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PRODAIRY Education & Applied Research

ANTIMICROBIAL RESISTANCE AND DAIRY MANURE SYSTEMS

2.B. Antibiotic resistant bacteria (ARB) - Measuring ARB in dairy manure

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Antibiotic resistant bacteria (ARB)

ARB are bacteria that have the ability to resist the effects of an antibiotic. They are naturally occurring, and can be found in dairy manure (See Fact Sheet 2.A.). Phenotypic [i.e. by growth patterns] or genotypic [i.e. by presence and/or expression of antibiotic resistance genes (ARGs)] determinations are used to verify ARB.

Phenotyping

There are several strategies to phenotypically verify that a bacterium is susceptibility to antibiotics. The disk diffusion test and broth micro-dilution test are the most common.

Disk diffusion test (Figure 1)[1]

A diluted culture (typ. 10^8 colony forming units per ml) of the bacterium of interest is evenly inoculated onto an agar plate suitable for its growth (typ. Mueller-Hinton agar). Commercially prepared wafers containing different antibiotics, or antibiotics of different concentrations, are then placed onto the agar. After a 12-16 h incubation (typ. at 37°C for pathogens) the growth on the plate is inspected. If the test bacterium is susceptible to the tested antibiotic, a clear zone of no growth will be observed around the particular wafer. This "zone of inhibition" measured in mm is compared to a standard interpretation chart used to categorize the isolate as susceptible, intermediately susceptible or resistant.

This test is *qualitative*, with the zone of inhibition depending on many factors, including the diffusion of the antibiotic within the agar medium, in addition to the effectiveness of the antibiotic at stopping the growth of the bacterium.

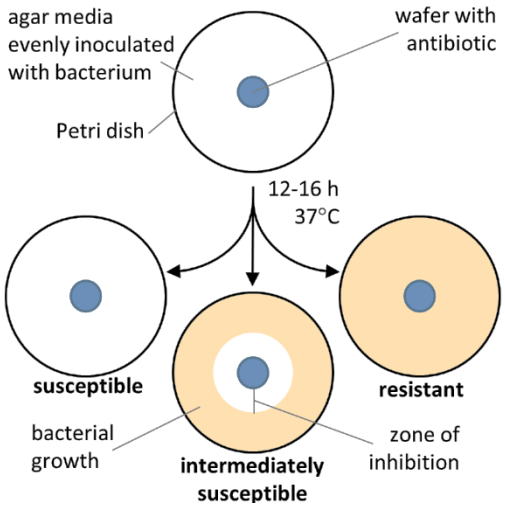

FIGURE 1. DIAGRAM OF THE DISK DIFFUSION TEST.

Broth micro-dilution test (Figure 2)[1]

Multi-well plates are filled with a broth media containing varying concentrations of antibiotics or no antibiotics (+ control). The bacterium of interest is add to each well and incubated for 12-16 h at 37°C. A spectrophotometer, which measures transmitted light, is used to check for visible growth in each well. Wells with growth appear cloudy and reduce light transmission. The test is *quantitative* and results are reported as a minimum inhibitory concentration (MIC).

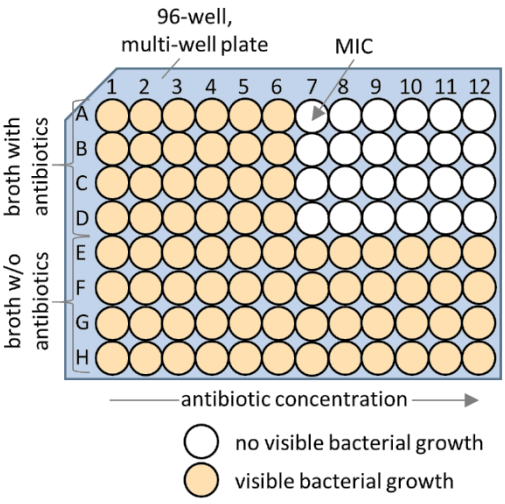

FIGURE 2. BROTH MICRO-DILUTION TEST.

Minimum Inhibitory Concentration (MIC)[2]

A MIC is the lowest concentration of an antimicrobial that will inhibit the visible, overnight growth of bacteria. MICs are not standardized, per se, but are based on suggested ranges of concentrations for combinations of different antibiotics and bacteria. There are also reference strains that can be used as positive controls for MIC testing.

Genotyping

Studies have revealed that specific genes across diverse functional categories of bacteria are associated with resistance to particular antibiotics. These ARGs (see Fact sheet 3.A.) can be used as a measure of a bacterium's ability to resist an antibiotic. There are many molecular approaches to determine the presence of an ARG in a bacterium. Quantitative polymerase chain reaction (qPCR), sometimes called real-time PCR, and genome sequencing are the most common.

Quantitative PCR[1]

Polymerase chain reaction is a technique used to generate thousands or millions of copies of a particular segment of DNA from just a single or few copies. Primers serve as a starting point for DNA replication, and can be used with PCR to targeted replicate particular genes or ARGs. In qPCR, florescent tags are used to label the targeted genes as they are replicated. This florescence can then be very accurately measured enabling the quantification of target genes. Small volume qPCR reactions targeting different ARGs can prepared commercially in multi-well plates. Sometimes referred to as a microarrays or biochips, this approach allows for the rapid screening of multiple genes from a single bacterium simultaneously.

Genome sequencing[1]

Genome sequencing is an approach where the entire set of DNA in a bacterium or cell is mapped. DNA is made up of four chemical compounds called bases. These are adenine (A), thymine (T), guanine (G), and cytosine (C). The specific four letter language that these bases make up, constitutes the specific genes in a cell. By replicated this 4 letter language and reading it a genome is sequenced.

There are many different ways to sequence a genome. Most fragment extracted DNA into manageable lengths. Then, DNA fragments are replicated using synthetic bases containing uniquely colored fluorescent tags so they can be read. Sophisticated algorithms are then used to align these fragment reads and recreate the sequence (Figure 3). The recreated genome can then be compared to databases so that genes (such as ARGs) can be identified.

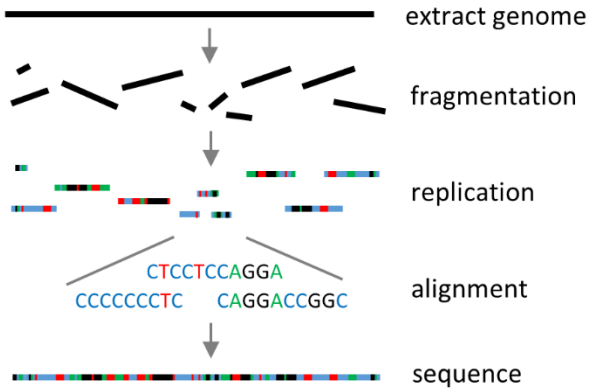

FIGURE 3. GENERALIZED SEQUENCING PROCEDURE.

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References

[1] Maria-Neto et al. 2015. *Biochimica et Biophysica Acta* **1848**, 3078–3088 [2] Andrews. 2001. *Journal of Antimicrobial Chemotherapy* **48**, 5-16.

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