent                        | N/A               |
| NSUI039 | 25            | Present                     | 1                 | Absent                           | N/A               | Absent                        | N/A               |
| NSUI040 | 25            | Present                     | 1                 | Absent                           | N/A               | Absent                        | N/A               |
| NSUI041 | 25            | Mostly present              | 2                 | Absent                           | N/A               | Absent                        | N/A               |
| NSUI042 | 25            | Mostly present              | 1                 | Absent                           | N/A               | Absent                        | N/A               |
| NSUI043 | 25            | Mostly present              | 1                 | Absent                           | N/A               | Absent                        | N/A               |
| NSUI044 | 25            | Mostly present              | 2                 | Absent                           | N/A               | Absent                        | N/A               |
| NSUI045 | 25            | Mostly present              | 2                 | Absent                           | N/A               | Absent                        | N/A               |
| NSUI046 | 25            | Mostly present              | 2                 | Absent                           | N/A               | Absent                        | N/A               |
| NSUI047 | 25            | Mostly present              | 1                 | Absent                           | N/A               | Absent                        | N/A               |
| NSUI048 | 25            | Present                     | 1                 | Absent                           | N/A               | Absent                        | N/A               |
| NSUI049 | 25            | Mostly present              | 1                 | Absent                           | N/A               | Absent                        | N/A               |
| NSUI050 | 25            | Absent*                     | N/A               | Absent                           | N/A               | Absent                        | N/A               |
| NSUI051 | 25            | Absent                      | N/A               | Absent                           | N/A               | Absent                        | N/A               |
| NSUI052 | 25            | Mostly present              | 1                 | Absent                           | N/A               | Absent                        | N/A               |
| NSUI053 | 25            | Mostly present              | 1                 | Absent                           | N/A               | Absent                        | N/A               |
| NSUI054 | 25            | Mostly present              | 1                 | Absent                           | N/A               | Absent                        | N/A               |
| NSUI055 | 25            | Mostly present              | 1                 | Absent                           | N/A               | Absent                        | N/A               |
| NSUI056 | 25            | Mostly present              | 1                 | Absent                           | N/A               | Absent                        | N/A               |
| NSUI057 | 25            | Mostly present              | 1                 | Absent                           | N/A               | Absent                        | N/A               |
| NSUI058 | 25            | Mostly present              | 1                 | Absent                           | N/A               | Absent                        | N/A               |
| NSUI059 | 25            | Mostly present              | 1                 | Absent                           | N/A               | Absent                        | N/A               |
| NSUI060 | 25            | Mostly present              | 1                 | Absent                           | N/A               | Absent                        | N/A               |
| NSUI061 | 25            | Mostly present              | 1                 | Absent                           | N/A               | Absent                        | N/A               |
| NSUI062 | 25            | Mostly present              | 1                 | Absent                           | N/A               | Absent                        | N/A               |
| NSUI063 | 25            | Mostly present              | 1                 | Absent                           | N/A               | Absent                        | N/A               |
| NSUI064 | 25            | Mostly present              | 1                 | Absent                           | N/A               | Absent                        | N/A               |
| NSUI065 | 25            | Mostly present              | 1                 | Absent                           | N/A               | Absent                        | N/A               |
| NSUI066 | 25            | Mostly present              | 1                 | Absent                           | N/A               | Absent                        | N/A               |
| NSUI067 | 25            | Mostly present              | 2                 | Absent                           | N/A               | Absent                        | N/A               |
| NSUI068 | 25            | Present                     | 1                 | Absent                           | N/A               | Absent                        | N/A               |
| NSUI069 | 25            | Present                     | 1                 | Absent                           | N/A               | Absent                        | N/A               |
| NSUI070 | 25            | Present                     | 1                 | Absent                           | N/A               | Absent                        | N/A               |
| NSUI071 | 25            | Mostly present              | 1                 | Absent                           | N/A               | Absent                        | N/A               |
| NSUI072 | 25            | Mostly present              | 3                 | Absent                           | N/A               | Absent                        | N/A               |
| NSUI073 | 25            | Present                     | 1                 | Absent                           | N/A               | Absent                        | N/A               |
| NSUI074 | 25            | Mostly present              | 1                 | Absent                           | N/A               | Absent                        | N/A               |
| NSUI075 | 25            | Mostly present              | 1                 | Absent                           | N/A               | Absent                        | N/A               |
| NSUI076 | 25            | Mostly present              | 1                 | Absent                           | N/A               | Absent                        | N/A               |
| NSUI077 | 25            | Mostly present              | 1                 | Absent                           | N/A               | Absent                        | N/A               |
| NSUI078 | 25            | Mostly present              | 1                 | Absent                           | N/A               | Absent                        | N/A               |
| NSUI079 | 25            | Absent                      | N/A               | Absent                           | N/A               | Absent                        | N/A               |
| NSUI080 | 25            | Present                     | 1                 | Absent                           | N/A               | Absent                        | N/A               |
| NSUI081 | 25            | Mostly present              | 1                 | Absent                           | N/A               | Absent                        | N/A               |
| NSUI082 | 25            | Mostly present              | 1                 | Absent                           | N/A               | Absent                        | N/A               |
| NSUI088 | 25            | Present                     | 1                 | Absent                           | N/A               | Absent                        | N/A               |
| NSUI089 | 25            | Mostly present              | 1                 | Absent                           | N/A               | Absent                        | N/A               |
| NSUI092 | 25            | Present                     | 1                 | Absent                           | N/A               | Absent                        | N/A               |
| NSUI093 | 25            | Mostly present              | 1                 | Absent                           | N/A               | Absent                        | N/A               |
| NSUI094 | 25            | Mostly present              | 1                 | Absent                           | N/A               | Absent                        | N/A               |
| NSUI096 | 25            | Mostly present              | 1                 | Absent                           | N/A               | Absent                        | N/A               |
| NSUI097 | 25            | Present                     | 2                 | Absent                           | N/A               | Absent                        | N/A               |
| Strain | Sequence type | Suicin 65       | Number of contigs | Suicin 90-1330 | Number of contigs | Suicin 3908 | Number of contigs |
|--------|---------------|-----------------|-------------------|----------------|-------------------|-------------|-------------------|
|        |               | Present or absent |                   | Present or absent |                   | Present or absent |                   |
| NSUI099| 25            | Present          | 1                 | Absent          | N/A               | Absent      | N/A               |
| NSUI100| 25            | Mostly present   | 3                 | Absent          | N/A               | Absent      | N/A               |
| NSUI102| 25            | Mostly present   | 1                 | Absent          | N/A               | Absent      | N/A               |
| NSUI103| 25            | Mostly present   | 1                 | Absent          | N/A               | Absent      | N/A               |
| NSUI002| 28            | Absent           | N/A               | Absent          | N/A               | Absent      | N/A               |
| NSUI003| 28            | Absent           | N/A               | Present         | 1                 | Absent      | N/A               |
| NSUI004| 28            | Absent           | N/A               | Absent          | N/A               | Absent      | N/A               |
| NSUI005| 28            | Absent           | N/A               | Present         | 1                 | Absent      | N/A               |
| NSUI007| 28            | Absent           | N/A               | Present         | 2                 | Absent      | N/A               |
| NSUI008| 28            | Absent           | N/A               | Absent          | N/A               | Absent      | N/A               |
| NSUI009| 28            | Absent           | N/A               | Present         | 1                 | Absent      | N/A               |
| NSUI010| 28            | Absent           | N/A               | Absent          | N/A               | Absent      | N/A               |
| NSUI011| 28            | Absent           | N/A               | Absent          | N/A               | Absent      | N/A               |
| NSUI013| 28            | Absent           | N/A               | Absent          | N/A               | Absent      | N/A               |
| NSUI014| 28            | Absent           | N/A               | Absent          | N/A               | Absent      | N/A               |
| NSUI015| 28            | Absent           | N/A               | Present         | 1                 | Absent      | N/A               |
| NSUI016| 28            | Absent           | N/A               | Present         | 1                 | Absent      | N/A               |
| NSUI017| 28            | Absent           | N/A               | Present         | 1                 | Absent      | N/A               |
| NSUI018| 28            | Absent           | N/A               | Mostly present  | 1                 | Absent      | N/A               |
| NSUI019| 28            | Absent           | N/A               | Absent          | N/A               | Absent      | N/A               |
| NSUI020| 28            | Absent           | N/A               | Absent          | N/A               | Absent      | N/A               |
| NSUI021| 28            | Absent           | N/A               | Absent          | N/A               | Absent      | N/A               |
| NSUI022| 28            | Absent           | N/A               | Mostly present  | 2                 | Absent      | N/A               |
| NSUI023| 28            | Absent           | N/A               | Mostly present  | 2                 | Absent      | N/A               |
| NSUI024| 28            | Absent           | N/A               | Mostly present  | 2                 | Absent      | N/A               |
| NSUI025| 28            | Absent           | N/A               | Present         | 2                 | Absent      | N/A               |
| NSUI026| 28            | Absent           | N/A               | Mostly present  | 1                 | Absent      | N/A               |
| NSUI027| 28            | Absent           | N/A               | Absent          | N/A               | Absent      | N/A               |
| NSUI028| 28            | Absent           | N/A               | Absent          | N/A               | Absent      | N/A               |
| NSUI029| 28            | Absent           | N/A               | Absent          | N/A               | Absent      | N/A               |
| NSUI030| 28            | Absent           | N/A               | Absent          | N/A               | Absent      | N/A               |
| NSUI031| 28            | Absent           | N/A               | Absent          | N/A               | Absent      | N/A               |
| NSUI032| 28            | Absent           | N/A               | Absent          | N/A               | Absent      | N/A               |
| NSUI036| 28            | Absent           | N/A               | Mostly present  | 1                 | Mostly present | 1                 |
| NSUI058| 28            | Absent           | N/A               | Absent          | N/A               | Absent      | N/A               |
| NSUI059| 28            | Absent           | N/A               | Absent          | N/A               | Absent      | N/A               |
| NSUI062| 28            | Absent           | N/A               | Absent          | N/A               | Absent      | N/A               |
| NSUI064| 28            | Absent           | N/A               | Absent          | N/A               | Absent      | N/A               |
| NSUI067| 28            | Present          | 1                 | Absent          | N/A               | Absent      | N/A               |
| NSUI073| 28            | Absent           | N/A               | Absent          | N/A               | Absent      | N/A               |
| NSUI074| 28            | Absent           | N/A               | Absent          | N/A               | Absent      | N/A               |
| NSUI076| 28            | Absent           | N/A               | Absent          | N/A               | Absent      | N/A               |
| NSUI079| 28            | Absent           | N/A               | Absent          | N/A               | Absent      | N/A               |
| NSUI080| 28            | Absent           | N/A               | Present         | 3                 | Absent      | N/A               |
| NSUI081| 28            | Absent           | N/A               | Absent          | N/A               | Absent      | N/A               |
| NSUI083| 28            | Absent           | N/A               | Absent          | N/A               | Absent      | N/A               |
Table 1: Continued.

| Strain   | Sequence type | Suicin 65 | Suicin 90-1330 | Suicin 3908 |
|----------|---------------|-----------|----------------|-------------|
|          | Present or absent | Number of contigs | Present or absent | Number of contigs | Present or absent | Number of contigs |
| NSUI084  | 28            | Absent    | N/A            | Absent       | N/A            | Absent    |
| NSUI085  | 28            | Present   | 1             | Absent       | N/A            | Absent    |
| NSUI086  | 28            | Present   | 1             | Absent       | N/A            | Absent    |
| NSUI087  | 28            | Absent    | N/A            | Absent       | N/A            | Absent    |
| NSUI090  | 28            | Absent    | N/A            | Absent       | N/A            | Absent    |
| NSUI091  | 28            | Absent    | N/A            | Present      | 1             | Absent    |
| NSUI095  | 28            | Present   | 2             | Absent       | N/A            | Absent    |
| NSUI098  | 28            | Absent    | N/A            | Absent       | N/A            | Absent    |
| NSUI101  | 28            | Absent    | N/A            | Absent       | N/A            | Absent    |

a. The majority of the genes are present, but some are missing or truncated.
b. The suicin gene cluster is present in its entirety.
c. The suicin gene cluster is completely absent.
d. The majority of the genes are absent, but some are still present.
e. N/A: not applicable.

Figure 1: Genetic organization of the suicin 90-1330 (a), 3908 (b), and 65 (c) gene clusters. sslA/suiA/sssA/sssA1: suicin precursor; sslB: dehydratase involved in suicin synthesis; sslC: cyclase involved in suicin synthesis; sslE/suiE/sssE, sslF/suiF/sssF, sslG/suiG/sssG, and sslI/suiI: immunity proteins; sslK/suiK/sssK: sensor histidine kinase; suiM/sssM: synthetase involved in lantibiotic modification; sslP: protease involved in proteolytic cleavage of the leader peptide; sslR/suiR/sssR: response regulator; sslT/suiT/sssT: ABC transporter.

genes similar to that of Figure 1 in a single contig of the de novo assembled genomes). On the other hand, in one of the ST28 strains that had all suicin 90-1330 genes, the genes were spread across 3 contigs, suggesting two transposon insertions in intergenic regions of the suicin 90-1330 gene cluster. In two other ST28 strains, genes were spread across two contigs, apparently by insertion of a transposon that truncated parts of gene sslT (one strain) or gene sslC (one strain). These transposon insertions should result in strains that are unable to express the suicin. In the six strains in which seven or more suicin 90-1330 cluster genes were only partially present (Figure 2), the gene specifically encoding the structural suicin (sslA) was missing, suggesting that this suicin cannot be produced by these strains. In addition, in 50% of strains with a partial gene cluster, the genes were spread across two contigs (Table 1), probably due to transposon insertions. None of the ST25 strains analyzed in this study possessed genes encoding suicin 90-1330 (Figure 2).

Examination of the genome data revealed that none of the ST25 strains possessed the suicin 3908 gene cluster. Similar results were also observed in 50 of the 51 ST28 isolates (Figure 3). One ST28 strain (NSUI036, isolated in Thailand) possessed some of the cluster genes (in a single contig, Table 1) but lacked the first two genes, suiA (structural gene) and suiM, in full and suiT in part.

The suicin 65 gene cluster was found in its entirety in 15 of the 51 ST25 strains and partially in 33 of the 51 ST25 strains (Figure 4). Only three ST25 strains did not possess any of the genes of the suicin 65 gene cluster. In the 33 ST25 strains that possessed a partial suicin 65 gene cluster, the
structural sssA1 gene and gene sssM were always absent, thus suggesting that these strains cannot produce active suicin 65. The majority of ST28 strains (42/51) did not possess any of the suicin 65 cluster genes. However, eight of the 51 strains possessed all cluster genes. One ST28 strain had only the sssK gene, which lies at the extremity of the cluster. Of the ST25 and ST28 strains that contained the partial or full suicin 65 cluster, 81% (ST25) and 88% (ST28) contained all genes in a single contig (Table 1). In two of the ST25 and ST28 strains that had all suicin 65 genes, the genes were spread across 2 contigs (Table 1).

The in silico analysis described above identified strains which possessed a partial suicin locus containing genes
encoding the immunity proteins but no gene encoding the structural suicin protein. More specifically, strain NSU1018 possessed immunity protein for suicin 90-1330, strain NSU1036 possessed immunity protein for suicin 3908, and strain NSU1060 possessed immunity protein for suicin 65. The genomes of these three strains were further analyzed for the presence of promoter-like regions upstream of the genes encoding the immunity proteins. This additional analysis revealed the presence of putative promoter sequences upstream of the gene encoding the immunity proteins in all three strains. However, as demonstrated by a plate diffusion assay, all three strains were found to be susceptible to the three strains. However, as demonstrated by a plate diffusion assay, all three strains were found to be susceptible to the strain producing the corresponding suicin (data not shown). Incorporation of CaCO3 ruled out the possibility that inhibition could be related to acid production. Thus, putative sequences identified in silico upstream of the immunity genes are not functional promoters or additional factors are required to allow immunity to suicins. With the perspective to use suicin-producing avirulent strains in prophylaxis, such results indicate that the presence of a partial suicin locus containing the immunity genes does not endow a strain with suicin resistance.

4. Conclusions

The gene clusters encoding suicin 65 (mostly in ST25 strains) and, to a lesser extent, suicin 90-1330 (exclusively in ST28 strains) were the most prevalent. None of the strains that had suicin 90-1330 had suicin 65 and vice versa.

Competing Interests

The authors declare that they have no competing interests.

Acknowledgments

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