[0006] As such, assays that generally require a minimum amount of growth or a minimum growth rate may not detect target cells that do not exhibit the required growth characteristics during the assay.

[0007] Related patent applications include: PCT/US2014/026536, filed on March 13, 2014, which is hereby incorporated by reference, in its entirety, for all purposes.

SUMMARY

[0008] Disclosed herein is a cellular detection method that operates independent of cell growth. While existing cellular detection methodologies benefit from cell growth, the methods disclosed herein demonstrate embodiments that are independent of cell growth.

[0009] The methods disclosed herein are generally independent of growth – which can be an important feature for detecting cells at a metabolic state that does not support adequate growth (e.g., cells encountered in clinical samples) and for strains of cells with lower than expected growth rates.

[0010] Disclosed herein are various methods, systems, and compositions for the growth independent detection of cells.

[0010a] In one aspect, the present disclosure refers to a growth-independent method for detecting a microorganism of interest in a sample, comprising: -contacting the sample with a plurality of non-replicative transduction particles (NRTPs), wherein the plurality of NRTPs are produced using a disruption/complementation method, wherein said methods do not produce NRTPs that contain viral genome, such that the plurality of NRTPs specifically transduces one or more viable microorganisms of interest in the sample, wherein the plurality of NRTPs are bacteriophages capable of delivering a non-viral nucleic acid molecule into the one or more viable microorganisms of interest without containing replicated viral genome in the transduction particle, said non-viral nucleic acid molecule comprising a reporter nucleic acid sequence including a reporter gene operatively linked to a promoter, and wherein the growth rate of the one or more viable microorganisms of interest is less than logarithmic phase; -providing conditions for activation of the reporter nucleic acid sequence and expression of a reporter molecule from said reporter gene; and -detecting a signal produced by the reporter molecule, wherein the presence of the signal indicates the presence of the one or more viable microorganisms of interest, and wherein the absence of the signal indicates the absence of the one or more viable microorganisms of interest. In one example, there is disclosed a growth-independent method for detecting a microorganism of interest in a sample, comprising: -contacting the sample with a plurality of non-replicative transduction particles (NRTPs) such that the plurality of NRTPs transduces one or more microorganisms of interest in the sample, wherein the plurality of NRTPs comprises a reporter nucleic acid sequence, and wherein the growth rate of the one or more microorganisms of interest is less than logarithmic phase;- providing conditions for activation of the reporter nucleic acid sequence; and - detecting a signal produced by the reporter nucleic acid sequence, wherein the presence of the signal indicates the presence of the one or more

microorganisms of interest, and wherein the absence of the signal indicates the absence of the one or more microorganisms of interest.

[0010b] In another example, the present disclosure refers to a sample or cell culture comprising a plurality of non-replicative transduction particles (NRTPs) produced using a disruption/complementation method, said NRTPs thereby not containing viral genome, and one or more viable microorganisms of interest, wherein the plurality of NRTPs are bacteriophages capable of delivering a non-viral nucleic acid molecule into the one or more viable microorganisms of interest without containing replicated viral genome in the transduction particle, said non-viral nucleic acid molecule comprising a reporter nucleic acid sequence including a reporter gene operatively linked to a promoter, and wherein the growth rate of the one or more viable microorganisms of interest is less than logarithmic phase. In one example, there is disclosed a sample or cell culture comprising a plurality of non-replicative transduction particles (NRTPs) and one or more microorganisms of interest, optionally wherein the plurality of NRTPs comprises a reporter nucleic acid sequence, and wherein the growth rate of the one or more microorganisms of interest is less than logarithmic phase.

[0011] For example, a method disclosed herein can include a growth-independent method for detecting a microorganism of interest in a sample, comprising: contacting the sample with a plurality of non-replicative transduction particles (NRTPs) such that the plurality of NRTPs transduces one or more microorganisms of interest in the sample, wherein the plurality of NRTPs comprises a reporter nucleic acid sequence, and wherein the growth rate of the one or more microorganisms of interest is less than logarithmic phase; providing conditions for activation of the reporter nucleic acid sequence; and detecting a signal produced by the reporter nucleic acid sequence, wherein the presence of the signal indicates the presence of the one or more microorganisms of interest, and wherein the absence of the signal indicates the absence of the one or more microorganisms of interest.

[0012] As a further example, a composition disclosed herein can include a sample or a cell culture comprising a plurality of non-replicative transduction particles (NRTPs) and one or more microorganisms of interest, optionally wherein the plurality of NRTPs comprises a reporter nucleic acid sequence, and wherein the growth rate of the one or more microorganisms of interest is less than logarithmic phase.

BRIEF DESCRIPTION OF THE SEVERAL VIEWS OF THE DRAWINGS

[0013] These and other features, aspects, and advantages of the present invention will become better understood with regard to the following description, and accompanying drawings, where:

CLAIMS

1. A growth-independent method for detecting a microorganism of interest in a sample, comprising:
    - contacting the sample with a plurality of non-replicative transduction particles (NRTPs), wherein the plurality of NRTPs are produced using a disruption/complementation method, wherein said methods do not produce NRTPs that contain viral genome, such that the plurality of NRTPs specifically transduces one or more viable microorganisms of interest in the sample, wherein the plurality of NRTPs are bacteriophages capable of delivering a non-viral nucleic acid molecule into the one or more viable microorganisms of interest without containing replicated viral genome in the transduction particle, said non-viral nucleic acid molecule comprising a reporter nucleic acid sequence including a reporter gene operatively linked to a promoter, and wherein the growth rate of the one or more viable microorganisms of interest is less than logarithmic phase;
    - providing conditions for activation of the reporter nucleic acid sequence and expression of a reporter molecule from said reporter gene; and
    - detecting a signal produced by the reporter molecule, wherein the presence of the signal indicates the presence of the one or more viable microorganisms of interest, and wherein the absence of the signal indicates the absence of the one or more viable microorganisms of interest.
2. The method of claim 1, wherein the growth rate of the one or more viable microorganisms of interest is less than 0.1 divisions per hour, and wherein the one or more viable microorganisms of interest is Methicillin Resistant *Staphylococcus aureus* (MRSA).
3. The method of any one claims 1 or 2, further comprising providing an antimicrobial agent to the sample and detecting for the signal produced by the reporter molecule to determine whether the one or more viable microorganisms of interest is susceptible or non-susceptible to the antimicrobial agent.
4. The method of any one of claims 1 to 3, wherein the one or more viable microorganism of interest is in stationary phase; or wherein the one or more viable microorganism of interest is undergoing no growth.
5. The method of any one of claims 1 to 4, wherein the growth rate of the one or more viable microorganisms of interest is negative or homeostatic.

6. The method of any one of claims 1 to 5, wherein the one or more viable microorganism of interest comprises a Methicillin Resistant *Staphylococcus aureus* (MRSA) cell, *Staphylococcus aureus*, *Staphylococcus spp.*, Enterobacteriaceae, *Enterococcus spp. Streptococcus spp.*, *Acinetobacter spp.*, *Pseudomonas spp.*, *Stenotrophomonas spp.*, or *Mycobacterium spp.*.
7. The method of any one of claims 1 to 6, wherein the sample is a clinical sample.
8. The method of any one of claims 1 to 7, wherein the providing step comprises contacting the sample with a fatty aldehyde bacterial luciferase substrate reagent, optionally wherein the reagent is tridecanal.
9. The method of claim 3, wherein the antimicrobial agent is cefoxitin, a β-lactam, an extended-spectrum β-lactam, an Aminoglycoside, an Ansamycin, a Carbacephem, Carbapenems, any generation of Cephalosporin, a Glycopeptide, a Lincosamide, a Lipopeptide, a Macrolide, a Monobactam, a Nitrofuran, an Oxazolidonone, a Penicillin, a Polypeptide, a Quinolone, a Fluoroquinolone, a Streptogramin, a Sulfonamide, a Tetracycline, a Rifampicin, a mycobacterial antibiotic, Chloramphenicol, and/or Mupirocin.
10. The method of any one of claims 1 to 9, wherein the reporter gene is a luciferase gene, optionally wherein the luciferase gene is *luxAB*, *luc*, *ruc*, or *nluc*.
11. The method of any one of claims 1 to 10, wherein the reporter gene is operatively linked to a constitutive promoter, optionally wherein the constitutive promoter is a *S. aureus clpB* promoter.
12. The method of any one of claims 1 to 11, wherein the reporter gene is selected from the group consisting of genes encoding enzymes mediating luminescence reactions (luxA, luxB, luxAB, luc, ruc, nluc), genes encoding enzymes mediating colorimetric reactions (lacZ, HRP), genes encoding fluorescent proteins (GFP, eGFP, YFP, RFP, CFP, BFP, mCherry, near-infrared fluorescent proteins), nucleic acid molecules encoding affinity peptides (His-tag, 3X-FLAG), and genes encoding selectable markers (ampC, tet(M), CAT, erm).
13. The method of any one of claims 1 to 12, wherein the reporter gene is operatively linked to a constitutive promoter.
14. The method of any one of claims 1 to 13, further comprising providing an antibiotic to the sample at a pre-determined concentration and detecting a presence or absence of the signal to determine whether the one or more viable microorganisms of interest is resistant or sensitive to the antibiotic.

15. The method of any one of claims 1 to 14, further comprising providing varying pre-determined concentrations of antibiotic to the sample and detecting the amount of the signal to determine the minimum inhibitory concentration of the one or more viable microorganisms of interest to the antibiotic.