

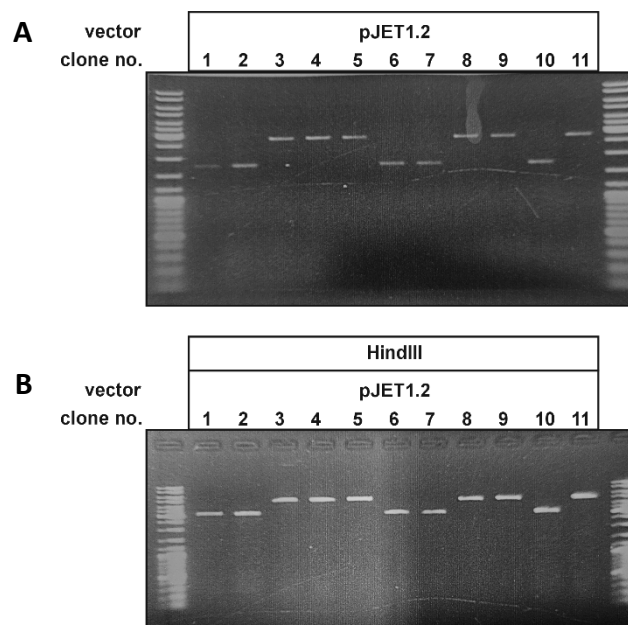
## Cloning of the *sanA* gene

The DNA fragment containing the *sanA* gene was amplified using primers Dnase980F and Dnase98R and genomic DNA of *S. anginosus* 980/01 as the template. The primers were designed in a way to generate KpnI and Sall sites at the ends of the PCR product. The size of the PCR product (2567 bps - the *sanA* ORF, 2211 bps, with its upstream promoter sequence) was verified by agarose electrophoresis and the DNA fragment was purified using an appropriate DNA purification kit.

Cloning of the *SanA* gene was done using two approaches. In the first approach, the gene was cloned in two shuttle vectors (pUSS11 and pUSS16) which can replicate in both Gram-positive and Gram-negative bacteria. One of them, pUSS16 also contains the EGfp gene which facilitates the identification of plasmid-caring cells. Both plasmids carry the spectinomycin-resistance gene which can be used to select plasmid-bearing cells. Before cloning in pUSS11 or pUSS16, the PCR product containing the *sanA* gene was digested using KpnI and Sall endonucleases. The same enzymes were used for the digestion of pUSS11 and pUSS16 vectors. Purified, linear vectors were used for ligation with the *sanA*-containing DNA fragment. The ligation products were used for the transformation of *E. coli* cells. Since one could expect that the transformation of bacterial cells with a plasmid encoding a DNase gene could affect cell viability, transformed cells were incubated not only at 37°C but also at 30°C and 23°C. The highest number of transformant colonies was observed on plates incubated at 23°C. Plasmid DNA was isolated from transformants and analyzed by restriction digestion and electrophoresis. Representative images of agarose gels are shown in **Figure 1**. After digestion with the HindIII enzyme pUSS11 and pUSS16 were linearized with DNA fragment sizes 5532 bps and 6670 bps, respectively. The expected size of DNA fragments generated from plasmids with the cloned fragment was 5977, 1289, 800 bps (vector pUSS11) and 7115, 1289, 800 bps (vector pUSS16). As shown in **Figure 1**, only three plasmids contained the cloned DNA fragment. The three plasmids (number 2, 9, and 15) were subject to DNA sequencing. Unfortunately, several mutations were found within the cloned *sanA* gene (**Table 1**).



Another approach was to use the ClonJet Kit from ThermoFisher, for easy cloning of PCR-amplified DNA fragments. The pJET1.2 vector molecule is designed in a way to be easily ligated with PCR products without restriction endonuclease treatment. The ligation products were used for the transformation of *E. coli* cells. Eleven transformants were obtained and plasmid DNA was isolated from these clones (**Figure 2A**). The presence of the *sanA* gene in isolated plasmids was verified using the HindIII endonuclease. The expected size of DNA fragments obtained after HindIII treatment of plasmids with the *sanA* gene was, depending on the insert orientation 3208, 1533, 800 bps, or 3997, 800, 744 bps. Restriction analysis with HindIII enzyme revealed DNA fragments representing empty pJET1.2 vectors (2974bps) or their dimers (**Figure 2B**) demonstrating that none of the isolated plasmids contained the *sanA* gene.



**Figure 2.** Analysis of plasmid DNA isolated from ampicillin-resistant transformants. *E. coli* cells were transformed with ligation mixtures containing pJET1.2 vector ligated with a PCR product encompassing the *sanA* gene: **A** – plasmids isolated from transformants; **B** – restriction analysis of isolated plasmid performed with the HindIII enzyme.