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"Tracking" a Strain Using the Sherlock Microbial Identification System (MIS)

INTRODUCTION

Clinical laboratories may use phage typing or plasmid/DNA electrophoretic typing of strains in epidemiological studies. In the QC operations of the pharmaceutical and food industries, the same type of strain "tracking" is extremely useful in determining the source of product-contaminating microbes. Criteria essential for efficiently tracing the origins of strains are the same in both situations: speed, precision, cost effectiveness, and ease of use. These criteria are met by Sherlock's multivariate clustering algorithm.

Gas chromatography of fatty acid methyl esters (FAME) using the Sherlock MIS has been shown to be an effective tool for identification of microbes important in medical research and industrial applications and closely parallels ribosomal RNA and DNA homologies. At the core of Sherlock are the databases of FAME profiles of yeast and aerobic and anaerobic bacteria. The Sherlock Library Generation Software (LGS) package extends the standard capabilities of Sherlock. LGS enables users to create a custom database of their unique or proprietary organisms. The LGS package also contains two cluster analysis packages that provide strain "tracking" abilities. The Dendrogram and 2-D Plot programs use data from fatty acid analyses of organisms sampled from the environment in question and graphically illustrate relationships between the organisms. To take advantage of this "tracking" feature, all samples must be grown under the same environmental conditions, on the same medium and to comparable physiological ages. Extraction and analysis are then performed using the standard Sherlock protocol.

STRAIN "TRACKING" VIA CLUSTER ANALYSIS

Cluster analysis uses mathematical techniques to display similarities among objects in a set. Using MIDI's cluster analysis tools, Dendrogram and 2-D Plot, samples with common fatty acid compositions can be identified and grouped. Based on the defined groups, a sample can be identified as a member of a strain if the sample is included in a cluster.

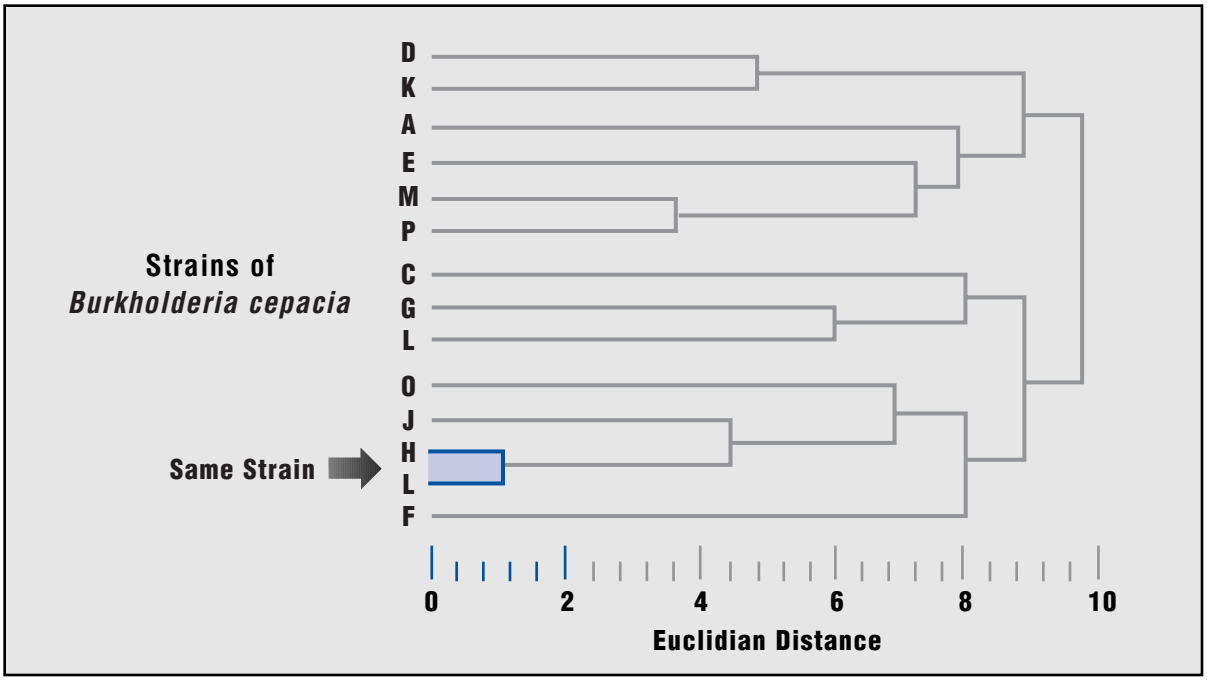

Figure 1: *Dendrogram*

DENDROGRAM

Dendrogram uses cluster analysis techniques to produce unweighted pair matching based on fatty acid compositions. The results are displayed graphically to depict the relatedness between organisms. Experience with this program has shown that multiple analyses of the same strain over a period of time results in linkages among samples of that strain at a level of 2 Euclidian distances or lower. As the term is used in the Sherlock programs, Euclidian Distance is the distance in n-dimensional space between two strains when their fatty acid compositions are compared. The "unweighed pair-grouping" method used in the Sherlock software is most effective at comparisons of isolates at the strain and subspecies levels.

Figure 1 shows a dendrogram from fatty acid compositions of various strains of *Burkholderia cepacia* isolated from a pharmaceutical product and from the manufacturing environment. Strain H was a contaminant isolated from the product, and strain L was isolated from the deionized water supply. The other strains were isolated from other raw materials. The linkage of strains H and L at less than 2 indicates that these two strains are probably identical and strain L is the likely source of contamination.

Thus, in an industrial QC situation, a contaminant in a product may be found to link closely to a strain coming from a water supply, but not to link closely with strains of the same species from other sources (e.g. raw material, surfaces in the filling room, etc.). Likewise, in clinical settings, this technique may be useful in "tracking" nosocomial infections because of its speed, minimal labor input, and power. Confirmation may be obtained using other typing techniques. Although the Euclidian distance scale permits quick determination of the relatedness of entries at the species and subspecies levels (approximately 10 and 6 Euclidian distances respectively), there is a strong dependence upon the current state of taxonomy in the taxa analyzed.

DENDROGRAM OPERATION

From the Sherlock software "CommandCenter" select the Analysis icon from the task bar. Under the "Selector Tab" choose the Calculation Method. Under the same tab, choose the samples to be analyzed. Finally select the Dendrogram Tab to view a copy of the generated graph. The Zoom In and Zoom Out buttons permit magnification of the Dendrogram to the desired level. While viewing the Dendrogram, samples may be selected and deselected to instantaneously see their impact on the graph.

2-D PLOT

In addition to Dendrogram, 2-D Plot is another cluster analysis tool that can be used to track a strain. In its normal operating mode, 2-D Plot uses a principal components analysis of FAME profiles to group entries in a two-dimensional space. The x-axis represents principal component 1, and the y-axis represents principal component 2. (These may be changed to plot 1 vs. 3, or 2 vs. 3 to gain additional perspective.)

The 2-D Plot is most useful for finding relationships among large numbers of organisms or for visualizing the relationships of distantly related organisms. Thus, large numbers of organisms can be analyzed, the data called into the 2-D Plot, and the resulting diagram(s) used to define groups of closely related organisms, even when the organisms are not members of species currently nameable by Sherlock.

Figure 2 is an example of a group of entries in which *Pseudomonas aeruginosa* and *Pseudomonas* fluorescens were distinguished using the 2-D plot clustering program.

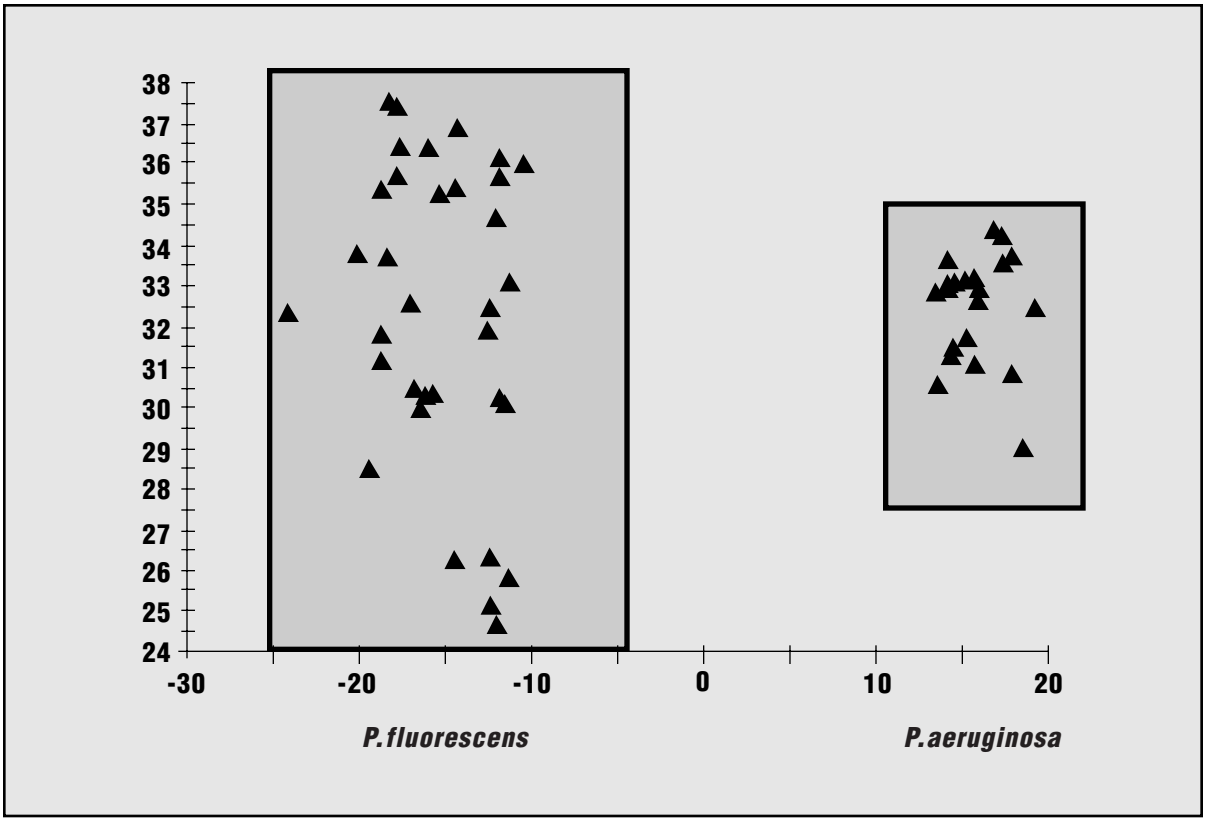

Figure 2: *2-D Plot*

2-D PLOT OPERATION

This program is also called from "CommandCenter" while in the Analysis view. Under the "Selector Tab" choose the Calculation Method. Under the same tab, choose the samples to be analyzed. Finally select the 2-D Plot tab to view a copy of the generated graph. While viewing the 2-D Plot, the axis can be altered to view the relationship between different principal components. Like Dendrogram, samples may be selected and deselected to view their impact on the 2-D Plot.

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