

## Bacteria in Metalworking Fluids

Miriam Kay Lonon

Department of Health and Human Services, United States Public Health Service  
Centers for Disease Control, National Institute for Occupational Safety and Health  
4676 Columbia Parkway, R-7, Cincinnati, Ohio, 45226

### ABSTRACT

Used metalworking fluids from a variety of sources were inoculated onto agar media for isolation and identification of bacterial species. Types of fluids examined were either synthetic or semisynthetic. Forty-five isolates were grown in pure culture. Thirty of these were identified to species. The most commonly isolated species belonged to the genus *Pseudomonas*. Other genera identified include *Acinetobacter*, *Rhodococcus*, *Variovorax*, *Bacillus*, *Serpens*, *Xanthomonas*, and *Providencia*.

### INTRODUCTION

Microbial degradation of metalworking fluids is a well known problem in the metalworking trades. Microbial enzymes may cause changes in viscosity, altering lubricity and cooling properties. Acid products of microbial metabolism may lower pH, causing corrosion. Growth of sulfate-reducing bacteria often produces foul odors, causing "souring" of the fluids. Excessive growth may clog filters and nozzles and may interfere with the metalworking operation.

Microbially contaminated metalworking fluids also may pose special hazards for workers exposed to them. Many of the organisms isolated from contaminated fluids are opportunistic pathogens that may infect susceptible individuals. In addition, the fluids may become contaminated by frank pathogens, that infect healthy as well as compromised hosts.<sup>(1,2)</sup> The aerosols generated by the machining processes are truly bioaerosols, containing many live and dead microorganisms. Inhalation of these aerosols may cause a serological or immunological response. Microbial enzymes and toxins accumulating in the fluids may act directly on the tissues of exposed workers

or may aggravate the effects of existing chemical irritants. Toxic metabolites, such as ammonia and hydrogen sulfide, may be evolved as gasses directly into the work space.

The bacteria that grow in metalworking fluids include many Gram negative species. Gram negative organisms produce endotoxin, which, in large doses, by injection or by gut absorption, is fatal, and in smaller doses, by either route, may result in fever, circulatory and blood pressure disturbances, depressed immune response, and hypersensitivity to drugs and chemicals<sup>(3)</sup>. Inhalation of endotoxin has been associated with the inflammatory disease, hypersensitivity pneumonitis.<sup>(4)</sup>

### METHODS

In this study, eighteen samples of used synthetic or semisynthetic metalworking fluids from a variety of sources were examined and inoculated onto agar media for isolation and identification of bacterial species. The fluids were streaked onto trypticase soy agar (TSA) and incubated at 28 degrees centigrade until colonies appeared. After that, the plates were kept at room temperature. Isolated colonies were picked and subcultured on TSA until only one kind of colony appeared and the Gram stain showed a homogenous population. Catalase and oxidase tests were performed on each isolate. These isolates were then analyzed, using the Biolog Microbial Identification System (Biolog, Inc., Hayward, CA). The Biolog system identifies bacteria based on a metabolic profile generated from a ninety-six well microtiter plate. The wells are filled with substrate containing ninety-five different carbon sources (the A1 well is a negative control with no carbon source) and a redox dye that undergoes a color change to indicate that respiration

(utilization of the carbon source) is taking place. The wells were filled with a suspension of the unknown organism in physiological saline and, after a suitable incubation time (4 or 24 hours), the plate was read in a microtiter plate reader. The Biolog system compares the metabolic profile generated by the unknown organism to those stored in a library of reference strains and identifies the unknown using a best fit analysis.

## RESULTS

Attempts to cultivate pure cultures resulted in the isolation of forty-five unknowns. Thirty of these strains were identified to species by Biolog (Table 1).

**Table 1.** Bacterial species identified in contaminated metalworking fluids.

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<i>Pseudomonas putida</i> Type B1
<i>Pseudomonas pseudoalcaligenes</i>
<i>Pseudomonas putida</i> Type A1
<i>Pseudomonas diminuta</i>
<i>Pseudomonas stutzeri</i>
<i>Pseudomonas vesicularis</i>
<i>Pseudomonas viridilivida</i> A
<i>Acinetobacter</i>
<i>radioresistens</i> /Genospecies 8
<i>Acinetobacter lwoffii</i> /Genospecies 8
<i>Acinetobacter johnsonii</i> /Genospecies 7
<i>Rhodococcus globerulus</i>
<i>Rhodococcus erythropolis</i>
<i>Alcaligenes faecalis</i> ss <i>faecalis</i>
<i>Variovorax paradoxus</i>
<i>Bacillus pasteurii</i>
<i>Bacillus brevis</i>
<i>Serpens flexilis</i>
<i>Xanthomonas maltophilia</i>
<i>Providencia rettgeri</i>

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Of these, twelve belonged to the genus *Pseudomonas*. Seven of the twelve were identified as *P. putida*. Other *Pseudomonas* species included *P. stutzeri*, *P. vesicularis*, *P. pseudoalcaligenes*, *P. diminuta*, & *P. viridilivida*.

Four of the isolates were identified as *Acinetobacter* and three are thought to be *Rhodococcus*. Other genera identified include *Variovorax*, *Bacillus*, *Serpens*, *Xanthomonas*, and *Providencia*.

## DISCUSSION

The results of this study are consistent with similar studies reported in the literature. The majority of the organisms isolated from metalworking fluids are aerobic, nonfermenting Gram negative rods with *Pseudomonas* being the genus most commonly reported.<sup>(2,5,6)</sup> The pseudomonads produce a great number of extracellular enzymes that enable them to degrade macromolecules into simpler substrates. They are able to grow on a wide variety of hydrocarbons from simple fatty acids to aromatics. Many are resistant to antimicrobial agents. They are ubiquitous in the environment and can be isolated readily from soil or water. They are important opportunistic pathogens, causing severe local and systemic infections in susceptible individuals.

*Acinetobacter*, another genus often reported from metalworking fluids,<sup>(7)</sup> is also able to use a wide variety of simple and complex hydrocarbons as an energy source. Like *Pseudomonas*, *Acinetobacter* is an opportunistic pathogen, causing septicemia, meningitis, pneumonia, and endocarditis. Clinical isolates are resistant to most antibiotics.

Three of the isolates in this study were identified as *Rhodococcus* species. The rhodococci also are able to use unusual hydrocarbon and nitrogen-containing compounds as substrates and often have been isolated from soils contaminated with petroleum.<sup>(8)</sup> Some rhodococci are pathogenic.

Some of the isolates from this study were not identified. This may have been due to the lack of a suitable reference in the library. In addition, some of the isolates grew very poorly on the isolation media, making it very difficult, in some cases, to harvest enough material for analysis. Furthermore, the Biolog system requires that the

unknown isolates be in pure culture. Although great care was taken to ensure the preparation of pure cultures, it is difficult, when working with environmental samples, to be sure that the cultures are, in fact, pure. Slow-growing contaminants may be passed from one culture to the next, unseen because they are overgrown by the predominating species. However, they may still produce metabolic products that interfere with the Biolog assay, resulting in a pattern that does not match anything in the reference library.

### CONCLUSIONS

This study is the preliminary investigation of a larger study to identify and characterize potential hazards associated with bacterial contamination of metalworking fluids. The species identified from these samples are characterized by great nutritional versatility as the result of their ability to produce a wide variety of extracellular enzymes. Many of these extracellular enzymes have been characterized as virulence factors, contributing to disease by enabling the organism to use the macromolecules found in human tissues as substrates for growth. Many of these enzymes cause damage when applied directly to tissues, especially lung tissues. This phase of the study confirmed the presence of organisms such as *Pseudomonas* and *Acinetobacter*. The next phase will investigate the possible accumulation of bacterial enzymes and toxins in the fluids and the potential hazards posed by exposure to fluid aerosols.

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