THE BACTERIAL FLORA OF USED EMULSION OILS

A Thesis

Presented to

the Faculty of the Department of Biology

University of Houston

In Partial Fulfillment

of the Requirements for the Degree

of

Master of Science in Biology

by

Carl O. Tant

June 1957

Abstract

Although several workers have studied the bacterial flora of emulsion oils, there is much inconsistency in their results. This inconsistency was thought to be the result of inadequate methods of studying the flora of these oils.

An adequate method for isolating bacteria from used emulsion oils was developed based upon concentration and inhibition techniques.

Following development of this method a study was conducted in a large industrial plant. Over an eleven weeks period, a total of twenty-seven different species of bacteria was isolated from seven different commercial cutting oils.

Growth studies of fifteen of these organisms in pure culture were conducted with six of the same coolants from which they were isolated. It was found that the growth of bacteria in emulsion oils is dependent upon both the nature of the organism and the composition of the coolant.

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APPENDIX

I. Introduction

Emulsion oils consist of petroleum oils emulsified with soaps of tall oil, rosin, petroleum sulfonate, and others, and such additives as rust inhibitors and coupling agents. These emulsions, commonly referred to as cutting oils or coolants, serve two principal functions: (1) to cool both the tools and the stock so that neither becomes overheated and thus subject to cracking and chipping, and (2) to lubricate the point of contact between tools and stock.

The cutting oil is sold to the customer as a water-free concentrate which is diluted according to the characteristics of the coolant and the type of machining operation involved. The diluted material forms a stable milk-white emulsion which thus combines the lubricating properties of petroleum and the cooling properties of water.

These emulsions are supplied to the cutting and grinding machines, either from individual tanks containing fifty to 100 gallons, or from large sumps containing thousands of gallons and supplying many machines. The coolant flows over the metal being worked and then returns to the individual tank or sump from which

it is recirculated.

The open circulation systems offer ample opportunity for the coolant to become contaminated with bacteria. The workers may place their hands in the coolant and inadvertantly contaminate it with the natural flora of their skin. Contamination may also occur when the workers expectorate, cough, urinate, or discard lunch scraps into the collecting troughs at the base of the machines.

There is more opportunity for contamination to occur in the case of machines served by coolants from large sumps than for machines with individual tanks. In some of the older installations, the return lines from the machine to the sump are open troughs which may or may not be partailly covered with a heavy sttel mesh or wooden slats. In walking over these troughs, the employees unavoidably brush particles of dirt from their shoes or the floor into the coolant. The sump itself is often only partially covered and the thousands of gallons of emulsion oil in it are thus exposed to direct aerial contamination.

Codkroaches, pieces of wood, cloth rags, and other debris

have been observed in the coolant, but these larger particles are usually prevented by a metal screen from returning to the machines. These particles remain in the sump until it is drained and cleaned, a procedure which is carried out once every six to eighteen months.

The bacteria that find their way into the emulsion constitute a serious problem from both the economic and the health standpoints. Some of the ways by which the bacteria produce these deleterious effects are as follows: (1) The emulsion may serve as a vehicle of disease transmission if pathogenic bacteria are present in it. (2) Some of the organisms are capable of oxidizing the emulsion with the production of nauseating odors. (3) A spoiled emulsion will not function properly and may result in damage to the machine bits or tools. Considerable loss in production as well as direct coolant expense is entailed by the company when a spoiled emulsion must be discarded, the machine cleaned, and new coolant added.

In recent years, the bacterial content of cutting emulsions has received considerable attention. Research has been directed toward understanding the mechanisms by which the bacteria spoil the emulsion, determining methods for controlling bacterial

spoilage, and determining whether, under industrial conditions, emulsion oils may contain disease producing microorganisms.

Rosenberger (1922) investigated the bacterial flora of oil emulsions and reported the presence of both sporeforming and non-sporeforming bacilli.

Schwartz (1941) found that comedones of the hands and fingers frequently occurred in workers handling cutting oils, and reported that folliculitis was the most frequent type of dermatitis. He isolated pathogenic staphylococci from these lesions, but felt that they were not from the oil itself, and stated,

> . . . soluble petroleum oils, when diluted for use, are not well suited as culture media because of the fact they consist mostly of water and a small percentage of the soluble oil. . . . The insoluble cutting oils as a class are not suitable for the growth of bacteria because they contain such a large percentage of petroleum oil and because many of them also contain an inhibitor which has antiseptic properties. . . .

During a five months investigation of used emulsion oils, Lee and Chandler (1941) found that a new species, Pseudomonas oleovorans, was present almost to the exclusion of all other species. Duffett, et al (1943) found that the majority of the species isolated from thirty different emulsion oil samples

belonged to the genus Pseudomonas. In addition to Pseudomonas oleovorans, Pseudomonas aeruginosa, two species of Achromobacter, Aerobacter aerogenes, Escherichia coli, Bacillus alvei, yeasts, and molds they isolated six new species of pseudomonads. Each emulsion sample usually contained several different species and genera.

Weirich (1943) found Escherichia coli to be the second or third most common organism found in cutting emulsions, and also noted the presence of micrococci and Pseudomonas aeruginosa. The C. B. Dolge Company (undated pamphlet) reported that Escherichia coli was present in large numbers and that streptococci and micrococci were occasionally present. Pivnick (1952) found micrococci, Sarcina, Flavobacterium, Vibrio, and many species of pseudomonads.

Bennett and Wheeler (1954) found large numbers of Micrococcus pyogenes var. aureus in a used sample from a plant where several workers were suffering from pyogenic infections. Pivnick and Fabian (1954) did not find Escherichia coli in samples they examined; however, they isolated Aerobacter aerogenes and coli-aerogenes intermediates as well as pseudomonads. Pivnick

(1955) isolated a new species, Pseudomonas rubescens, from emulsion oils. Bennett (1956) reported the isolation of a Paracolobactrum species and a Salmonella species from used cutting emulsions.

Okawaki (1953) reported that several species of intestinal pathogens survived and also multiplied in oil emulsions. Pivnick et al (1954) confirmed Okawaki's work. Wheeler and Bennett (1954) and Bennett and Wheeler (1954) showed that Gram-positive organisms were not capable of surviving in oil emulsions, but that many Gram-negative pathogens remained viable for as long as 250 days in two different emulsions. Wheeler and Bennett (1956) conducted laboratory studies which showed that most commercial cutting oil inhibitors were of no value in inhibiting the bacterial flora of emulsions.

The results obtained by other workers have been somewhat inconsistent. The literature, however, generally indicates that aerobic bacteria are capable of surviving and growing in emulsion oils under laboratory conditions. In view of the lack of agreement concerning the normal flora of cutting oils, it was believed that failure of other workers to isolate more than two or three

species of bacteria from used emulsion samples might be due to the limitations of the techniques employed. From many standpoints, it was also believed that if the organisms were present in the emulsions under industrial conditions, it would be important to know whether they were present merely as temporary contaminants or whether they actually survived and multiplied in the oils. Consequently, this investigation was undertaken to develop a technique that would demonstrate the presence of bacteria in small quantities in emulsion oils. If such species existed in used emulsions, a method should be developed to determine whether the organisms merely survived or whether they could multiply in the various cutting compounds.

II. Experimental Procedure for Isolating Bacteria from Used Emulsions

Attempts were made to isolate organisms from used emulsion oils by streaking loopsful of the sample directly onto nutrient agar, blood agar, and S S agar (Difco) plates. In all cases the plates, incubated at 37°C either showed little growth or *Proteus* and *Pseudomonas* species swarmed and overgrew the other colonies, making it impossible to obtain pure cultures.

Failure to obtain organisms from other genera agreed with the report of officials of the plant cooperating in the research. On several occasions over a period of years they had sent coolant samples to a commercial laboratory several hundred miles away. Although the samples had been packed in dry ice and shipped by the most rapid transportation available, no conclusions regarding the cause of deterioration could be made. Bennett (1956) reported that freezing samples of emulsion reduced the bacterial flora as much as ninety-nine per cent.

After considerable experimentation the following procedure was developed for isolating bacteria from emulsion oils.

1. Four ml of the sample was centrifuged for fifteen

minutes at 20,000 rpm. The supernatant was immediately asceptically pipetted off and discarded, and the sediment was used to streak nutrient and blood agar plates.

2. A 20.0 ml amount of the uncentrifuged sample was inoculated into 200.0 ml of Selenite Broth (Difco). S S and E M B plates were streaked from the Selenite Broth after it had incubated for eighteen hours at 37°C.

3. The addition of 0.1 per cent chloral hydrate and/or 0.004 per cent potassium tellurite (Schaub and Foley, 1952) to all solid media controlled overgrowth of Pseudomonas and Proteus species.

Table 1 indicates that two factors are involved in studying the bacterial flora of a used cutting oil: (1) speed in inoculating the primary isolation media, and (2) concentration and inhibition techniques to control overgrowth and swarming of Pseudomonas and Proteus species.

Thirty cultures were isolated from these six coolants by using the new technique, while only eleven were isolated by using the old method.

Table 1 indicates a possible reason for the failure of other workers to obtain consistent results in studying the flora

of used emulsions. Even using the concentration techniques and enrichment media, speed in processing the sample is essential if reliable results are to be obtained. Only a few hours delay may result in practically uncontrollable overgrowth of Proteus and Pseudomonas species. The time factor involved in shipping used coolant samples to a distant laboratory (Pivnick, 1955) probably accounts for the failure to isolate few species other than the pseudomonads.

III. The Flora of Used Emulsions from a Large Machine Shop

Samples of seven different commercial coolants were obtained at weekly intervals for eleven weeks from a large machine shop. These samples were obtained from the following nine collecting points:

Collecting point no. 1. Machine served by individual tank, capacity 60 gallons. Oil is not changed, although one to three gallons is added daily to make up for loss due to evaporation, etc.

Collecting point no. 2. Machine supplied from a 6,500 gallon sump (listed below as collecting point no. 4) serving six large machines.

Collecting point no. 3. Machine served by individual tank, capacity 80 gallons. Coolant added and changed every 3-4 months as needed.

Collecting point no. 4. Sample obtained from 6,500 gallon sump serving six large machines. Coolant changed every 3-4 months, and lines to machines flushed and cleaned approximately once yearly

Collecting point no. 5. Sample obtained from 7,000 gallon sump serving approximately fifty machines. Sump cleaned and coolant added approximately once yearly.

Collecting point no. 6. Sample obtained from machine served by sump described as collecting point no. 5.

Collecting point no. 7. Machine served by individual tank, capacity 50 gallons. Coolant changed and added as needed. Because of objectionable odor, two ounces of "Rancrid" deodorant is added weekly.

Collecting point no. 8. Machine served by individual tank, capacity 70 gallons. Coolant changed and added as needed. Machine is only in intermittant use.

Collecting point no. 9. Machine served by individual tank, capacity 72 gallons. Coolant changed approximately once each month.

A 100.0 ml sample of each coolant was obtained weekly from each collecting point over a period of eleven weeks. Isolation and identification of organisms was accomplished by the technique described in Part II.

While this study was in progress, a record was kept of all

coolant changes, machine cleaning, etc.

Table 2 shows that twenty-seven species were identified from the 100 coolant samples. Additional organisms which could not be identified were encountered.

Machines (collecting points no. 2 and no. 6) supplied by large sumps (collecting points no. 4 and no. 5 respectively) with coolants B and D were found to contain a much larger number of species. The reason for the high number might have been due to the type of system or to the kind of coolant used.

The number of species isolated varied widely from one coolant to another, ranging from only four species in the sample obtained at collecting point no. 1 to twenty-two species in the sample obtained at collecting point no. 5.

There was no significant change in the bacterial flora present following addition or change of coolants in the machines, as shown in table 5.

Furthermore, it was noted that the same species were usually present from week to week (table 4) at each collecting point, although a few species were highly erratic in their appearance.

The relative consistency in the composition of the flora isolated from each collecting point indicated that either these species were multiplying in the coolant, or that frequent and heavy contamination was occurring. Consequently an attempt was made to determine whether fifteen selected species isolated from used emulsions were capable of reproducing in pure cultures in six of the emulsions tested.

V. Experimental Procedure for Growth Studies

The following procedures were employed for studying the growth of bacteria in emulsion oils:

1. Pure cultures of organisms were isolated from used emulsion oils as previously described.

2. Concentrates of coolants A, B, C, D, E, and F were diluted as used in industry.

3. A 19.5 ml aliquot of each coolant was placed in standard water-analysis test tubes, which were plugged with gauze-wrapped cotton and autoclaved for 15 minutes at 15 lb pressure.

4. A suspension of the pure culture to be studied was made in physiological saline solution, and 0.5 ml was inoculated into each coolant.

5. At the time of inoculation a count was made by routine plate count procedure.

6. The tubes were incubated at room temperature for sixteen days with constant agitation on an automatic shaking machine.

7. Plate counts were made daily.

8. Normally, three strains of each species were studied; however, if inconsistency of results was apparent, four strains were

used. Only one or two strains of the rarely isolated organisms were studied.

VI. Results and Discussion

Table 6 shows that some organisms were able to multiply in all six coolants whereas others varied widely in their ability to multiply or survive. Bennett and Wheeler (1956) reported that the Gram-negative organisms constituted the most serious problem in emulsion oils. This study is confirmatory, as all of the organisms which grew with any frequency were gram-negative. However, as table 6 shows, there were also many of the Gram-negative organisms which did not survive.

Achromobacter species

Members of this genus are considered to be common inhabitants of the soil and water (Bergey, et al, 1948). The presence of Achromobacter in emulsion oils has beenreported previously by Duffett et al (1943). Since these organisms were isolated from 12 per cent of the emulsions studied, growth studies were carried out on two strains. All strains gradually declined in number in the coolants tested.

This study indicates that these organisms do not multiply in emulsions, and that they play no role in the deterioration process. They are probably present as temporary contaminants

from the water used in the coolant, or from dirt which has fallen into the system.

Aerobacter aerogenes

Aerobacter aerogenes is widely distributed in nature and is normally found on plants and to a varying degree in the intestinal tract of man and other animals.

This organism has been found in emulsion oils by Duffett, et al (1943) and Pivnick and Fabian (1954). Fabian and Pivnick (1953) reported that it was capable of growing in pure culture in emulsion oil. Bennett and Wheeler (1954) reported that these organisms had a survival time of from 30 to 59 days in two different emulsions. Bennett (1956) reported this organism in almost pure culture from one industrial emulsion.

As can be seen from table 6, Aerobacter aerogenes was capable of surviving in four of the six emulsions studied. This consistent survival indicates that under some conditions the organism might be capable of growing in the coolant.

If such be the case, the report of Hunter and Weiss (1938) that Aerobacter aerogenes is capable of producing hydrogen sulfide under certain conditions is of considerable interest. If

this occurs to any extent, it is possible that the organisms could be a factor in odor production.

Aerobacter cloacae

Aerobacter cloacae was isolated only once during the initial stages of this investigation. It could not again be found; therefore no growth studies were conducted. The organism, which is normally found in the feces and sewage cannot therefore be considered an important constituent of the flora of emulsion oils, and was probably present only as a temporary contaminant.

Bacillus cereus and Bacillus subtilis

These organisms have not been found in emulsions before, although the presence of Bacillus alvei has been reported by Duffett, et al (1943). Bennett and Wheeler (1954) reported that Bacillus subtilis did not survive for any appreciable time in emulsion oils. It will be noted from tables 3 and 4 that the occurrence of these organisms in a particular emulsion was sporadic.

During the second stage of this investigation, Bacillus cereus was not again isolated; however, Bacillus subtilis was, and growth studies were conducted with it. In no case did the organisms multiply in the coolants. However, following an

initial drop in count, the organisms seemed to remain fairly static, showing a very gradual decrease in number in all emulsions except Coolants E and F. In these two coolants the drop in viable count was more rapid.

On the basis of this data and previous reports in the literature, it would seem safe to assume that these organisms are not an important part of the normal flora of emulsion oils.

Diplococcus pneumoniae

Diplococcus pneumoniae is normally found in the respiratory tract of man and animals. It is the most common cause of lobar pneumonia and has been implicated in meningitis, mastoiditis, peritonitis, pericarditis, endocarditis, and many other diseases. It is also found in the respiratory tract and salivary secretions of normal individuals.

The presence of Pneumococcus in emulsion oils has not been reported before. Bennett and Wheeler (1954) reported that this organism did not survive in emulsion oils. The evidence indicates that it was present as a result of recent contamination. As the organism was not again found following the beginning of the growth studies, further information could not be obtained.

Escherichia coli

Escherichia coli is a normal inhabitant of the intestinal tract of man and other vertebrates. The organism is widely distributed in nature. Although generally considered non-pathogenic it may cause infections of the genito-urinary tract and has been reported to be the etiological agent of infant diarrhea.

Escherichia coli is considered important as an index of fecal contamination. The purity of water is based on the number of these organisms present. Consequently, its presence in large numbers in industrial emulsion oils can be considered of some importance.

The presence of Escherichia coli in emulsion oils has been reported by Duffett, et al (1943), Weirich (1943), and the C. B. Dolge Company (undated pamphlet). Weirich (1943) reported this organism to be the second or third most common encountered in used emulsions. Fabian and Pivnick (1953) did not find it in used emulsions, but reported that pure cultures of the organism grew in emulsion oils, while Bennett and Wheeler (1954) reported this organism was not capable of surviving for any appreciable length of time. The variation in results may have been due to a strain

variation.

In this study Escherichia coli was isolated from 41 per cent of all samples studied and showed some consistency from week to week in several of the used emulsions.

The results of the growth study show that the organism does not grow to any great extent in any of the coolants studied. However, as can be seen from table 6, one strain of the organism multiplied slightly in Coolants B and E, and two strains multiplied slightly in Coolant D. It is interesting to note that Strain 20-E-7d did not survive more than six days in any of the coolants, while Strains 21-D-5b and 23-B-2a multiplied slightly or survived in Coolants B, D, E, and F. None of the three strains survived more than five days in coolants A and C. This indicates that growth or failure to grow is dependent not only upon strain variation, but also upon the formulation of the coolant.

Escherichia intermedium

Escherichia intermedium is frequently found in the soil, water, and intestinal tract of man and other animals. It was isolated twice during the initial study of flora in emulsion oils, and once for the growth study. As table 6 indicates, the organism

did not survive more than one day in any of the six coolants.

Escherichia freundii

Escherichia freundii is normally found in the soil and water and occasionally in the intestinal tract of vertebrates. It was isolated only seven times from two coolants during the early part of this study, and could not again be isolated for growth study.

In view of the rarity with which this species was isolated, it cannot be considered an important part of the flora of emulsion oils.

Klebsiella pneumoniae

Klebsiella pneumoniae is considered a normal inhabitant of the intestinal tract of man and other vertebrates. It is occasionally associated with infections of the genito-urinary tract and has been implicated in infant diarrhea. It is the second most common cause of pneumonia, producing a form of the disease which rapidly terminates fatally if not quickly diagnosed and treated.

Pivnick, et al (1954) reported that one culture was capable of growing in emulsion oils, while another culture decreased in numbers and disappeared entirely after 21 days incubation. Bennett and Wheeler (1954) reported that the organism did not survive

in either of the two emulsions they studied. Pivnick, et al (1954) reported that after recovery from the emulsion, the strain which grew was capable of killing mice.

In this study Klebsiella pneumoniae was isolated thirty-two times from the 100 samples studied. As can be seen from table 5, these isolations were fairly evenly distributed among five of the seven coolants.

As can be seen from table 6 and plate 2, Klebsiella pneumoniae exhibited extreme variation in its ability to multiply in the various coolants. With the exception of Coolant A in which few species multiplied, the ability of Klebsiella to grow seems dependent upon strain variation. It is interesting to note that Strain 25-D-6a survived for the entire sixteen days in Coolant A. It is possible that the high count which Strain 23-B-4a obtained from Coolant B from which it was originally isolated was the result of adaptation to the coolant, although the results of other strains do not support such theory.

The results obtained in this study confirm both those of Pivnick, et al (1954) and Bennett and Wheeler (1954), indicating that survival and multiplication of this species in commercial cut-

ting oils is dependent upon strain variation.

Micrococcus species

The pyogenic micrococci are normally found on the skin of the human body and in the oral and nasal cavities. Micrococcus pyogenes var. aureus is found more commonly than Micrococcus pyogenes var. albus or Micrococcus pyogenes var. citreus.

The micrococci have often been reported in used emulsion oils. Schwartz (1941) found that no significant number of these organisms could be found in emulsion oils. The C. B. Dolge Company (undated pamphlet) reported that they were occasionally present in used emulsion oils. Pivnick (1952) reported the presence of these organisms in emulsion oils. Bennett and Wheeler (1954) found large numbers of Micrococcus pyogenes var. aureus in a used sample from a plant in which several workers were suffering from pyogenic infections. The organism could not be recovered from the emulsion after the sample had been allowed to stand for one week. These same workers could not show survival for any significant period of time in two different emulsions. From these observations Bennett and Wheeler (1954) concluded that the micrococci are not capable of growing in emulsions, but that the organism could be isolated

where considerable numbers were being inoculated into the coolant by infected workers.

Tables 4 and 5 show that the presence of these organisms in emulsions is very sporadic. Table 6 indicates that Micrococcus pyogenes var. aureus generally failed to survive more than a few days in any of the emulsions tested. The one exception, Culture 17-D-4a in Coolant D, was originally isolated from that emulsion, and when reinoculated into it in pure culture attained a maximum count of 1,200,000 organisms per ml in five days. These results provide basis for assuming that this species may be able to adapt or mutate and multiply in emulsion oils.

Paracolobactrum intermediates

The paracolon bacilli are normally found in the intestinal tract of man. Stuart, et al (1943) reported that they were found in greated numbers in the feces during outbreaks of gastroenteritis. Morgan and Cheever (1952) suggested, however, that they are not primary etiological agents of disease since they are found in the normal intestinal tract, especially in tropical areas.

Bennett (1956) reported the isolation of a Paracolobactrum organism from a used emulsion. In this study members of this genus

were isolated in 47 per cent of the samples studied. When this frequency is considered along with the high rate of other members of the family Enterobacteriaceae, further evidence for heavy fecal contamination of emulsion oils is presented.

Pure cultures of the genus exhibited wide variation in their ability to grow in emulsion oils (table 6; plate 1). Culture 25-B-4b which was isolated from Coolant B multiplied in Coolants B, D, E, and F. Culture 25-F-8a isolated from Coolant F multiplied in Coolants B, D, and F. Culture 26-D-5a isolated from Coolant D did not grow in that oil, but did survive; unlike any of the other cultures, however, it did multiply in Coolant C. Culture 26-C-3b, isolated from Coolant C survived, but failed to grow in that coolant, while multiplying rapidly in Coolants B, D, E, and F. None of the four cultures survived in Coolant A.

Thus, it would appear that, like Klebsiella pneumoniae, the growth or survival of Paracolobactrum intermediates is dependent upon the nature of the coolant as well as strain variation. In view of the fact that 50 per cent of the cultures multiplied and 12 per cent survived, the paracolon organisms can probably be considered an important part of the flora of emulsion oils. The

fact that 38 per cent failed to survive, considering that almost one-half of the origianl samples studied contained the organisms is indicative of frequent fecal contamination.

Proteus species

Proteus vulgaris is the type speceis of the genus *Proteus*, and was the fourth most frequently isolated organism in the study of used emulsion oils. The organism has a wide range of habitation in nature. It is frequently found in putrifying organic matter as a saprophyte. It is a common inhabitant of sewage and manure, and is frequently found in the normal human stool (Morgan and Cheever, 1952). It has been implicated as the etiological agent in some cases of gastroenteritis (Cooper, *et al*, 1941). Morgan and Cheever (1952) reported that it is frequently the cause of peritonitis and wound abscesses. Pierson and Honke (1941) found that *Proteus vulgaris* played a definite etiologic role in from six to thirteen per cent of all human urinary tract infections.

Proteus vulgaris is a facultative anaerobe which grows rapidly and exhibits swarming motility over a large temperature range. The organism is capable of extreme proteolytic action. It may play some role in the spoilage of emulsion oils, as one

of its characteristic biochemical reactions is the production of H_2S from sulfur and thiosulfates. On one or two occasions a definite putrifactive odor was noticed, and Proteus vulgaris was isolated in large numbers from that sample. The organism is also capable of reducing nitrates to nitrites, and may in this way cause deterioration of nitrate-containing emulsion oils.

Bennett and Wheeler (1954) reported that Proteus vulgaris was capable of surviving in one emulsion for longer than 150 days. In the growth study this organism multiplied rapidly and consistently in all of the emulsions studied with the exception of Coolant A.

Proteus morganii, Proteus mirabilis, and unidentifiable Proteus species were isolated sporadically from the emulsions studied (table 5). Failure of one strain of Proteus morganii to survive in pure culture in the six emulsions (table 6) confirms the suspicions of Tant and Bennett (1956) that these species do not constitute an important part of the bacterial flora of emulsion oils.

Pseudomonas species

The presence of Pseudomonas species in emulsion oils has

been reported by many workers (Lee and Chandler, 1941; Duffett, et al, 1943; Weirich, 1943; Pivnick, 1952; Pivnick and Fabian, 1954; and Pivnick, 1955).

These organisms have been shown by many workers to be capable of utilizing cutting oils as nutrient and energy sources. Lee and Chandler described a new species, Pseudomonas oleovorans which they found in almost pure culture in several oil samples obtained from a large machine shop. Stone, Fenske, and White (1942) described several species as capable of attacking hydrocarbon and petroleum fractions.

Sabina and Pivnick (1956) isolated Pseudomonas formicans from a used sample obtained in Illinois, and Pseudomonas oleovorans from a sample obtained from England. They showed that these two species were able to oxidize soluble oil emulsions and emulsifiers, but that they exhibited wide variability in this activity, depending upon the chemical composition of the emulsion. Bennett and Wheeler (1954) reported that Pseudomonas aeruginosa was capable of surviving for longer than 250 days in one emulsion.

The results of this study indicate that Pseudomonas oleovorans and Pseudomonas aeruginosa are equally common in emulsion

oils (tables 4 and 5). The growth study shows that each is equally capable of multiplying over a wide range of coolants (table 6). In addition to these two species, other pseudomonads which could not be readily identified as to species were encountered in 34 per cent of the samples examined.

Organisms of the genus Pseudomonas were, with the exception of Klebsiella pneumoniae (table 4), the only ones isolated from collecting point no. 1 and were the only ones which multiplied to any extent in this coolant (table 6). It has been shown (Fabian and Pivnick, 1953; Pivnick and Fabian, 1954) that growth of pseudomonads in soluble oil emulsions inhibits the growth of other organisms. This study indicates that such is not the case. The time factor involved in shipping used coolant samples to a distant laboratory probably accounts for the failure of Pivnick (1955) to isolate but few organisms other than those belonging to the genus Pseudomonas. Table 1 supports this fact.

On the basis of this study organisms of the genus Pseudomonas constitute an important part of the flora of emulsions, and are probably responsible in part for aerobic deterioration.

Sarcina species

Members of the genus Sarcina are microaerophilic to an-

aerobic gram-positive cocci found in the soil, sand, mud, and water. Pivnick (1952) reported the presence of Sarcina organisms in emulsion oils. One sample obtained in this study contained the organism, but it could not be again isolated for growth study. In view of this, the genus is probably not an important part of the flora of industrial cutting oils.

Shigella madampensis (Shigella dispar)

This is the first time that organisms of the genus Shigella have been reported in used emulsion oils. Bennett and Wheeler (1954) reported that Shigella paradysenteriae (flexneri) was capable of surviving in two different emulsions for as long as 76 days. Shigella sonnei was capable of surviving for only 19 days in one of the emulsions studied. Pivnick, et al (1954) reported that four strains of Shigella sonnei and five strains of Shigella dysenteriae rapidly decreased in numbers and could not be detected after the tenth day of incubation. It is possible that this organism may be introduced into emulsion oils, but it probably dies very quickly. The organism could not again be isolated for growth study in different emulsions.

Streptococcus pyogenes

Although normally found in the human throat and on the skin, the pyogenic streptococci are, upon gaining entrance into the body, the etiological agents of a wide host of diseases ranging from a minor sore throat or cutaneous infection to endocarditis and meningitis.

The C. B. Dolge Company (undated pamphlet) reported that the streptococci were occasionally present in emulsion oils. Bennett and Wheeler (1954) could not show survival of these organisms in two different emulsion oils even for twenty-four hours.

In this study the alpha and beta hemolytic streptococci were isolated a total of seventeen times from the 100 coolant samples studied. Growth study of the organisms inoculated into six different emulsions (table 6) indicates that this frequency was the result of constant inoculation, since in no case were any of the strains capable of surviving more than four days.

Yeasts

Duffett, *et al* (1943) reported the presence of yeasts in emulsion oils. In this study unidentified yeasts were observed in 41 per cent of the samples studied. Failure of the organisms

to grow when reinoculated in pure culture (table 6) indicates that they are not an important part of the normal flora of emulsions, and are probably present as temporary contaminants. Eisenberg and Bennett (unpublished data) however, found organisms having the morphology of yeasts growing in great abundance in laboratory emulsion oil samples. The exact significance of these organisms in commercial cutting oils remains unknown.

Salmonella typhosa

Salmonella typhosa has not previously been isolated from used emulsion oils. However, Bennett (1956) reported the isolation of a Salmonella species from a used emulsion oil sample.

Bennett and Wheeler (1954) reported that a laboratory culture of Salmonella typhosa inoculated into an emulsion oil apparently maintained its virulence as the junior author of that report accidentally contracted a severe case of typhoid fever from contact with the organism.

Pivnick, et al (1954) showed that laboratory cultures of Salmonella schottmuelleri, Salmonella typhimurium, Salmonella oranienburg, and Salmonella pullorum grew readily in oil emulsions. but that all six strains of Salmonella paratyphi tested and three

of the four strains of Salmonella typhosa tested failed to grow.

Bennett and Wheeler (1954) reported that Salmonella typhosa survived for ten days in one of the five commercial cutting oils tested, while Salmonella typhimurium survived only nine days and Salmonella schottmuelleri survived a maximum of six days.

During the initial stages of this investigation no member of the genus Salmonella was isolated. Later, while obtaining samples for the growth study of other organisms, Salmonella typhosa was found in Coolant D, collecting point 4. The organism gave characteristic biochemical reactions of the species, including the production of H_2S. Diagnosis was confirmed by the use of Salmonella Typing Antiserum (Lederle). On the basis of positive reactions to O antigens IX, XII, and Vi, and H antigen d, the organism was placed in Kauffmann-White Group D.

Following identification, a growth study was conducted. The organism multiplied in four of the six oils tested (table 6). Here again is strong evidence that the growth of bacteria in commercial cutting oils is to a great extent dependent upon the composition of the coolant or inhibitor it contains.

VII. Significance of Results

The results of this investigation are highly significant for the following reasons: (1) They increase our knowledge of the bacterial flora of emulsion oils, furnishing a basis for future understanding of the mechanisms of bacterial deterioration of the oils. This has important economic implications for the manufacturer and consumer of cutting oils. (2) They are important from the industrial health standpoint.

For the first time a complete study of the true bacterial flora of emulsion oils has been made. The presence in used emulsions of Aerobacter cloacae, Bacillus subtilis, Diplococcus pneumoniae, Escherichia freundii, Escherichia intermedium, Micrococcus citreus, Micrococcus pyogenes var. albus, Proteus mirabilis, Proteus morganii, Shigella madampensis, and Salmonella typhosa have not been previously reported.

From the results of this study, it is obvious that commercial cutting oils have a more extensive bacterial flora than has been heretofore suspected. The incompleteness of previous reports appears to be the result of isolation methods employed (table 1). The isolation procedures developed in this study now

give us a knowledge of the true flora of emulsion oils. The importance of this lies in the fact that without a complete understanding of the types of organisms present, we cannot hope to control emulsion spoilage with efficiency.

The fact that twenty-seven different species of organisms were isolated, many with consistency, during the course of this investigation is even more remarkable when one considers the nature of the industrial plant cooperating in this research. This machine shop is generally acknowledged to have an exceptionally good industrial hygiene and safety program. The shops are kept clean and are air-conditioned. The presence of insects and small rodents is kept at a minimum by an exterminating firm. The cutting oils are watched carefully, and any complaint of odor or spoilage is immediately acted upon by plant management. Every effort is made to control contamination of the cutting oils. It is logical to suspect that the number and species of bacteria is infinitely higher in a less progressively managed industrial plant where the coolants are changed only when they begin to break down completely or employees refuse to work because of odor.

Despite these efforts to reduce contamination of the oils, it appears that changing coolants and cleaning machines has little

effect on the bacterial flora present (table 2). This fact implies two things: (1) Present methods of cleaning machines and sumps are inadequate and should be improved. (2) Frequent and practically uncontrollable contamination of the emulsion occurs. On the basis of reports by Lee and Chandler (1941), Liberthson (1945), and this study, it seems that the methods of cleaning machines and changing the oils leaves a residual flora which serves as an inoculum for the new wmulsion. Users of cutting oils could reduce their coolant expense considerably by adequate cleaning.

Of the fifteen different species observed in the growth study, seven were able to multiply in at least some of the coolants. The growth of these organisms in pure culture does not necessarily imply that similar results will be obtained when they are studied in the presence of other species. The fact that symbiosis or antagonism between two or more species may occur must be considered. However, it is safe to assume that the organisms which multiply in emulsion oils (tables 6 and 7) are those which are most likely responsible for aerobic deterioration of the emulsion.

Previous reports indicate that the bacterial flora of

emulsion oils is principally of a Gram-positive character. Both the isolation and growth studies of this research support this fact, as Gram-positive organisms were isolated irregularly and failed to grow in pure cultures in the six different emulsions.

A possible explanation for the predominance of Gram-negative organisms involves (1) the nature of the organisms themselves, and (2) the formulation of the cutting oils.

It is known that the bacterial flora of an emulsion oil exists in the water phase of the emulsion, and that the site of bacterial attack of the hydrocarbons is probably on the emulsifying agent at the oil-water interface. Gram-positive organisms are generally more susceptible to detergent toxicity because of the phospholipid envelope surrounding the cell (Wyss, 1951). Hotchkiss (1946), Gale and Taylor (1947), and Mitchell and Crowe (1947) found that cytolytic injury occurs more readily to the Gram-positive organisms, and that amino-acids and other compounds leak from the cell.

The pH range of most commercial cutting oils is slightly alkaline. In view of the fact that the isoelectric point of most Gram-positive bacteria is in the range of pH 2-3, while that of

the Gram-negative bacteria is in the range of pH 4-5, it is possible that insoluble salts are formed in the Gram-positive cells by metal ions present in the alkaline emulsions.

Table 6 demonstrates that Coolant A and Coolant C are relatively insusceptible to bacterial attack. This may be the result of both formulation of these two coolants or the inhibitor used in them. Although these two coolants are the most expensive of all the emulsions studied, they may well be more economical for the consumer since they are more resistant to bacterial attack. Even organisms which showed definite strain variation in their ability to grow in emulsions (plates 1 and 2) were unable to multiply in these two oils. The manufacturer of such oils gains considerable advantage in sales, and the user would probably save money over a period of time by using resistant products. Since conditions vary in different plants, it might be worthwhile for large consumers to conduct bacteriological studies of their own situation to determine which cutting oils are most resistant.

This study gives further support to our knowledge of the ability of bacteria to adapt and mutate to utilize unusual environments; nevertheless many workers fail to realize its im-

portance and possible applications. With particular reference to cutting oils, adaptation and mutation are of importance. The manufacturer and user of emulsion oils must consider the fact that an emulsion which will not support bacterial growth originally may do so within a short period of time. Likewise, an emulsion which will not support growth of one strain of an organism may be an adequate media for multiplication of another strain of the same species.

The importance of this study from the industrial health standpoint cannot be ignored. The presence of extremely high numbers of coliform organisms as well as the intestinal pathogens is indicative of fecal contamination. It is important to remember that the presence of pathogenic bacteria in emulsion oils is potentially dangerous whether or not they are capable of multiplying in the oil. The presence of even small numbers of micrococci and streptococci could be the cause of infections among machine tool workers. Attempts should be made to determine if correlation exists between the potentially pathogenic flora of emulsion oils and employee illnesses in machine shops.

Officials of the plant cooperating in this research feel that if even "run-of-the-mill" cutaneous, upper respiratory, and

gastro-intestinal infections were to be correlated with contaminated coolant solutions, industry would be forced to shoulder a heavy additional financial burden of workman's compensation claims by employees.

In view of the results obtained employing the techniques described, the following future investigations are indicated:

1. Examination of soluble and emulsion oil coolants in many industries to determine how widespread are the conditions found to date.

2. Examination of the same coolant as used in varying conditions as a determination of the extent to which contamination is related to conditions of use.

3. Study of the growth and survival of the isolated organisms in mixed rather than pure cultures to determine exactly what effect one species has upon another.

4. Attempts should be made to trace the source of contamination if possible.

5. Attempts should be made to determine if a correlation exists between illnesses of workers and contamination of the coolants they use.

6. Virulence studies should be carried out to determine if these organisms normally found in emulsions are pathogenic for animals.

7. Studies of the organisms which grow in these oils would be of value in selection of good inhibitors.

* * * * * * *

Acknowledgment

The author would like to express his appreciation to the industry which cooperated in this investigation.

Summary

A new technique for the isolation of aerobic bacteria from used emulsions is described. Failure of other workers to achieve similar isolations is ascribed to their techniques.

Twenty-seven different species of organisms were isolated.

The frequency and variety of organisms was found to vary with different emulsion oils, but was always higher in samples obtained from sumps serving more than one machine.

Inadequacy of the usual methods of cleaning machines and sumps and changing coolants is pointed out.

Growth of bacteria in commercial cutting oils is a factor of both formulation of the coolants and characteristics of the organism.

Some evidence that adaptation by some species to the inhibitor and/or other constituents of the coolant is presented.

The significance of this investigation from the economic, industrial-hygiene, and public health standpoints is discussed.

Future investigations which the author feels should be undertaken are outlined.

APPENDIX

TABLE 1

Effects of Method of Isolation and Time on the Bacterial Flora of Six Used Emulsions

SAMPLE NO.	ORGANISM	TIME FROM COLLECTION OF SAMPLE									
		Immediate		12 hr.		24 hr.		3 days		5 days	
		Old Mtd.	New Mtd.	Old Mtd.	New Mtd.	Old Mtd.	New Mtd.	Old Mtd.	New Mtd.	Old Mtd.	New Mtd.
1	Pseudomonas oleovorans	+	+	+	+	+	+	+	+	+	+
2	Escherichia coli		+		+						
	Klebsiella pneumoniae	+	+	+	+		+				
	Micrococcus pyogenes var. aureus		+								
	Proteus vulgaris	+	+	+	+	+	+	+	+	+	+
	Pseudomonas aeruginosa	+	+	+	+		+		+		+
	Yeast species				+						+
3	Aerobacter aerogenes		+								
	Escherichia coli		+		+		+				
	Micrococcus pyogenes var. aureus		+								
	Paracolobactrum species	+	+		+						
	Proteus vulgaris	+	+	+	+	+	+	+	+	+	+
	Pseudomonas aeruginosa		+		+		+				
	Pseudomonas oleovorans		+		+						
	Yeast species					+					

TABLE 1 (Continued)

SAMPLE NO.	ORGANISM	TIME FROM COLLECTION OF SAMPLES									
		Immediate		12 hr.		24 hr.		3 days		5 days	
		Old Mtd.	New Mtd.	Old Mtd.	New Mtd.	Old Mtd.	New Mtd.	Old Mtd.	New Mtd.	Old Mtd.	New Mtd.
4	Aerobacter aerogenes		+								
	Escherichia coli		+		+		+		+		
	Klebsiella pneumoniae		+	+	+		+		+		+
	Micrococcus pyogenes var. aureus		+								
	Pseudomonas aeruginosa	+	+		+		+	+	+	+	+
	Pseudomonas oleovorans		+	+	+	+	+	+	+		+
5	Escherichia coli		+								
	Paracolobactrum species	+	+		+						
	Proteus vulgaris	+	+	+	+	+	+	+	+	+	+
	Pseudomonas aeruginosa		+		+		+		+		+
	Streptococcus pyogenes, alpha hemolytic		+								
6	Escherichia coli		+								
	Klebsiella pneumoniae		+		+		+				
	Proteus vulgaris	+	+	+	+	+	+		+		+
	Pseudomonas aeruginosa	+	+	+	+	+	+	+	+	+	+
	Pseudomonas oleovorans		+		+		+		+		+

TABLE 2

Organisms isolated from one hundred samples of used emulsion oils

Organisms	Total number of times organisms isolated
Achromobacter species	12
Aerobacter aerogenes	18
Aerobacter cloacae	1
Bacillus cereus	7
Bacillus subtilis	3
Diplococcus pneumoniae	1
Escherichia coli	41
Escherichia freundii	7
Escherichia intermedium	2
Klebsiella pneumoniae	32
Micrococcus citreus	1
Micrococcus pyogenes var. albus	5
Micrococcus pyogenes var. aureus	17
Paracolobactrum intermediates	47
Proteus mirabilis	2
Proteus morganii	6
Proteus species	4
Proteus vulgaris	46
Pseudomonas aeruginosa	57
Pseudomonas oleovorans	64
Pseudomonas species	34
Sarcina species	1
Shigella madampensis (Shigella dispar)	1
Streptococcus pyogenes, alpha hemolytic	11
Streptococcus pyogenes, beta hemolytic	6
Yeast species	14

TABLE 3

Comparison of the microbial flora of seven commercial metal coolants*

Commercial coolant	Organisms	Number of times isolated
Coolant A (Collecting point 1) (Emulsion oil)	Pseudomonas aeruginosa	5
	Pseudomonas oleovorans	9
	Pseudomonas species	4
	Klebsiella pneumoniae	4
Coolant B (Collecting point 2) (Emulsion oil)	Achromobacter species	1
	Bacillus cereus	2
	Escherichia coli	5
	Klebsiella pneumoniae	6
	Micrococcus pyogenes var. aureus	2
	Paracolobactrum species	7
	Proteus vulgaris	7
	Pseudomonas aeruginosa	7
	Pseudomonas oleovorans	5
	Pseudomonas species	3
	Shigella madampensis (Shigella dispar)	1
	Streptococcus pyogenes, alpha hemolytic	3
Coolant B (Collecting point 4)	Achromobacter species	3
	Aerobacter aerogenes	6
	Bacillus cereus	2
	Escherichia coli	6
	Escherichia freundii	2
	Klebsiella pneumoniae	6
	Micrococcus pyogenes var. albus	2
	Micrococcus pyogenes var. aureus	2
	Paracolobactrum species	6

TABLE 3 (Continued)

Commercial coolant	Organisms	Number of times isolated
	Proteus vulgaris	10
	Pseudomonas aeruginosa	9
	Pseudomonas oleovorans	7
	Pseudomonas species	2
	Streptococcus pyogenes, alpha hemolytic	3
	Streptococcus pyogenes, beta hemolytic	2
	Yeasts	4
Coolant C (Collecting point 3) (Soluble oil)	Escherichia coli	5
	Paracolobactrum species	5
	Proteus morganii	3
	Pseudomonas aeruginosa	5
	Pseudomonas oleovorans	11
Coolant D (Collecting point 5) (Emulsion oil)	Achromobacter species	4
	Aerobacter aerogenes	6
	Bacillus cereus	2
	Bacillus subtilis	2
	Escherichia coli	6
	Escherichia freundii	3
	Diplococcus pneumoniae	1
	Klebsiella pneumoniae	6
	Micrococcus pyogenes var. albus	1
	Micrococcus pyogenes var. aureus	4
	Micrococcus pyogenes var. citreus	1
	Paracolobactrum species	6
	Proteus morganii	2
	Proteus vulgaris	7
	Pseudomonas aeruginosa	5

TABLE 3 (Continued)

Commercial coolant	Organisms	Number of times isolated
	Pseudomonas oleovorans	6
	Pseudomonas species	1
	Sarcina species	1
	Streptococcus pyogenes, alpha hemolytic	4
	Streptococcus pyogenes, beta hemolytic	2
	Yeasts	5
Coolant D (Collecting point 6)	Achromobacter species	3
	Aerobacter aerogenes	2
	Bacillus cereus	1
	Bacillus subtilis	1
	Escherichia coli	4
	Escherichia freundii	2
	Klebsiella pneumoniae	5
	Micrococcus pyogenes var. albus	1
	Micrococcus pyogenes var. aureus	4
	Paracolobactrum species	6
	Proteus morganii	1
	Proteus vulgaris	5
	Pseudomonas aeruginosa	7
	Pseudomonas oleovorans	5
	Pseudomonas species	3
	Streptococcus pyogenes, alpha hemolytic	1
	Streptococcus pyogenes, beta hemolytic	2
	Yeasts	4

TABLE 3 (Continued)

Commercial coolant	Organisms	Number of times isolated
Coolant E (Collecting point 7) (Emulsion oil)	*Achromobacter* species	1
	Aerobacter aerogenes	2
	Escherichia coli	8
	Klebsiella pneumoniae	4
	Micrococcus pyogenes var. *albus*	1
	Micrococcus pyogenes var. *aureus*	3
	Paracolobactrum species	8
	Proteus species	1
	Proteus vulgaris	7
	Pseudomonas aeruginosa	8
	Pseudomonas oleovorans	5
	Pseudomonas species	3
	Yeasts	1
Coolant F (Collecting point 8) (Emulsion oil)	*Aerobacter aerogenes*	2
	Escherichia coli	4
	Escherichia intermedium	2
	Klebsiella pneumoniae	1
	Micrococcus pyogenes var. *aureus*	2
	Paracolobactrum species	4
	Proteus vulgaris	4
	Pseudomonas aeruginosa	5
	Pseudomonas oleovorans	9
	Pseudomonas species	6
Coolant G (Collecting point 9) (Emulsion oil)	*Aerobacter cloacae*	1
	Escherichia coli	3
	Proteus mirabilis	2
	Proteus species	3

TABLE 3 (Continued)

Commercial coolant	Organisms	Number of times isolated
	Proteus vulgaris	6
	Pseudomonas aeruginosa	6
	Pseudomonas oleovorans	8
	Pseudomonas species	5

*Eleven samples of each coolant were tested, with the exception of Coolant C, which is represented by twelve samples.

TABLE 4

The weekly frequency of the microbial flora of used emulsion oils for eleven weeks

ORGANISM	COLLECTING POINT 1 Week 1 2 3 4 5 6 7 8 9 10 11	COLLECTING POINT 2 Week 1 2 3 4 5 6 7 8 9 10 11	COLLECTING POINT 3 Week 1 2 3 4 5 6 7 8 9 10 11	COLLECTING POINT 4 Week 1 2 3 4 5 6 7 8 9 10 11	COLLECTING POINT 5 Week 1 2 3 4 5 6 7 8 9 10 11	COLLECTING POINT 6 Week 1 2 3 4 5 6 7 8 9 10 11	COLLECTING POINT 7 Week 1 2 3 4 5 6 7 8 9 10 11	COLLECTING POINT 8 Week 1 2 3 4 5 6 7 8 9 10 11	COLLECTING POINT 9 Week 1 2 3 4 5 6 7 8 9 10 11
Achromobacter species									
Aerobacter aerogenes									
Aerobacter cloacae									
Bacillus cereus									
Bacillus subtilis									
Diplococcus pneumoniae									
Escherichia coli									
Escherichia freundii									
Escherichia intermedium									
Klebsiella pneumoniae									
Micrococcus pyogenes var. albus									
Micrococcus pyogenes var. aureus									
Micrococcus pyogenes var. citreus									
Paracolobactrum intermediates									
Proteus mirabilis									
Proteus morganii									
Proteus species									
Proteus vulgaris									
Pseudomonas aeruginosa									
Pseudomonas oleovorans									
Pseudomonas species									
Sarcina species									
Shigella madampensis (S. dispar)									
Streptococcus pyogenes, alpha hemolytic									
Streptococcus pyogenes, beta hemolytic									
Yeasts									

TABLE 5

Effects of Changing Coolant and Cleaning Machine on the Bacterial Flora

ORGANISM	WEEK					
	Before change			After change		
	1	2	3	4	5	6
Aerobacter aerogenes			+			
Escherichia coli		+	+	+		+
Klebsiella pneumoniae		+			+	
Micrococcus pyogenes var. albus		+				
Micrococcus pyogenes var. aureus						+
Paracolobactrum species	+	+	+	+	+	
Proteus vulgaris	+	+		+		+
Pseudomonas aeruginosa		+	+	+	+	+
Pseudomonas oleovorans	+	+			+	
Pseudomonas species						+
Yeast species			+			

TABLE 6

Growth of bacteria in six commercial cutting oils

ORGANISM	COOLANT A	COOLANT B	COOLANT C	COOLANT D	COOLANT E	COOLANT F
Achromobacter species						
Strain 25-B-4d	±	±	±	±	±	±
Strain 26-D-6c	±	±	±	±	±	±
Aerobacter aerogenes						
Strain 22-F-8b	-	±	-	±	±	±
Strain 23-B-2c	-	±	-	±	±	±
Strain 25-D-5a	-	±	-	±	±	±
Bacillus subtilis						
Strain 21-D-5a	±	±	±	±	±	±
Strain 22-B-2a	-	±	±	±	±	±
Strain 23-D-6a	-	±	±	±	±	±
Escherichia coli						
Strain 20-E-7d	-	-	-	-	-	-
Strain 21-D-5b	-	±	-	+	±	±
Strain 23-B-2a	-	+	-	+	+	±
Escherichia intermedium						
Strain 26-A-4d	-	-	-	-	-	-

The capital letter in the strain number indicates the coolant from which the organism was isolated.
Growth is indicated by the symbols:

- \- Did not survive 7 days
- ± Survived at least 7 days, but did not multiply.
- \+ To 500,000 per ml.
- ++ 500,000 to 5,000,000 per ml.
- +++ 5,000,000 to 50,000,000 per ml.
- ++++ 50,000,000 to 300,000,000 per ml.
- +++++ Over 300,000,000 per ml.

TABLE 6 (Continued)

ORGANISM	COOLANT A	COOLANT B	COOLANT C	COOLANT D	COOLANT E	COOLANT F
Klebsiella pneumoniae	-					
Strain 20-C-3a	-	+	++++	-	-	+++
Strain 22-E-7a	-	+++	++++	-	+	++++
Strain 23-B-4a	-	++++	-	-	++++	+++
Strain 25-D-6a	±	±	-	±	++++	++++
Micrococcus pyogenes var. aureus						
Strain 16-B-8a	-	-	-	-	-	-
Strain 17-D-4a	-	-	-	++	-	-
Strain 19-B-2a	-	-	-	-	-	-
Paracolobactrum species						
Strain 25-B-4b	-	++++	-	+++	++++	++++
Strain 25-F-8a	-	+	-	+	±	+++++
Strain 26-D-5a	-	-	++	±	-	-
Strain 26-C-3b	-	+++++	±	++++	+++++	++++
Proteus morganii						
Strain 26-C-3c	-	-	-	-	-	-
Proteus vulgaris						
Strain 22-C-3a	-	+++	++	+++++	++++	+++++
Strain 23-D-6a	-	+++	++++	+++++	+++++	+++++
Strain 25-B-2a	-	++++	++++	+++++	++++	+++++

TABLE 6 (Continued)

ORGANISM	COOLANT A	COOLANT B	COOLANT C	COOLANT D	COOLANT E	COOLANT F
Pseudomonas aeruginosa						
Strain 26-A-1a	++++	+++++	++++	+++++	+++++	+++++
Strain 27-E-7a	++++	+++++	++++	+++++	+++++	+++++
Strain 28-D-2b	++++	+++++	+++++	+++++	+++++	+++++
Pseudomonas oleovorans						
Strain 26-A-1c	++++	+++++	++++	++++	+++++	++++
Strain 27-C-7c	++++	+++++	++++	+++++	+++++	++++
Strain 28-D-4b	++++	+++++	++++	+++++	+++++	+++++
Salmonella typhosa						
Strain 27-D-4d	-	+++++	-	+++++	++++	++++
Streptococcus pyogenes, alpha hemolytic						
Strain 18-D-5c	-	-	-	-	-	-
Strain 24-F-8a	-	-	-	-	-	-
Strain 25-B-2a	-	-	-	-	-	-
Yeast species						
Strain 25-B-4a	-	±	-	-	-	-
Strain 25-D-5c	-	±	-	-	-	-

TABLE 7

Organisms which grow in emulsion oils

Klebsiella pneumoniae
Paracolobactrum species
Proteus vulgaris
Pseudomonas aeruginosa
Pseudomonas oleovorans
Salmonella typhosa

TABLE 8

Organisms which do not grow in emulsion oils

Achromobacter species
Aerobacter aerogenes
Bacillus subtilis
Escherichia coli*
Escherichia intermedium
Micrococcus pyogenes var. aureus
Proteus morganii
Streptococcus pyogenes, alpha hemolytic
Yeast species

*Grows to a limited extent in some oils.

PLATE 1

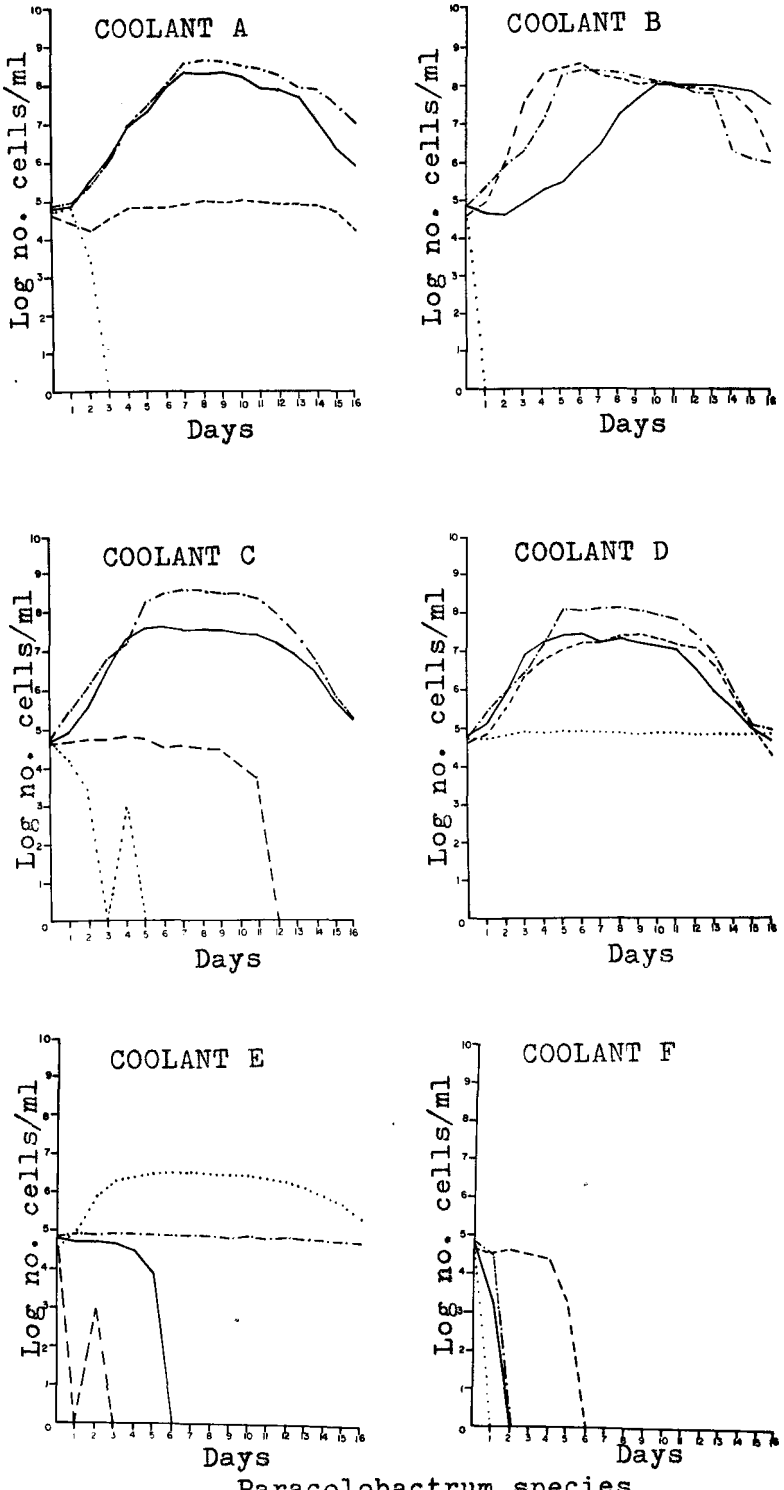
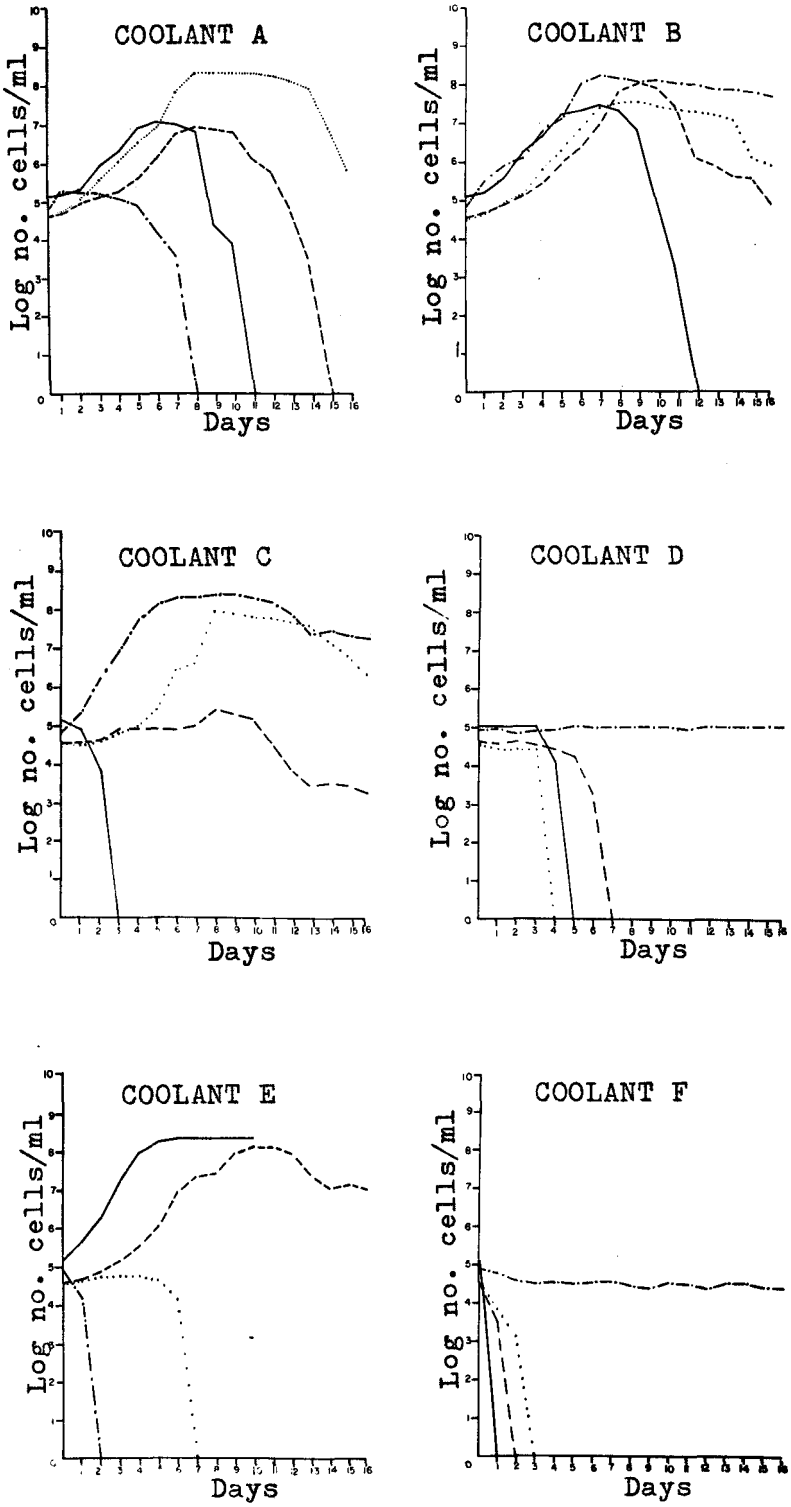

COOLANT A
COOLANT B
COOLANT C
COOLANT D
COOLANT E
COOLANT F
Log no. cells/ml
Days
Paracolobactrum species
25-B-4b
26-C-3b
25-F-8a
26-D-5a

PLATE 2


COOLANT A
COOLANT B
COOLANT C
COOLANT D
COOLANT E
COOLANT F
Log no. cells/ml
Days
Klebsiella pneumoniae
20-C-3a
22-E-7a
25-D-6a
23-B-4a

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