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Resistance to Daptomycin, a Newly Marketed Antibiotic

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The problem of antibiotic resistance in potentially life-threatening pathogens is becoming more serious. In particular, methicillin-resistant *Staphylococcus aureus* (MRSA), the widespread "hospital superbug", and vancomycin-resistant enterococci (VRE) are major causes for concern. Daptomycin is a 13 amino acid cyclic lipopeptide produced by *Streptomyces roseosporus* with calcium-dependent bacteriocidal activity. It is the first commercial cyclic lipopeptide antibiotic, approved by the FDA and marketed in November 2003. Daptomycin is very effective for treatment of skin and soft tissue infections and endocarditis, and in the treatment of VRE and MRSA, but the mechanism of daptomycin action is not clear. The study of daptomycin resistance may provide insights into the mechanism of daptomycin action and prolong the useful lifespan of the drug. I have investigated *Streptomyces coelicolor*, which shows very high resistance to daptomycin, similar to the producer strain *Streptomyces roseosporus* (MIC > 256 μg/ml). A Bac library of *S. coelicolor* DNA was introduced into a daptomycin-sensitive mutant of *Streptomyces ambofaciens* (MIC 2~4 μg/ml) and two non-overlapping Bac clones, clone 1 and clone 2, were identified that confer daptomycin resistance. Subcloning from clone 1 identified a candidate resistance gene, *dapR1,* including 14 integral membrane domains and one kinase domain. From Blast analysis, *dapR1* homologues only occur in actinomycetes. Southern blotting identified homologues in six different *Streptomyces* spp. (*S. avermitilis*, *S. lividans*, *S. roseosporus*, *S. scabies*, *S. venezuelae*, and *S. coelicolor*) and, surprisingly, in a daptomycin-sensitive mutant of *S. ambofaciens*. Real-time quantitative PCR (qPCR) showed that transcription of *dapR1* and its orthologue was induced by daptomycin in both *S. coelicolor* and *S. ambofaciens*.