ASM AND SIM MEETINGS

NEW APPROACHES TO MONITORING CELLS

SAN DIEGO and BALTIMORE—As biotechnology processes advance to commercial reality, more attention is being paid to cell culture monitoring and quality control. This increased emphasis was in evidence at meetings held this summer by two key groups in the field: the American Society for Microbiology (ASM) and the Society for Industrial Microbiology (SIM).

Charles Keese and his collaborators at the General Electric Research and Development Center (Schenectady, NY) have been developing a sensitive technique that may provide a means for monitoring cultured cells in an industrial setting. As he explained in San Diego this past June at the Second Annual ASM Conference on Biotechnology, the new method depends on microelectrodes to detect weak changes in current as cells undergo locomotion.

And at SIM's annual meeting, held in Baltimore in August, Barry Bochner of Biolog Inc. (Hayward, CA) described an approach for rapidly subjecting microorganisms to a full and unequivocal battery of biochemical tests to measure catabolic activity. This soon could prove useful in quality control efforts for a wide segment of the biotech industry, where it is being introduced commercially. The method can be adapted, for instance, to screen a "producer strain" repetitively before use in a production run to ensure that it has not undergone any significant genetic change. The development of this new microtiter-plate screening procedure was aided by the Small Business Innovation Research program administered by the National Institutes of Health.

Traditional methods for identifying bacteria depend largely on pH dye development after differential fermentation by an organism on a wide array of sugars. According to Bochner, color changes sometimes can be subjective, transitory, or so slight as to be virtually impossible to read. Other detection methods, such as those based on DNA probes or monoclonal antibodies, tend to focus on single traits, so negative results may be difficult to interpret. Yet another method, this one based on differentiating fatty acid patterns between organisms and being developed by Hewlett-Packard (Palo Alto, CA), involves expensive and frequently temperamental gas-chromatographic detection devices. Similarly, protein pattern analysis on electrophoretic gels also entails considerable technical expertise and

can be time consuming.

"We feel our technology, which uses a reducible tetrazolium dye in microtiter plates to detect catabolic activities of microbes, has distinct advantages over all that," Bochner explains. The crux of the new technology is the unambiguous detection of reduced nicotinamide adenine dinucleotide, which builds up in virtually all cells undergoing catabolic activity. This cellular by-product is detected by converting the tetrazolium detector dye to the reduced formazan. An extensive analysis of biochemicals that may or may not be metabolized by different species of microorganisms—mainly bacteria so far—renders the method highly selective. Although slight contamination of the test inoculant can be tolerated, Bochner notes, the system is really designed for identifying clonally derived cells.

Early on, his research group began running nearly 500 biochemical compounds in tests as potential catabolites for any bacterium being identified. By discarding unstable chemicals and those that interfered with the dye's activity, this range was narrowed to just under 100 chemicals. Even with this decrease, a single battery of tests offers 2^{95} (or 4×10^{28}) outcomes. Because of the distinctive patterns of positive and negative results, this number of tests has proved adequate for identifying all of the approximately 200 prototype species tested so far. Any two species of bacteria are differentiated by an average of 38 of the tests. In the worst cases so far, the hardest-to-distinguish pairs behave differently in at least 6 of the tests, Bochner says.

The method being developed by Keese and his collaborators at General Electric (GE) has not yet been examined so exhaustively with different cell types. However, it is proving useful for mammalian cells, a type outside the range for which the Biolog methods are being honed. As with the Biolog test, Keese envisions his new method eventually playing a quality control role in industrial settings.

The GE research group has focused so far on ensuring that their method genuinely detects cellular signals. "You can't just stick a culture medium into a field" to obtain reliable measurements of electrical changes, Keese says, because the medium bathing the cells damps out most meaningful electrical signals. Thus, 100-micron gold electrodes, which are connected by thin copper

Charles Keese (left) and colleague Ivar Giaever use a weak electric current to monitor the movement of cells.

wires and insulated with small wads of wax, had to be fabricated before a.c. signal changes of cells could be reliably detected in the microampere range. The electrodes lie on the bottom of the culture dish; the electric fields applied are so low as not to interfere with the cells' normal behavior, he notes.

When mammalian fibroblast cells are inoculated onto such surfaces, the impedance signal rises sharply after a brief lag. This indicates the attachment and spreading of cells on the medium's surface, Keese says. After they have flattened out along the surface, the signal continues to fluctuate with time, reflecting cell motility. It is as if the cells "stand on small feet" while growing in monolayer cultures, with some cell movements resembling those of an athlete "doing push-ups."

The technique is so sensitive that it enables observers to visualize cellular behavior "below the resolution of the light microscope," Keese says. He suggests the method may be adapted to biosensor applications. Here the electrode could be used to monitor cells being mass cultured, as in an industrial facility where productivity may depend on maintaining optimal conditions for delicate cell types. "We need to learn how to interpret [signs of] cell health," Keese says. He envisions such a system providing an early warning of any critical changes in growth conditions and thus serving a valuable role for quality control in mammalian tissue culture applications.

—Jeffrey L. Fox