Comprehensive Microbial Identification with Odin

Introduction

Microbial identification has been the cornerstone of microbiology from the beginning of the field. Now, with a dramatic increase in the availability of DNA sequencing and other methods such as MALDI-TOF, researchers can reliably identify microbes like never before. While modern methods like DNA sequencing have become widely used, identification is often constrained by the availability of assembled reference genomes and the ability to extract sufficient quantities of high-quality genomic DNA. Phenotypic identification methods are still therefore widely used, due in no small part to their comparative ease of use and interpretation relative to genomic or proteomic methods, as well as the complementarity of the data they provide.

The Odin™ platform (Figure 1) gives researchers the ability to identify a wide array of prokaryotic and eukaryotic microbes by generating a phenotypic profile which serves as a unique metabolic fingerprint. Each identification panel consists of a single 96-well microplate containing a set of metabolic and chemical sensitivity assays specifically selected for identification of gram-negative and gram-positive bacteria (GEN III), yeast (YT), filamentous fungi (FF), and anaerobes (AN). With the addition of a redox reporter dye to each well, a microbe grown on one of these plates will have a unique pattern of color development that can be used to match it against Biolog's database of over 2,900 unique identification profiles to identify the unknown anaerobic or aerobic bacteria, yeast, or filamentous fungi. Organisms included in the database were selected based on their prevalence and utility in environmental monitoring, industrial applications, and microbiome studies.

Identification of an unknown organism with the GEN III microplate is straightforward. A single suspension of cells in a pre-portioned tube of Inoculating Fluid (IF) is all that is required to add to the GEN III plate prior to incubation. Once inoculated, the plate is then incubated in Odin

Figure 1. Odin is the all-in-one platform for cellular characterization. Up to 50 plates can be incubated and read in one experiment. Odin is ideal for identifying unknown microbes, monitoring growth curves, and measuring cell respiration kinetics.

and measured every 20 minutes until an ID is automatically called, which can be as fast as 2-3 hours depending on the strain. YT, FF, and AN microplates utilize the same dye reduction based, single plate method with simple sample preparation. For slower growing organisms, Odin will continue incubating and reading plates for up to 72 hours.

This application note shows that Odin, coupled with GEN III, YT, FF, and AN test panels, is able to rapidly, easily, and reproducibly identify a variety of microbes tested including eight diverse gram-positive and gram-negative bacteria, four yeast species, three filamentous fungi, and four anaerobes respectively.

Methods

Identification with GEN III Microplates

The Biolog GEN III Microplate characterizes each microorganism using 94 phenotypic tests, including 71 carbon source utilization assays and 23 chemical sensitivity assays to assign an identity. All necessary nutrients, biochemicals, and a tetrazolium-based dye are dried to the bottom of each well of the GEN III microplate. Each bacterial strain was cultured on BUG+B (Biolog Universal Growth medium plus Blood) at 36 °C for 24 hours prior to GEN III plate inoculation after which a cell suspension was made according to Biolog GEN III ID Protocol A. In brief, cells from an approximately 3 mm diameter colony area were transferred to inoculating fluid A (IF-A) using a Biolog InoculatorZ™ swab to a cell density of 95% transmittance (T) as measured by a Biolog turbidimeter. The resulting cell suspension was transferred to the GEN III microplate at 100 µL per well. GEN III plates were placed in Odin for incubation at 33 °C and read every 20 minutes for the duration of the experiment. After each read, the plate profile was compared to the Biolog GEN III ID database to determine if an ID could be called.

Identification with YT Microplates

The Biolog YT Microplate characterizes yeast using 94 carbon-source utilization assays, including 35 substrates selected to probe oxidation using the tetrazolium-based dye reduction and 59 substrates where uptake by the cell is measured through cell proliferation. All necessary nutrients, biochemicals, and the tetrazolium-based dye are dried to the bottom of each well of the YT microplate. Each yeast strain was cultured on BUY (Biolog Universal Yeast medium) at 26 °C for 24 hours prior to YT plate inoculation after which a cell suspension was made according to Biolog YT ID Protocol. In brief, cells from an approximately 3 mm diameter colony area were transferred to Biolog yeast inoculating fluid (YT-IF) using a Biolog InoculatorZ™ swab to a cell density of 47% T as measured by a Biolog turbidimeter. The resulting cell suspension was then transferred to the YT microplate at 100 µL per well. YT plates were then incubated at 26 °C and read on the Odin instrument at 24, 48, and 72 hours or until an ID was called.

Identification with FF Microplates

The Biolog FF Microplate characterizes filamentous fungi using 94 carbon-source utilization assays to assign an identity according to color development in response to metabolism. All necessary nutrients, biochemicals, and iodonitrotetrazolium violet-based dye are dried to the bottom of each well of the FF microplate. Each filamentous fungal strain was cultured on 2% malt extract agar at 26 °C for up to 10 days prior to inoculation to allow for sufficient spore/conidial development, after which a cell suspension was made according to Biolog FF ID Protocol. In brief, spores from a large colony area were transferred to filamentous fungi inoculating fluid (FF-IF) using a Biolog InoculatorZ™ swab to a cell density of 75% T as measured by a Biolog turbidimeter. The resulting cell suspension was then transferred to the FF microplate at 100 µL per well. FF plates were incubated at 26 °C and read on the Odin instrument at 24, 48, and 72 hours or until an ID was called.

Identification with AN Microplates

The Biolog AN Microplate characterizes anaerobic bacteria using 95 different biochemical tests. All necessary nutrients, biochemicals, and tetrazolium-based dye are dried to the bottom of each well of the AN microplate. Each anaerobic strain was cultured on BUA+B (Biolog Universal Anaerobe medium + Blood) at 36 °C in an anaerobic chamber for 24 hours prior to AN plate inoculation after which a cell suspension was made according to Biolog AN ID Protocol. In brief, cells from an approximately 3 mm diameter colony area were transferred to inoculating fluid (AN-IF) using a Biolog InoculatorZ™ swab to a cell density of 65% T as measured by a Biolog turbidimeter. The resulting cell suspension was then transferred to the AN microplate at 100 µL per well. AN microplates were sealed in an airtight container with a non-hydrogen producing oxygen absorbing sachet and resazurin indicator, incubated at 36 °C for 22 hours and read on the Odin instrument to ID.

Microplate ID Determination

ID calls were made in real time by Odin software, which evaluates the pattern of color development using an algorithm to calculate a distance between the test pattern and those for each ID taxa stored in the Biolog database. These curated ID taxa include species-level as well as sub-species or strain level information. Importantly, the Biolog ID database contains sufficient pattern information for possible timepoints and protocols for all ID taxa so that an ID can be determined regardless of day-to-day variation in time to ID. Distance values are then used to rank and calculate a similarity coefficient between 0 and 1 for each ID taxa relative to the unknown organism. From this resulting ranked list, the top ranked four ID taxa are evaluated for a similarity coefficient of >0.5. If this threshold is met or exceeded, an ID is called for the test sample. If none of the top four ID taxa meet the threshold, then "No ID" is called for that read period, and another ID attempt will be made after the subsequent read.

Results

GEN III Microplate

The GEN III Microplate for the identification of bacteria functions by generating a unique phenotypic fingerprint for the unknown and comparing it against our database of 1,568 distinct taxa. Odin reads GEN III microplates every 20 minutes, and after each read an ID attempt was made until the calculated "similarity" value reached a threshold of 0.5 at which point an ID was called. The Odin instrument was employed to successfully identify, to the species level, four diverse microbes including both gram-positive and gram-negative species. *Escherichia coli* is a well-known gram-negative rod which is commonly isolated from environmental samples in a wide array of settings. Using a GEN III Microplate, Odin successfully called an ID at 9.5 hours post-inoculation (Table 1). *Paenibacillus polymyxa* is a gram-positive, nitrogen fixing bacteria which is often isolated from soil and plant samples. Odin successfully called an ID at 5.5 hours post-inoculation (Table 1). *Staphylococcus epidermidis* is a gram-positive member of the human skin microflora and a facultative anaerobe. Odin successfully called an ID within 6.5-7.25 hours post-inoculation (Table 1). *Stenotrophomonas maltophilia* is an aerobic gram-negative species which is ubiquitous in soil and often found in aquatic environments. Odin successfully called an ID within 9.75-13.75 hours post-inoculation (Table 1).

YT Microplate

The YT Microplate for the identification of yeast functions by generating a unique phenotypic fingerprint for the unknown and comparing it against our database of 289 distinct taxa. Odin reads YT microplates every 24 hours, and after each read an ID attempt was made until the calculated "similarity" value reached a threshold of 0.5 at which point an ID was called. Odin successfully identified to the species level, four diverse yeast species including clinically- and industrially-relevant strains. *Candida albicans* is an opportunistic pathogenic fungus which is one of the leading causes of fungal nosocomial infections. Odin successfully called an ID at 24 hours post-inoculation (Table 1). *Candida geochares* is also a potential pathogen while growing commensally on many people. Odin successfully called an ID at 48 hours post-inoculation (Table 1). *Kluyveromyces marxianus* has been isolated from dairy products, sisal leaves, and some plants like corn, and this species is regarded highly in the biofuel industry for its ability to process whey waste. Odin successfully called an ID at 72 hours post-inoculation (Table 1).

FF Microplate

The FF Microplate for the identification of filamentous fungi functions generates a unique phenotypic fingerprint for the unknown and comparing it against our database of 649 distinct taxa. Odin reads plates every 24 hours, and after each read an ID attempt was made until the calculated "similarity" value reached a threshold of 0.5 at which point an ID was called. Odin was employed to successfully identify, to the species level, three diverse filamentous fungal species which are commonly isolated from contaminated environments and spoiled food. *Galactomyces geotrichum* (the filamentous telomorph of *G.* candidus) has widespread usage in the cheese industry where it is cultured on the surface of cheese as it ages, to add unique flavor profiles. Odin successfully called an ID at 24 hours post-inoculation (Table 1).

Aspergillus niger is best known as "black mold" which can be isolated from a wide array of spoiled foods, and it is also capable of causing infection. Odin successfully called an ID at 24 hours post-inoculation *Penicillium chrysogenum* is a mold which is commonly found in areas with extensive water damage and whose spores are a known human allergen. Odin successfully called an ID within 24 hours post-inoculation.

AN Microplate

The AN Microplate for the identification of anaerobic bacteria functions by generating a unique phenotypic fingerprint for the unknown and comparing it against our database of 393 distinct taxa. Odin reads plates at 22 hours; an ID attempt was then made to determine the calculated "similarity" value which needed to pass a threshold of 0.5 at which point an ID was called. Odin was employed to successfully identify, to the species level, four diverse anaerobes including pathogenic, probiotic, and commensal strains. *Bifidobacterium breve* and *Lactobacillus casei* are prominent probiotic bacteria which are typically found in the human gut, where they produce lactic and acetic acids. *Clostridium sordelii* is a rare spore-forming rod that can cause severe pneumonia and endocarditis, among other conditions, and is extremely difficult to treat. *Micromonas micros* is a commonly isolated oral anaerobe which is typically commensal in the mouth and has been documented to cause infections at prosthetic joints. Odin successfully called an ID for all four species at 22 hours post-inoculation (Table 1).

Table 1:

Species	ATCC number	Biolog ID protocol	Plate type	Time to ID (hrs)
Escherichia coli	11775	A	GEN III	9.5
Paenibacillus polymyxa	842	A	GEN III	5.5
Staphylococcus epidermidis	12228	A	GEN III	6.5-7.25
Stenotrophomonas maltophilia	13637	A	GEN III	9.75-13.75
Candida albicans	10231	YT	YT	48
Candida geochares	36852	YT	YT	48
Kluyveromyces marxianus	2512	YT	YT	72
Galactomyces geotrichum	34614	FF	FF	24
Aspergillus niger	10106	FF	FF	24
Pennecillium chrysogenum	15700	FF	FF	24
Bifidobacterium breve	9714	AN	AN	22
Clostridium sordelii	393	AN	AN	22
Lactobacillus casei	2512	AN	AN	22
Micromonas micros	33270	AN	AN	22

Conclusion

The Odin instrument provides the ideal platform for high-throughput identification of thousands of microbes through the use of GEN III, YT, FF, and AN microplates. With less than 10 minutes of hands-on prep work, we utilized a combination of cell density and metabolic profile quantification by redox dye reduction to generate a unique phenotypic fingerprint for each unknown organism. This profile was automatically monitored and matched to our database of 2,900 cumulative profiles to achieve identification in as little as 5.5 hours. The Odin system for identification of microorganisms is a powerful tool which can be employed with significantly less bench-side work, less specialized training, and lower capital equipment costs than is required for other identification methods like DNA sequencing or MALDI-TOF. These advantages along with the flexibility of identifying bacteria and fungi provide a marked benefit for labs across many areas including environmental monitoring where processing hundreds of samples quickly is required, agricultural labs seeking to understand the soil microbiome, and research labs conducting population analyses.

00A 097 Rev A - June 2023