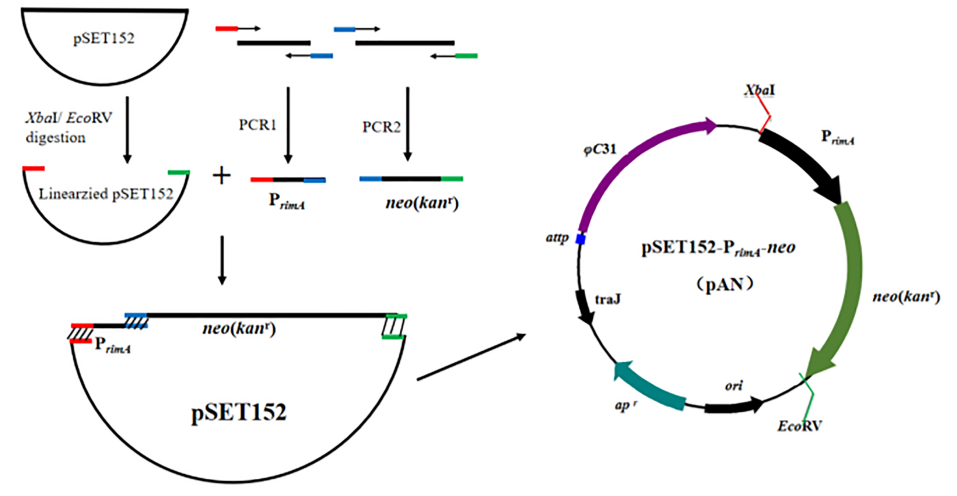
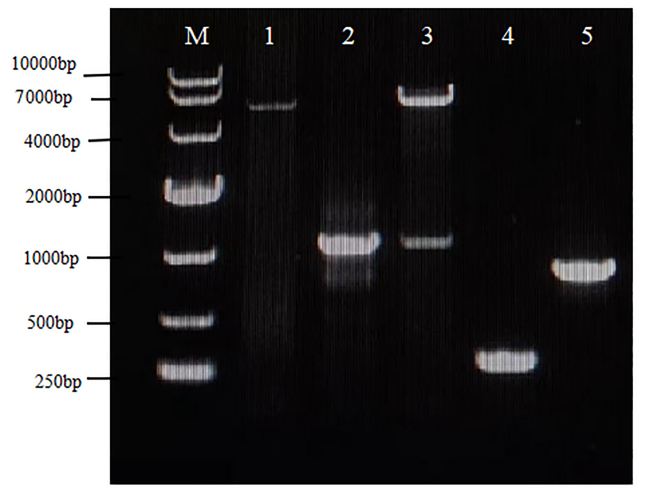
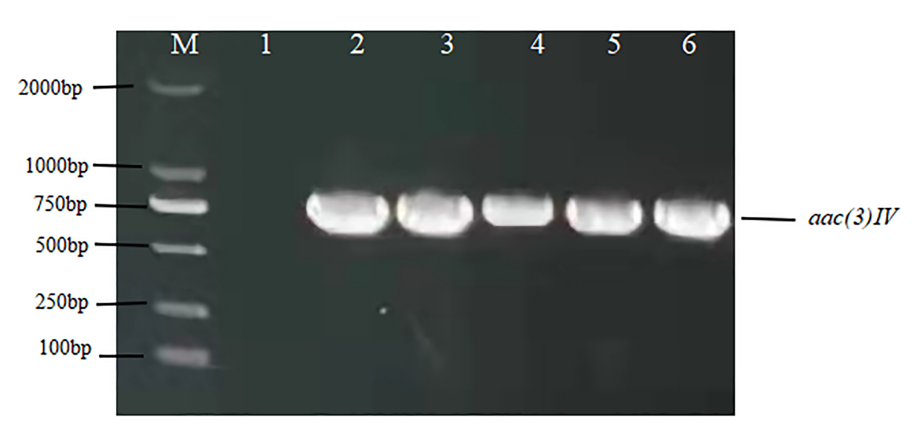
**Supplementary materials**



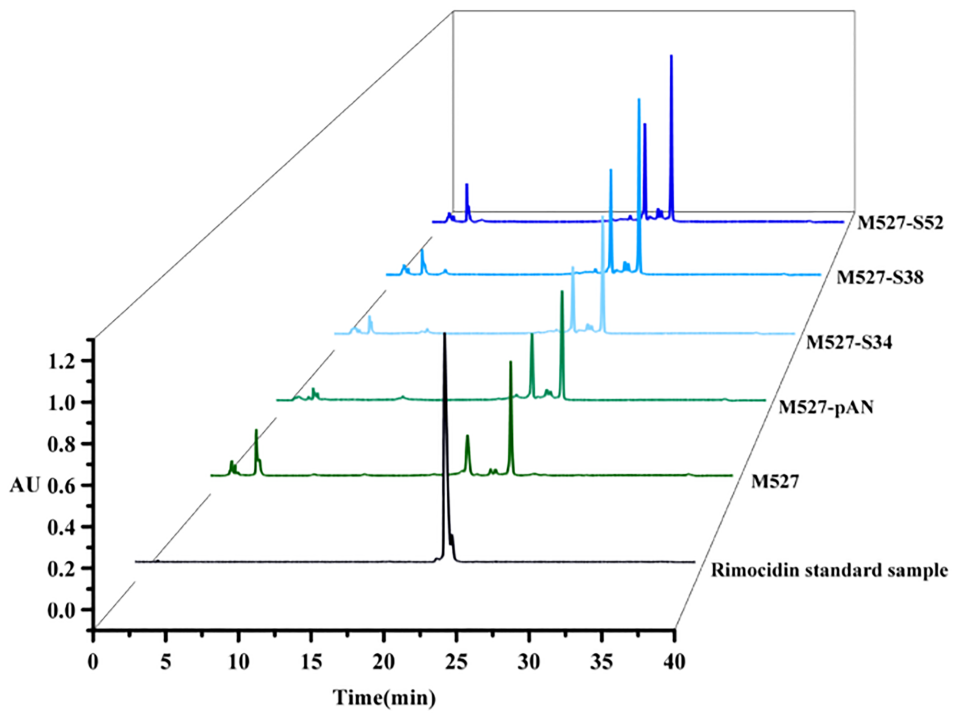
**Figure S1** Construction of single-reporter plasmid pAN.Native promoter of *rimA* gene (P*rimA*) and kanamycin resistance gene (*neo*) were obtained by PCR using corresponding primers. A 255-bpP*rimA* and 816-bp *neo* gene fragment were inserted into the *Xba* Iand *Eco*R V sites of plasmid pSET152 based on DNA seamless cloning technology, yielding reporter plasmid pSET152-P*rimA*- *neo* (pAN). 5' and 3' ends of the two PCR fragments have the homologous sequence as the two ends of the linearized pSET152 or upstream/downstream genes.

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**Figure S2** Identification of single-reporter plasmid pAN constructed in this study. Plasmid pAN was digested, or the genes in the plasmids were amplified by PCR. DNA fragments from the digestion and PCR products were separated on agarose gel. M: DL10000 DNA marker. Lane 1, empty plasmid pSET152 digested with *Xba* I and *Eco*R V; lane 2, PCR product of P*rimA-neo* from plasmid pAN; lane 3, plasmid pAN digested with *Xba* I and *Eco*R V; lane 4, PCR product of 255-bpP*rimA* from plasmid pAN; lane 5, PCR product of 816-bp *neo* fragment from plasmid pAN.



**Figure S3** PCR analysis of apramycin resistance gene *(aac(3)IV*) from initial strain *S. rimosus* M527-pAN. DL DNA 2000 marker was used (M). Lane 1: PCR product of *aac(3)IV* gene from WT strain *S. rimosus* M527; lane 2: PCR product of *aac(3)IV* gene from plasmid pAN; lane 3-5: PCR product of *aac(3)IV* gene from four randomly strains *S. rimosus* M527-pAN.



**Figure S4** HPLC analysis of rimocidin production from fermentation extracts of the wild-type strain *S. rimosus* M527, the initial strain M527-pAN, three mutants M527-pAN-S34, S38, and S52*.*

**Figure S5**

**Figure S5** Lethal rate of *S. rimosus* M527-pAN by ARTP mutagenesis. The lethal rates of *S. rimosus* M527-pAN treated by different times (20, 40 and 60 s). When the samples were treated for 60 s or even longer, no spores were able to survive.