reporter molecules in cells, while eliminating replication-competent progeny virus. Effective and accurate methods for detecting molecules in cells using the expressed reporter molecules are also needed.

SUMMARY

The present describes methods to detect *Staphylococcus aureus* (*S.aureus*) in a sample. These methods may be used to detect both Methicillin Resistant *S. aureus* (MRSA) and Methicillin Sensitive *S. aureus* (MSSA). The present disclosure also describes compositions comprising non-replicative transduction particles (NRTPs) used to detect *S. aureus* in a sample and methods of producing the NRTPs.

Therefore, in one aspect, the present disclosure refers to a method for detecting *S. aureus* in a sample, the method comprising the steps of: (a) contacting the sample with a lysate comprising a plurality of non-replicative transduction particles (NRTPs) such that the plurality of NRTPs transduces *S. aureus*, if present in the sample, wherein the plurality of NRTPs are produced by (i) inducing a lytic phase of a bacterial cell packaging system, wherein said bacterial cell packaging system comprises: a host bacteria cell; a φ80α bacteriophage genome having a disrupted packaging initiation site sequence existing as a lysogen within the host bacteria cell; a reporter nucleic acid molecule separate from the φ80α bacteriophage genome, having a luxAB reporter gene and a non-disrupted packaging initiation site sequence for facilitating packaging of a replicon of the reporter nucleic acid molecule into the NRTP, wherein the reporter nucleic acid molecule comprises a sequence selected from SEQ ID NO: 3, SEQ ID NO: 4, and SEQ ID NO:5; (ii) allowing the replicon of the reporter nucleic acid molecule to be packaged to produce the NRTPs; (b) providing conditions for expression of the luxAB reporter gene; and (c) detecting the presence or absence of light produced by the luxAB reporter gene, wherein the presence of light indicates the presence of *S. aureus*.

In one example, the method further comprises a step prior to step (b) of providing an antimicrobial agent to the sample and detecting for the presence or absence of light produced by the luxAB reporter gene to determine whether the sample contains *S. aureus* that is resistant or susceptible to the antimicrobial agent. In one example, the reporter nucleic acid molecule comprises the sequence of SEQ ID NO: 3. In another example, the reporter nucleic acid molecule comprises the sequence of SEQ ID NO: 4. In still another example, the reporter nucleic acid molecule comprises the sequence of SEQ ID NO: 5. In one example, the φ80α bacteriophage genome comprises the sequence of SEQ ID NO: 1.

In another aspect, the present disclosure refers to a bacterial cell packaging system for packaging a reporter nucleic acid molecule into a non-replicative transduction particle (NRTP) for introduction into *S. aureus*, comprising a host bacteria cell; a φ80α bacteriophage genome having a disrupted packaging initiation site sequence existing as a lysogen within the host bacteria cell; a reporter nucleic acid molecule separate from the φ80α bacteriophage genome, having a luxAB reporter gene and a non-disrupted packaging initiation site sequence for facilitating packaging of a replicon of the reporter nucleic acid molecule into the NRTP, wherein the reporter nucleic acid molecule comprises a sequence selected from SEQ ID NO: 3, SEQ ID NO: 4, and SEQ ID NO:5. In one example, the reporter nucleic acid molecule comprises the sequence of SEQ ID NO: 3. In another example, the reporter nucleic acid molecule comprises the sequence of SEQ ID NO: 4. In still another example, the reporter nucleic acid molecule comprises the sequence of SEQ ID NO: 5. In one example, the φ80α bacteriophage genome comprises the sequence of SEQ ID NO: 1.

In yet another aspect, the present disclosure refers to a method for producing and collecting a plurality of non-replicative transduction particles (NRTPs) for detecting S. aureus in a sample, comprising: (a) inducing a lytic phase of a bacterial cell packaging system, wherein said bacterial cell packaging system comprises: a host bacteria cell; a φ80α bacteriophage genome having a disrupted packaging initiation site sequence existing as a lysogen within the host bacteria cell; a reporter nucleic acid molecule separate from the φ80α bacteriophage genome, having a luxAB reporter gene and a non-disrupted packaging initiation site sequence for facilitating packaging of a replicon of the reporter nucleic acid molecule into the NRTP, wherein the reporter nucleic acid molecule comprises a sequence selected from SEQ ID NO: 3, SEQ ID NO: 4, and SEQ ID NO:5; (b) allowing the replicon of the reporter nucleic acid molecule to be packaged to produce the NRTPs; and (c) collecting a lysate comprising the plurality of NRTPs. In one example, the reporter nucleic acid molecule comprises the sequence of SEQ ID NO: 3. In another example, the reporter nucleic acid molecule comprises the sequence of SEQ ID NO: 4. In still another example, the reporter nucleic acid molecule comprises the sequence of SEQ ID NO: 5. In one example, the φ80α bacteriophage genome comprises the sequence of SEQ ID NO: 1.

In another aspect, the present disclosure refers to a reporter nucleic acid molecule comprising a sequence selected from SEQ ID NO: 3, SEQ ID NO: 4, and SEQ ID NO:5. In some examples, the reporter nucleic acid molecule is a plasmid or a vector.

CLAIMS

1. A method for detecting *S. aureus* in a sample, the method comprising the steps of:
   (a) contacting the sample with a lysate comprising a plurality of non-replicative transduction particles (NRTPs) such that the plurality of NRTPs transduces *S. aureus*, if present in the sample, wherein the plurality of NRTPs are produced by
      (i) inducing a lytic phase of a bacterial cell packaging system, wherein said bacterial cell packaging system comprises: a host bacteria cell; a φ80α bacteriophage genome having a disrupted terS packaging initiation site sequence existing as a lysogen within the host bacteria cell; a reporter nucleic acid molecule separate from the φ80α bacteriophage genome, having a luxAB reporter gene and a non-disrupted packaging initiation site sequence for facilitating packaging of a replicon of the reporter nucleic acid molecule into the NRTP, wherein the reporter nucleic acid molecule comprises a sequence selected from SEQ ID NO: 3, SEQ ID NO: 4, and SEQ ID NO:5;
      (ii) allowing the replicon of the reporter nucleic acid molecule to be packaged to produce the NRTPs;
   (b) providing conditions for expression of the luxAB reporter gene; and
   (c) detecting the presence or absence of light produced by the luxAB reporter gene, wherein the presence of light indicates the presence of *S. aureus*.

2. The method of claim 1 further comprising a step prior to step (b) of providing an antimicrobial agent to the sample and detecting for the presence or absence of light produced by the luxAB reporter gene to determine whether the sample contains *S. aureus* that is resistant or susceptible to the antimicrobial agent.

3. The method of any one of claims 1 or 2, wherein the reporter nucleic acid molecule comprises the sequence of SEQ ID NO: 3.

4. The method of any one of claims 1 or 2, wherein the reporter nucleic acid molecule comprises the sequence of SEQ ID NO: 4.

5. The method of any one of claims 1 or 2, wherein the reporter nucleic acid molecule comprises the sequence of SEQ ID NO: 5.

6. The method of any one of claims 1 to 5, wherein the φ80α bacteriophage genome having a disrupted terS packaging initiation site sequence comprises the sequence of SEQ ID NO: 1.

7. A bacterial cell packaging system for packaging a reporter nucleic acid molecule into a non-replicative transduction particle (NRTP) for introduction into *S. aureus*, comprising:

(a) a host bacteria cell;

(b) a φ80α bacteriophage genome having a disrupted packaging initiation site sequence existing as a lysogen within the host bacteria cell; and

(c) a reporter nucleic acid molecule separate from the φ80α bacteriophage genome, having a luxAB reporter gene and a non-disrupted packaging initiation site sequence for facilitating packaging of a replicon of the reporter nucleic acid molecule into the NRTP, wherein the reporter nucleic acid molecule comprises a sequence selected from SEQ ID NO: 3, SEQ ID NO: 4, and SEQ ID NO:5.

8. The cell packaging system of claim 7 wherein the reporter nucleic acid molecule comprises the sequence of SEQ ID NO: 3.

9. The cell packaging system of claim 7 wherein the reporter nucleic acid molecule comprises the sequence of SEQ ID NO: 4.

10. The cell packaging system of claim 7 wherein the reporter nucleic acid molecule comprises the sequence of SEQ ID NO: 5.

11. The cell packaging system of any one of claims 7 to 10, wherein the φ80α bacteriophage genome having a disrupted terS packaging initiation site sequence comprises the sequence of SEQ ID NO: 1.

12. A method for producing and collecting a plurality of non-replicative transduction particles (NRTPs) for detecting *S. aureus* in a sample, comprising:

(a) inducing a lytic phase of a bacterial cell packaging system, wherein said bacterial cell packaging system comprises:

(i) a host bacteria cell;

(ii) a φ80α bacteriophage genome having a disrupted packaging initiation site sequence existing as a lysogen within the host bacteria cell;

(iii) a reporter nucleic acid molecule separate from the φ80α bacteriophage genome, having a luxAB reporter gene and a non-disrupted packaging initiation site sequence for facilitating packaging of a replicon of the reporter nucleic acid molecule into the NRTP, wherein the reporter nucleic acid molecule comprises a sequence selected from SEQ ID NO: 3, SEQ ID NO: 4,

and SEQ ID NO:5;

(b) allowing the replicon of the reporter nucleic acid molecule to be packaged to produce the NRTPs; and

(c) collecting a lysate comprising the plurality of NRTPs.

13. The method of claim 12, wherein the reporter nucleic acid molecule comprises the sequence of SEQ ID NO: 3.

14. The method of claim 12, wherein the reporter nucleic acid molecule comprises the sequence of SEQ ID NO: 4.

15. The method of claim 12, wherein the reporter nucleic acid molecule comprises the sequence of SEQ ID NO: 5.

16. The method of any one of claims 12 to 15, wherein the φ80α bacteriophage genome having a disrupted terS packaging initiation site sequence comprises the sequence of SEQ ID NO: 1.

17. A reporter nucleic acid molecule comprising a sequence selected from SEQ ID NO: 3, SEQ ID NO: 4, and SEQ ID NO:5.