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Antibiotic Activity of *Escherichia coli* against Multiresistant *Staphylococcus aureus*

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The *pks* genomic island present in *Escherichia coli* encodes polyketides (PK) and nonribosomal peptide (NRP) synthases. As discussed in an elegant study published recently (1), this leads to the synthesis of numerous and still not fully purified and characterized PK-NRP compounds that confer to *pks*-harboring bacteria the ability to hijack host cell responses and to persist in the gut microbiota (2–5). Although the PK-NRP chemical family includes compounds used as antibiotics, such as β -lactams, glycopeptides, or daptomycin (6), there have been no studies of the antibiotic activity conferred by the *pks* island.

We studied bacterial growth inhibition using an antibiogram method derived from agar disc diffusion (7). We used as putative antibiotic sources three laboratory *E. coli* DH10 β strains hosting the empty bacterial artificial chromosome pBeloBAC11 (*E. coli* DH10 β) (2), pBeloBAC11 in which the *pks* island had been cloned (*E. coli* DH10 β *pks*⁺) (2), or pBeloBAC11 containing a *pks* island

with a deletion of *clbP* (*E. coli* DH10 β *pks* Δ *clbP*) (8), which encodes an enzyme required for *pks* island biological activities (1, 9). One hundred twenty-eight clinical strains belonging to 30 species were tested as reporter strains of antibiotic activity. These clinical strains were spread on Mueller-Hinton agar on which bacterial

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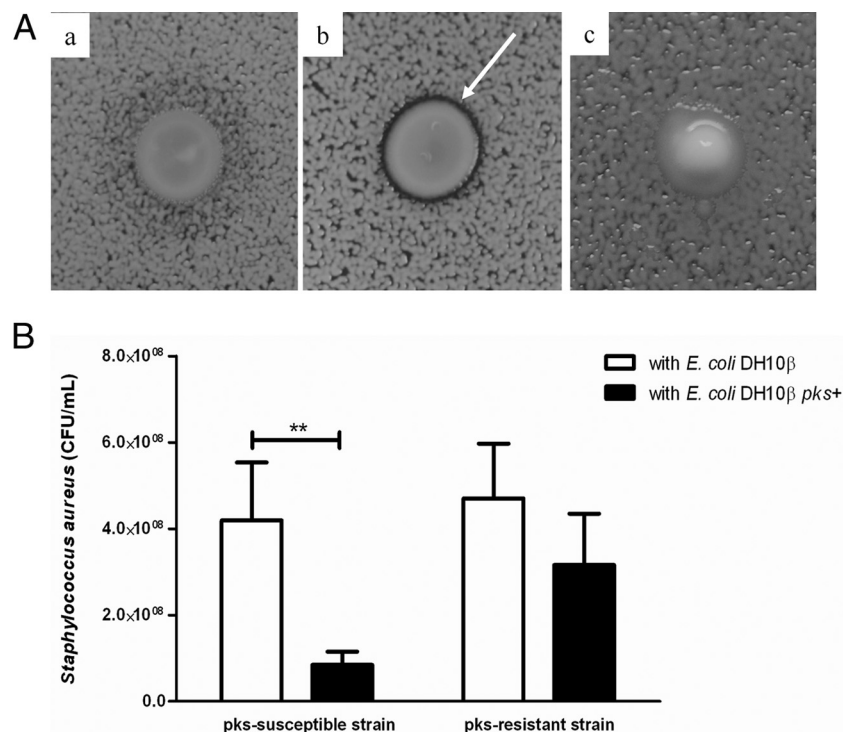


FIG 1 *pks*-harboring *E. coli* strains exhibit antibiotic activities in an agar diffusion test (A) and in growth competition experiments (B). (A) The tested *S. aureus* strains were spread on LB agar, and bacterial pellets of *E. coli* DH10 β (a), *E. coli* DH10 β *pks*⁺ (b), and *E. coli* DH10 β *pks* Δ *clbP* (c) were spotted. The white arrow indicates the growth inhibition zone. (B) Growth competition between *E. coli* DH10 β *pks*⁺ or *E. coli* DH10 β and *S. aureus* strains susceptible or resistant to the antibiotic activity conferred by the *pks* island. After 24 h of coculture in LB medium, serial dilutions were spread on LB agar and *S. aureus* colonies were counted. Data are expressed as numbers of CFU per milliliter. **, $P < 0.01$.

TABLE 1 Antibiotic activities of the *pks* island against several bacterial species

Bacterial species ^a	No. of tested strains	<i>pks</i> -susceptible strains	
		No.	%
<i>Staphylococcus aureus</i>	54	52	96.3
<i>Staphylococcus aureus</i> strains that are not multiresistant	18	18	100.0
Multiresistant <i>Staphylococcus aureus</i> strains that are:	36	34	94.4
Oxacillin R	32	30	93.8
Glycopeptide I/R	7	7	100.0
Linezolid R	3	2	66.7
Daptomycin R	6	5	83.3
Non- <i>aureus</i> staphylococci	19	0	0.0
<i>Staphylococcus epidermidis</i>	14		
<i>Staphylococcus haemolyticus</i>	1		
<i>Staphylococcus hominis</i>	2		
<i>Staphylococcus lugdunensis</i>	1		
<i>Staphylococcus saprophyticus</i>	1		
Streptococci and enterococci	16	0	0.0
<i>Enterococcus faecium</i>	1		
<i>Enterococcus faecalis</i>	3		
<i>Streptococcus agalactiae</i>	3		
<i>Streptococcus gallolyticus</i>	2		
<i>Streptococcus pneumoniae</i>	4		
<i>Streptococcus pyogenes</i>	3		
Enterobacteria	26	0	0.0
<i>Citrobacter braakii</i>	1		
<i>Citrobacter freundii</i>	1		
<i>Citrobacter koseri</i>	1		
<i>Enterobacter aerogenes</i>	1		
<i>Enterobacter cloacae</i>	2		
<i>Escherichia coli</i>	9		
<i>Hafnia alvei</i>	3		
<i>Klebsiella oxytoca</i>	2		
<i>Klebsiella pneumoniae</i>	2		
<i>Pantoea agglomerans</i>	1		
<i>Proteus mirabilis</i>	1		
<i>Proteus vulgaris</i>	1		
<i>Serratia marcescens</i>	1		
Other bacteria	13	0	0.0
<i>Acinetobacter baumannii</i>	1		
<i>Bacillus simplex</i>	1		
<i>Bacillus subtilis</i>	1		
<i>Clostridium difficile</i>	8		
<i>Pseudomonas aeruginosa</i>	2		
Total	128		

^a R, resistant; I, intermediate.

pellets of *E. coli* DH10 β strains had been spotted. The presence of an inhibition zone around the pellet was assessed after 18 h of incubation at 37°C.

Interestingly, growth inhibition around the *E. coli* DH10 β *pks*⁺ pellet was observed for 96% ($n = 52/54$) of *Staphylococcus aureus* strains but was not observed around *E. coli* DH10 β or *E. coli* DH10 β *pks* Δ *clbP* pellets (Table 1 and Fig. 1A). Inhibition was restricted to *S. aureus* species. To provide further evidence of this antibiotic activity, growth competition experiments were performed using resistant and susceptible *S. aureus* isolates. Unlike *E. coli* DH10 β , *E. coli* DH10 β *pks*⁺ decreased the growth of susceptible *S. aureus* strains (Fig. 1B).

Our results indicate that some of the compounds synthesized from the *pks* island exhibit antibiotic activity against pathogenic *S. aureus* species, including multiresistant isolates (see Tables S1 and S2 in the supplemental material). Interestingly, antibiotic activity was observed for 94% of methicillin-resistant *S. aureus* strains ($n = 30/32$). The growth of all *S. aureus* strains presenting reduced susceptibility to glycopeptides was also inhibited by the *E. coli* DH10 β *pks*⁺ strain ($n = 7/7$). Finally, the growth of strains resistant to “last-resort” antistaphylococcal antibiotics, such as linezolid or daptomycin, was also affected by *E. coli* DH10 β *pks*⁺ ($n = 2/3$ and $5/6$, respectively).

Antibiotic activity conferred by the *pks* island required live

bacteria, since *E. coli* DH10 β *pks*⁺ bacteria treated by chemical (chloroform or antibiotics) or physical (heat or sonication) processes did not inhibit *S. aureus* growth (data not shown). Furthermore, no antistaphylococcal effect was observed with the *E. coli* DH10 β *pks* Δ *clbP* strain, suggesting that the antibiotic compound needs to be matured by ClbP peptidase.

All together, our data suggest that the *pks* island produces antibiotics with enhanced activities against *S. aureus* strains, including multiresistant strains. We cannot exclude the possibility that the purified compound has a wider action spectrum than that discussed here. The challenge now is to purify those compounds in order to identify those responsible for this activity and to characterize more deeply their antistaphylococcal properties.

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