

Identification of Bacteria. II.
Nitrate Reduction on Tryptic Nitrate Medium.

Introduction

Another of the various diagnostic tests one may perform to identify bacteria is the determination of bacterial nitrate (NO_3^-) reduction on tryptic nitrate medium (TNM). This medium contains a small amount of KNO_3 (potassium nitrate). Bacteria which reduce nitrate reduce it to either nitrite (NO_2^-) or to gaseous nitrogen (N_2). The determination of nitrate reduction on the TNM is used for the determination of the reduction of nitrate to nitrite.

The basic premise of this test is that the N,N-dimethyl- α -naphthylamine reacts with nitrite imparting a red-violet color. The sulfanilic acid heightens the color response. If there is no color change upon addition of the two reagents, this does not necessarily mean that this is a true negative reaction. One must then add a pinch of zinc (Zn) dust to the reagents on the colonies. If this turns red-violet, then that means that nitrate is still in the medium and was NOT reduced. This latter test is due to the ability of the Zn dust to reduce the nitrate to nitrite. Some examples of gram negative bacteria and their Nitrate Reduction on Tryptic Nitrate medium reaction are shown in Table 1.

Genus	Tryptic Nitrate Reduction Reaction
<i>Pasturella</i>	+ (red-violet without Zn)
<i>Pseudomonas</i>	+/-
<i>Escherichia</i>	- (red-violet with Zn dust)
<i>Campylobacter</i>	Mostly +
<i>Helicobacter pylori</i>	-
<i>Mycobacterium tuberculosis</i>	+
<i>MAC complex</i>	-
<i>Serratia</i>	+
<i>Bacillus</i> *	-

Table 1. Tryptic Nitrate Reduction test reactions of several enteric bacteria.

* In Table 1, as well as in the tables in the previous experiments, Bacillus (Gram positive rod) is included for variety.

The purpose of this experiment is to learn how to perform and interpret the Reduction of Nitrate for various bacteria on solid TNM.

Materials and Methods

Materials

TNM	Sulfanilic acid reagent	N,N-dimethyl- α -naphthylamine reagent
Zn dust	Bacteria	Bunsen burner
Disposable pipets	Loop	Incubator
Paper towels	Disinfectant	

Method

Aseptically streak your TNM with one of the bacteria (per your professor's directions). Incubate for 48 hours. Finish this experiment at your next lab period.

Add 1 drop of each reagent directly to the colony under study on the medium. If the colony turns red-violet, this is a positive test. If the colonies do not turn red-violet, add a pinch of Zn dust. If the resulting mixture turns red-violet, this is conclusive for a negative test for nitrate reduction on TNM. How do these results compare with the table above?

Draw and label your observations for your bacterium below:

REFERENCES

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