THE PRODUCTION OF CAROTENOID PIGMENTS FROM MINERAL OIL BY BACTERIA

H. F. HAAS AND L. D. BUSHNELL

Received for publication March 27, 1944

During the course of studies of the utilization of petroleum products by microorganisms, several Mycobacterium cultures were isolated which grew well in a mineral salts medium containing mineral oil, and as a result of their metabolism, "oil-soluble" pigments were produced which colored the oil yellow or orange.

Since previous investigators have not described the production of "oil-soluble" pigments by hydrocarbon-utilizing bacteria, a study was made to isolate and characterize the pigments.

The ability of Mycobacterium species, such as M. lacticola, to utilize hydrocarbons and petroleum products in their metabolism has been substantiated by the work of Sohngen (1913), Wagner (1914), Büttner (1926), Haag (1926, 1927), and Jensen (1934).

The importance of this microbial activity in nature, particularly in the breakdown of hydrocarbons, is attested by the universal distribution of these agents in soils and water, since these are the sources of most cultures studied by various workers. Their presence has also been detected by Haas, Yantzi and Bushnell (1941) in oil-field sedimentation ponds and water bottoms of various storage tanks containing petroleum products. Their ubiquity is emphasized further by a recent publication by ZoBell, Grant and Haas (1943) which describes their presence in the sea and marine sediments. Studies by Ingraham and Bauman (1934) with various organisms revealed that Mycobacterium and related species have a particularly high carotene content.

Cryptoxanthin, α-carotene, β-carotene, γ-carotene, lycopene, lutein, zeaxanthin, azafin and dehydro-β-carotene (leprotin) are examples of the carotenoid pigments reported by the following workers as having been produced by various Mycobacterium species [Ingraham and Steenbock (1935), Chargaff and Lederer (1935) and Takeda and Ohta (1939)]. These investigations were made with bacteria grown on fairly complex media containing peptones and other organic compounds such as glycerol and glucose.

CULTURAL METHODS

The culture of M. lacticola employed in this investigation was isolated from mud obtained from a crude-oil sedimentation pond in an oil field near Peabody, Kansas.

1 Contribution No. 218. Department of Bacteriology, Kansas Agricultural Experiment Station. This paper covers a part of the dissertation submitted to the Graduate School of Kansas State College by the senior author as part requirement for the degree of Doctor of Philosophy.

2 Refined light mineral oil having a specific gravity of 0.84 at 25 C and a viscosity of about 105 (Saybolt) at 100 F.
An enrichment technic was used in the isolation of this culture by inoculating 500 ml Erlenmeyer flasks containing 100 ml of mineral salts medium with the following composition:

\[
\begin{align*}
\text{MgSO}_4 \cdot 7\text{H}_2\text{O} & \quad 0.2 \text{ g} \\
\text{CaCl}_2 & \quad 0.02 \text{ g} \\
\text{KH}_2\text{PO}_4 \cdot 3\text{H}_2\text{O} & \quad 1.0 \text{ g} \\
\text{K}_2\text{HPO}_4 & \quad 1.0 \text{ g} \\
\text{NH}_4\text{NO}_3 & \quad 1.0 \text{ g} \\
\text{(NH}_4\text{)}_2\text{SO}_4 & \\
\text{Distilled water} & \quad 1000 \text{ ml}
\end{align*}
\]

The medium was adjusted to pH 7.0 to 7.2 with dilute NaOH. Five per cent sterilized mineral oil was added aseptically to each vessel. The flasks were incubated at room temperature and, when growth was evident, subcultures were made by successive transfers into additional flasks containing the same medium. After several transfers, the organisms were plated on nutrient agar and pure cultures were selected for this study. The strain selected for this investigation possessed the major characteristics of the species *M. lacticola* as described in Bergey's Manual (1939).

The growth of *M. lacticola* in the mineral oil medium was compared with that of stock cultures of *M. phlei*, *M. leprae* and *M. smegmatis*. It was observed that all of the stock culture species could grow in media containing n-decane, light mineral oil, heavy mineral oil and paraffin wax, but they failed to grow in the presence of "Skelly-Solve," gasoline or kerosene. Although the stock cultures were pigmented when grown on ordinary media, they failed to yield pigments when cultured with hydrocarbons. The growth of *M. lacticola* in this medium differed only in its ability to produce pigments.

The growth of *M. lacticola* in mineral oil media first appeared as a thin, opaque, white film at the interface between the oil and water. Pigmentation of the cells became evident at the time the film thickened and a pale yellow color was imparted to the oil. Due to the nature of the surface of the organisms, the cells passed into the oil phase forming an emulsion just above the oil-water interface. As a result the water phase contained few organisms as indicated by the absence of turbidity or pigmentation. As the culture developed the oil became more heavily pigmented and the color deepened to a bright orange.

In order to obtain adequate amounts of pigments for study, *Mycobacterium lacticola* was cultured in large five-gallon, pyrex bottles containing four L of the mineral salts medium and 100 ml of mineral oil (light medicinal grade). These bottles were initially inoculated with the growth from agar slants. Subsequent transfers were made of the oil-bacteria emulsion from one bottle to the next. By employing this technic it was possible to increase the rate of growth and to reduce the period of incubation for maximum growth and pigment production from approximately 40 to 15 days.

The bottles were incubated in a horizontal position so as to increase the amount of surface exposed. As soon as there was visible growth the bottles
were agitated daily. This resulted in the formation of a bacterial emulsion in the oil and accelerated the development of the organisms. The cultures were incubated at a temperature of 37°C, the period varying from several weeks to nine months, in order to study the influence of time on pigmentation.

The isolation and characterization of oil-soluble pigments is complicated by the fact that carotene cannot be removed from oil by the conventional methods used in the analysis of plant tissues. Extraction methods employing various solvents were not successful because the pigments were either not removed or, if removed, were accompanied by some of the oil. Several different procedures were attempted but the most successful was a modification of the Peterson, Hughes, and Freeman (1937) method for the determination of carotene in plant tissues.

Since preliminary experiments indicated no qualitative differences in the pigments found in the oil and bacterial cells (separated by centrifugalization), no attempt was made to make separate quantitative determinations on the oil and bacterial cells. The objective of this investigation was restricted to a determination of the total amount of pigments produced from the mineral-oil medium and their identity.

The pigmented oil-phase, containing emulsified bacterial cells, was separated from the mineral salts solution by siphoning off the lower aqueous phase. Then the pigmented oil and bacterial emulsion was transferred to Erlenmeyer flasks, and equal volumes of freshly prepared, 10 per cent solution of KOH in ethyl alcohol were added. The flasks were then fitted with reflux condensers and boiled on a steam bath for thirty minutes to saponify the pigmented material.

After the flasks were allowed to cool the contents were transferred to separatory funnels and mixed with approximately 100 ml of distilled water. The red, alkaline alcohol-water solution was then drawn off, diluted with an equal volume of distilled water and re-extracted three or more times with 30 ml portions of "Skelly-Solve" until the extractions were colorless. The pigmented, alkaline, aqueous, alcoholic phase was analyzed for astacin as described later in this paper. The "Skelly-Solve" extracts were combined with the pigmented mineral oil and the mixture was washed with distilled water until free from alkali, as indicated by the absence of color in the wash water when tested with phenolphthalein.

The petroleum mixture was then extracted with 25 ml of 90 per cent methyl alcohol to remove any xanthophylls that might be present. Since no xanthophylls were detected, additional extractions with methyl alcohol were not necessary. The methyl alcohol was removed from the petroleum mixture by washing several times with distilled water in a separatory funnel.

The pigmented mineral oil "Skelly-Solve" solution was transferred to a large Erlenmeyer flask and the "Skelly-Solve" carefully distilled off under vacuum on a steam bath, leaving the saponified pigments in mineral oil.

The pigments were then separated from the oil by employing a modification of the Tswett (1906) chromatographic adsorption technic as described by Haas et al. (1942), in which a column of MgO (Micron Brand No. 2641) in large glass filters was used. After adsorption, the pigmented portion was removed me-
chanically and dissolved in petroleum ether. The pigments were again chro-
matographed on a column containing 50% MgO and 50% siliceous earth. Three
distinct pigmented bands were obtained in this manner. The color of the suc-
cessive bands on the developed chromatogram from the lowest to the highest
were: (I) yellow, (II) orange, and (III) pinkish orange.

![Absorption Spectra of β-Carotene and the Carotenoid Pigments of Myco-
bacterium lacticola in Petroleum Ether](image)

**FIG. 1. ABSORPTION SPECTRA OF β-CAROTENE AND THE CAROTENOID PIGMENTS OF MYCOBACTERIUM LACTICOLA IN PETROLEUM ETHER**

STUDY OF THE PIGMENTS

The absorption spectrum was determined for each pigment by means of a
Bausch and Lomb No. 2750 visual photometer and No. 2700 spectrometer. This
revealed that all three of the pigments had absorption maxima similar to that
of pure β-carotene (fig 1). This phenomenon of having three carotene pigments
with similar spectrometric properties has also been observed by Chargaff (1934)
with *Mycobacterium phlei* which had been cultured on ordinary nutrient agar.
Ingraham and Steenbock (1935) believed that this was due to the presence of
cryptoxanthin and zeaxanthin, since these were not differentiated spectrophoto-
metrically and were found to be present in the same culture.
CAROTENOID PIGMENTS FROM MINERAL OIL

It is no doubt possible for such a phenomenon to occur in the presence of β-carotene, cryptoxanthin and zeaxanthin, but the presence of the latter two pigments was ruled out by the fact that repeated attempts to detect xanthophylls were unsuccessful. It is therefore improbable that two of these pigments were xanthophylls.

Takeda and Ohta (1939, 1940) reported that M. phlei produced leprotin (dehydro-β-carotene) which has the spectrometric properties of β-carotene, and stated that this is the material reported by the other workers as β-carotene. This pigment was found to have provitamin A activity (20µg per day cured rats of xerophthalmia).

The variation in the reports of various workers concerning M. phlei regarding both carotene and xanthophyll pigments indicates the difficulties that may be encountered in studies of this nature. The possibility of encountering isomers of β-carotene as reported by Zechmeister and Tuzon (1938) must also be taken into consideration. Since leprotin and isomers of β-carotene have some vitamin A potency, a biological assay was made to determine the vitamin A activity of the pigments in order to characterize them more fully.

BIOLOGICAL ASSAY

It was necessary to obtain relatively large quantities of bacterial carotenes for feeding experiments in order to carry out a biological assay on their vitamin A value. This was achieved by inoculating a large number of bottles containing a total of 500 ml of mineral oil. These bottles were cultured for two weeks at 37°C, and the pigments isolated from the oil according to the procedure previously outlined.

The concentration of each pigment (calculated as β-carotene) was determined by means of the photoelectric photometer and the visual spectrophotometer according to the method outlined by Peterson, Hughes and Payne (1939). The concentration of each of these pigments per 500 ml of oil is shown in table 1.

The pigments were then dissolved in sufficient Wesson oil to give 1.2µg (equivalent to two units of vitamin A) per drop of oil. This oil was then used as a supplement in the rat-feeding experiments.

The biological assay was carried out with 48 young white rats especially bred and depleted of vitamin A for use in vitamin A assay. These rats were placed on the "vitamin A-free" diet prescribed by the United States Pharmacopoeia XI (1936) for the official assay of cod liver oil.

The rats were weighed at weekly intervals until a majority ceased to show any gain in weight, after which they were weighed for three consecutive days, then carefully sorted into eight groups of six rats each, and segregated in individual cages. These groups were fed the supplements indicated in table 2.

By this method it was possible to compare the vitamin activity of the bacterial carotene pigments with that of known β-carotene. Group VIII was introduced as a check on the method of isolating the pigments in order to determine whether any loss in the vitamin potency could be attributed to the technic employed. A known amount of S.M.A. β-carotene was dissolved in mineral
oil and the \( \beta \)-carotene was again separated by employing the same procedure as outlined in this paper.

### TABLE 1

**Concentration of carotenoid pigments produced from mineral oil, measured as \( \beta \)-carotene**

<table>
<thead>
<tr>
<th>PIGMENT</th>
<th>MILLIGRAMS PER 500 ML OF OIL</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.3678</td>
</tr>
<tr>
<td>2</td>
<td>0.4351</td>
</tr>
<tr>
<td>3</td>
<td>1.3400</td>
</tr>
</tbody>
</table>

### TABLE 2

**Groups of rats employed in the biological assay for vitamin A activity of bacterial carotenoid pigments**

<table>
<thead>
<tr>
<th>RAT GROUP</th>
<th>EQUIVALENT UNITS OF VITAMIN A FED DAILY</th>
<th>MICROGRAMS OF PIGMENTS FED DAILY</th>
<th>SUPPLEMENTS DISSOLVED IN WESSON OIL</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>0</td>
<td>0.0</td>
<td>None</td>
</tr>
<tr>
<td>II</td>
<td>1</td>
<td>0.6</td>
<td>S.M.A. ( \beta )-carotene</td>
</tr>
<tr>
<td>III</td>
<td>2</td>
<td>1.2</td>
<td>S.M.A. ( \beta )-carotene</td>
</tr>
<tr>
<td>IV</td>
<td>2</td>
<td>1.2</td>
<td>Pigment No. 1</td>
</tr>
<tr>
<td>V</td>
<td>2</td>
<td>1.2</td>
<td>Pigment No. 2</td>
</tr>
<tr>
<td>VI</td>
<td>2</td>
<td>1.2</td>
<td>Pigment No. 3</td>
</tr>
<tr>
<td>VII</td>
<td>2</td>
<td>1.2</td>
<td>S.M.A. ( \beta )-carotene from mineral oil†</td>
</tr>
</tbody>
</table>

* These feeding tests were conducted under the direction of Dr. W. J. Peterson, formerly with the Department of Chemistry of this college.
† The \( \beta \)-carotene employed in this study was obtained from S.M.A. (\( \beta \)-carotene and 10 per cent \( \alpha \)-carotene). The \( \alpha \)-carotene was removed by the chromatograph absorption technic employing MgO and petroleum ether.

### TABLE 3

**Average weekly gain in weight of both male and female rats**

<table>
<thead>
<tr>
<th>GROUP</th>
<th>SUPPLEMENT FOR EACH GROUP</th>
<th>AMOUNT FED DAILY</th>
<th>AVERAGE GAIN IN WEIGHT</th>
<th>COMBINED AVERAGE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>( \mu g )</td>
<td>( \xi ) ( \eta )</td>
<td>( \zeta )</td>
</tr>
<tr>
<td>I</td>
<td>Negative control</td>
<td>0.0</td>
<td>2.9 -5.7</td>
<td>-4.1</td>
</tr>
<tr>
<td>II</td>
<td>( \beta )-carotene</td>
<td>0.6</td>
<td>10.7 6.3</td>
<td>8.9</td>
</tr>
<tr>
<td>III</td>
<td>( \beta )-carotene</td>
<td>1.2</td>
<td>13.2 12.9</td>
<td>13.0</td>
</tr>
<tr>
<td>IV</td>
<td>Pigment No. 1</td>
<td>1.2</td>
<td>6.8 8.1</td>
<td>7.5</td>
</tr>
<tr>
<td>V</td>
<td>Pigment No. 2</td>
<td>1.2</td>
<td>13.8 10.5</td>
<td>12.2</td>
</tr>
<tr>
<td>VI</td>
<td>Pigment No. 3</td>
<td>1.2</td>
<td>-2.2 -7.3</td>
<td>-4.2</td>
</tr>
<tr>
<td>VII</td>
<td>( \beta )-carotene control</td>
<td>1.2</td>
<td>11.2 11.7</td>
<td>12.0</td>
</tr>
</tbody>
</table>

The average weekly gains in weight of both male and female rats are listed in table 3 and fig. 2.

These data are interpreted as showing that: (1) pigment 3 has no vitamin A activity, (2) pigment 2 has approximately the same vitamin A potency as \( \beta \)-
carotene, (3) pigment 1 has approximately one-half the potency of \( \beta \)-carotene, and that (4) the technic employed in this study for the isolation of the bacterial carotenoid pigments did not appreciably decrease the vitamin A value of the pigments.

The red alkaline alcohol-water phase remaining after saponification during the carotene determinations was found to contain a single red acidic carotenoid pigment which exhibited chemical and spectrographic properties similar to astacin.

Karrer, Loewe and Hübner (1935) found astacin to be tetraketo-\( \beta \)-carotene which is capable of acting as a weak acid due to tautomeric keto-enol equilibrium conditions. Thus, the pigment following saponification with alkali is converted into a salt which is soluble in basic aqueous-alcoholic solutions. This salt is readily hydrolyzed in slightly acidic solutions, yielding the free pigment which is soluble in petroleum ether.
These chemical characteristics were observed with this bacterial pigment. Partitioning experiments revealed that the potassium salt of the pigment in alkaline solution was insoluble in petroleum ether but soluble in diethyl ether. When the alkaline alcoholic phase was acidulated slightly, the red potassium salt was hydrolyzed and the color of the solution changed from red to yellow. The free pigment was soluble in petroleum ether to which it imparted a yellow color. It was also noted that during acidulation some of the pigment precipitated in the form of a red powder at the interface.

![Absorption Spectra of Astacin](image)

**FIG. 3. THE ABSORPTION SPECTRA OF ASTACIN OBTAINED FROM THE LOBSTER SHELL AND FROM MYCOBACTERIUM LACTICOLA**

The free pigment could be extracted from the petroleum ether by washing with 5 per cent KOH in 50 per cent ethanol.

Chromatographic experiments with the pigment in petroleum ether indicated that the pigment was tenaciously adsorbed at the top of MgO columns. The pigment could not be eluted with 1 per cent alcohol in petroleum ether, but required the use of KOH in 95 per cent alcohol.

In addition to these chemical properties, the pigment in CS₂ was found to have a single absorption maximum at 5000 Å which is the same as that given for astacin by Strain (1935).

For comparative purposes astacin was isolated from the shell of a fresh lobster by saponification with 10 per cent alcoholic KOH solution for one hour. The
highly pigmented red solution was then diluted with an equal volume of distilled water and extracted with petroleum ether which yielded only a trace of a yellow pigment. This was discarded.

The diluted alcoholic KOH solution was acidified and the pigment extracted with petroleum ether. The petroleum ether was washed repeatedly with distilled water, dried by passing through anhydrous Na₂SO₄, and evaporated under vacuum. The astacin was redissolved in CS₂ and was found to have a single absorption maximum similar to that of the bacterial astacin (fig. 3).

There is no evidence as to whether this pigment occurs naturally in the culture in the form of an ester, such as astaxanthin, or as the free pigment. Kuhn and Sorensen (1938) found that astacin from crustaceans is derived from astaxanthin. If such an ester did exist originally, the saponification employed in this analysis would convert it to astacin. It is noteworthy that attempts to demonstrate the presence of astacin in cultures grown on nutrient agar were unsuccessful, thus indicating that astacin is not produced by this organism when grown in the usual manner. A study of the carotenoids produced by this organism when grown on nutrient agar is discussed in the latter part of this paper.

EFFECT OF INCUBATION TIME

During the course of study on the production of carotenoid pigments from mineral oil by M. lactiloca, some qualitative and quantitative differences were observed which were apparently due to the time of incubation.

Most of the earlier investigations on the carotenoid pigments produced from mineral oil were carried out with bacterial cultures that had been incubated one month or longer, and the spectrometric curves obtained were not characteristic for any of the known carotenes. Pigments from such cultures revealed only one absorption maximum, at approximately 4500 A when petroleum ether (BP30–60 C) was used as a solvent. These curves were similar to those reported by Peters, Hughes and Payne (1939) for the carotene fractions of dehydrated oats and for β-carotene which had been exposed to the action of sunlight; this indicates that prolonged incubation brings about partial destruction of the carotene pigments.

Cultures in four large pyrex bottles were prepared, inoculated as previously described, and incubated at 37 C. At 15-day intervals a bottle was removed and analyzed. The relative concentration of the carotene pigments in the combined mineral oil and bacterial emulsion was determined by measuring the transmission of light by means of a KWSZ photometer, using a light source of 4600 A.

The red, alkaline alcohol-water phase was then extracted with diethyl ether to remove the astacin, which also was measured electrophotometrically. The results of this study are given in table 4.

Despite the fact that an insufficient number of samples was studied, it is possible to follow a certain trend of change in the concentration of the pigments. The carotene content appears to be highest at fifteen days, whereas the astacin appears to increase slightly with longer incubation. This change in pigmentation was also apparent with repeated observations of the cultures during incu-
bation; the initial yellow appearance of the oil gradually changed to dark orange upon longer incubation.

Additional evidence for this trend is given by a chromatographic study of the pigments from one-month-old and nine-month-old cultures. These pigments in petroleum ether were subjected to a chromatographic study by passage through columns of equal parts of MgO and siliceous earth.

A study of the developed chromatograms revealed that the top band (astacin) increased in width and intensity with the time of incubation, whereas the lower carotene pigments (pigments 1, 2, and 3 described in this study) decreased.

**TABLE 4**

<table>
<thead>
<tr>
<th>PERIOD OF INCUBATION</th>
<th>PERCENTAGE TRANSMISSION OF LIGHT</th>
<th>PERCENTAGE TRANSMISSION OF LIGHT</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Astacin (dissolved in 250 ml diethyl ether)</td>
<td>Carotene pigments (dissolved in 100 ml petroleum ether)</td>
</tr>
<tr>
<td>days</td>
<td></td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>44.0</td>
<td>27.1</td>
</tr>
<tr>
<td>30</td>
<td>24.0</td>
<td>45.4</td>
</tr>
<tr>
<td>45</td>
<td>35.0</td>
<td>34.8</td>
</tr>
<tr>
<td>60</td>
<td>32.0</td>
<td>48.3</td>
</tr>
</tbody>
</table>

**PRODUCTION OF CAROTENOID PIGMENTS BY MYCOBACTERIUM LACTICOLA ON NUTRIENT AGAR**

For comparative purposes, a chromatographic study was made of the carotenoid pigments produced by this organism when cultured on nutrient agar. Eight Roux bottles containing nutrient agar were inoculated with a suspension of cells removed from agar slants and were incubated for two weeks at 37 C. The resulting orange growth was carefully harvested by washing with minimum amounts of 10 per cent alcoholic KOH. This material was then transferred to Erlenmeyer flasks and refluxed for 30 minutes. It was then cooled, diluted with an equal volume of distilled water, and extracted repeatedly with petroleum ether. The pigmented petroleum ether solution was washed until free of alkali, dried by passing through anhydrous sodium sulfate, and a portion evaporated down to a volume of approximately 1.0 ml.

A chromatogram was made by passing the pigments through a column of equal parts of MgO and siliceous earth. Six pigmented bands were detected in the column. The colors of these bands in descending order were: purple violet, reddish-orange, orange, light orange, and yellow. It was observed that only the lower three bands could be washed through the column, while the top three bands did not change their respective positions. The lower three bands resembled those found in the oil, but due to insufficient quantities, they could not be studied spectrometrically. The tenacity with which the upper three bands were adsorbed suggested the presence of xanthophylls. The presence of xanthophyll pigments was substantiated since they were removed by extraction of the original petroleum ether with 90 per cent methanol.
Another interesting observation was that the alkaline KOH phase following saponification of the pigmented oil did not contain any red pigment, thus indicating that astacin was not formed under these conditions.

The results of this study indicate that the type of medium has a marked influence on the type of carotenoid pigments produced by *M. lacticola*. When cultured on nutrient agar, carotenes and xanthophylls were produced but not astacin, whereas, cultures of the organism on mineral oil media yielded carotene and astacin but no xanthophylls.

**DISCUSSION**

The observations made during the course of pigment study with numerous cultures grown at various intervals over a two-year period may be summarized as follows:

In actively growing cultures during the first two or three weeks of incubation, the three carotene pigments retain their spectrometric property which is similar to \( \beta \)-carotene. Upon continued incubation (30 days or more) they lose this characteristic spectrometric property, revealing a marked decline in their absorption at 4800 Å. As a result, an absorption curve with only a single maximum at 4500 Å is shown. Quantitative determinations of whole cultures reveal a reduction in the total amount of carotene pigments and a consistent rise in the quantity of astacin as the cultures undergo prolonged incubation.

These changes and the failure to detect xanthophylls suggest that carotene pigments under these conditions may undergo oxidation, giving rise to polyketo derivatives such as astacin, which is tetra-keto-carotene. This type of oxidation of hydrocarbons by microorganisms has been suggested by Hopkins and Chibnall (1932). Their studies of the oxidation of synthetic paraffin by *Aspergillus versicolor* indicated that the primary product of oxidation was a ketone or polyketone.

The production of oil-soluble pigments such as carotenoids and their derivatives by hydrocarbon-oxidizing bacteria, as noted in this study, may be of practical significance in view of the fact that one of the problems of the petroleum industry is the prevention of “off-colors” in water-white petroleum products during storage. The organism described in this paper produced approximately 2.14 mg of carotenoid pigments (calculated as \( \beta \)-carotene) in 500 ml of oil within two weeks. This suggests the possibility that the slight yellowish “off-colors” commonly observed in petroleum products during storage may in part be of microbial origin. This speculation has added significance when one considers that the bacterial content of water commonly present at the bottom of large storage tanks has been found to be quite high (Haas, Yantzi and Bushnell, 1941).

**SUMMARY**

A strain of *Mycobacterium lacticola* isolated from mud in contact with crude oil was found to produce carotenoid pigments when cultured in a mineral salts medium with mineral oil as the sole organic substrate.
Analysis of the pigmented oil medium revealed the presence of four carotenoid pigments. Three were carotene pigments having spectrographic properties similar to that of \( \beta \)-carotene but different degrees of chromatographic behavior and vitamin A activity. Although one of the pigments may be identified as \( \beta \)-carotene, the other two could not be identified, and are believed to be isomers of \( \beta \)-carotene.

A fourth pigment was found to possess chemical and spectrographic properties of astacin, an acidic carotenoid found primarily in crustaceans and hitherto never before associated with carotenoids of \( \text{Mycobacterium} \) species.

Prolonged incubation was found to effect spectrometric and quantitative changes in the pigmentation of mineral oil cultures. The quantity of carotene pigments decreased while the amount of astacin increased.

REFERENCES


Wagner, R. 1914 Benzol bacteria which can utilize benzol, toluol and xylol as a source of carbon. Z. Gärungsphysiol., 4, 289-319. (Chem. Abstracts, 8, 3193)
