IDENTIFICATION OF UNKNOWN BACTERIAL SPECIES: GENERAL ASPECTS

I. OBJECTIVES

• To demonstrate a capacity to utilize previous laboratory experiences to accurately interpret tests conducted to identify a certain organism.

• To know specifically which diagnostic tests are required to identify a bacterial species.

• To demonstrate skill in coordinating the usual laboratory work with that of identifying an unknown organism.

II. INTRODUCTION

In the majority of cases, when we want to embark on the process of identifying an organism, we find that the organism of interest is usually mixed with many other organisms. For example, to identify the pathogen that is causing a disease in a patient, we find that there are many different species of bacteria present in the stool or tissue sample that we have obtained from the patient. So, if we know that the organism is not in pure form, our first step would be to separate it from all the others. This is accomplished in two stages.

The first stage, which is called primary isolation, is to select the appropriate media, time, and temperature of incubation to ensure that the organism of interest has a good chance of being isolated. Knowledge of the specimen source and a microscopic description of the mixture are essential in choosing the proper media and growth conditions. For example, if a stool specimen is being examined for a possible pathogen, a MacConkey plate must be included for use in the primary isolation. Since pathogens in stool samples are primarily Gram negative rods, the MacConkey would increase the likelihood of their isolation because it selects for this group of organisms.

The second stage is the secondary isolation where the subculturing of each colony type from the primary isolation onto non-inhibitory media is accomplished. Sometimes multiple subculturings are necessary to obtain a pure secondary culture. Upon isolation of a pure culture, each organism is described in detail according to cellular and colonial morphologies, optimum growth characteristics, and Gram stain reactions.

Growth on a plate can distort the characteristic three-dimensional cell arrangement, whereas a broth allows the direction of a cell division to occur naturally in different planes. Thus, cellular descriptions of size, shape, and arrangement must be made from a broth culture. Also, an accurate measure of cell length and width is very important because it may be the only definitive characteristic available to distinguish one bacterium from another. Thus to have a basic framework to start from, we need information on shape (rod, coccus, spirillum) and Gram stain reaction (positive, negative) of the species of interest.
In our lab, identification of unknown bacteria is divided into 3 projects; EU, OU and PCR-ID projects. For EU (identification of a bacterial species belonging to the Enterobacteriaceae family) and OU (identification of a non-Enterobacteriaceae bacterial species), we use the conventional microbiological diagnostic techniques to identify the species. For the PCR-ID project, we will be using modern molecular biology techniques whereby a specific part of the genomic DNA is amplified and its sequence of bases is determined and compared to those of different bacterial species to find a match.

You will be dealing with each one of these unknown projects in their proper time and place but for each one, we require that you write a paper on the subject. It is possible that your instructor may decide that you perform the PCR-ID on the same sample that you obtained as your OU or EU, in which case only two reports will suffice.

Some General Rules and Regulations

- All tests and work on your unknown species should be performed during the regular lab periods.
- All your tubes and plates must be clearly marked with your initials, section number, date, and unknown number.
- Diagnostic test materials must be discarded after results are read and recorded.
- Your lab instructor and TA are not to assist you in plotting the course of your work or confirming your conclusions. These are your independent projects, so they should be done without any help from others.
- The reports that are due at the end of the semester on your EU, OU and/or PCR-ID are analogous to scientific papers that should include Introduction, Materials and Methods, Results, Discussion, Summary and References. Refer to each specific project to learn more about the way you are expected to present your report.

Use of any section of this Lab Manual without the written consent of Dr. Eby Bassiri, Dept. of Biology, University of Pennsylvania is strictly prohibited.