Comparative Utilization of Paraffins by a 
*Trichosporon* Species

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Utilization of normal and isoparaffins, separately and in mixtures, by a *Trichosporon* sp. was investigated. From a mixture of normal paraffins and isoparaffins, the organism consumed straight-chain paraffins, leaving the branched paraffins relatively unchanged. When offered separately, the highest utilization of *n*-alkanes by the organism was obtained in the range of undecane to octadecane; *n*-pentadecane was poorly utilized. From a mixture of *n*-alkanes, the rate of consumption of shorter-chain alkanes, *n*-decane to *n*-dodecane, was found to be relatively faster and more uniform than that of longer-chain alkanes.

Considerable attention has been paid in recent years to the cultivation of yeast on gas oil for the production of single-cell protein (2, 3, 11, 15). Yeast utilizes predominantly the normal paraffinic components of the gas oil for growth. There are various reports on the assimilation characteristics of individual *n*-alkanes by yeast during which each *n*-alkane is present alone as carbon source (10, 13, 16, 18), but they do not give sufficient information on the interaction of various *n*-alkanes on their capacity to be utilized by microorganisms. As gas oil contains a mixture of *n*-alkanes of different carbon chain lengths, it is desirable to know the order and extent of utilization by yeast of individual *n*-alkanes in the mixture. Some reports have been published on the nature of utilization of mixtures of *n*-alkanes by microorganisms. Miller and Johnson (11) cultivated a mixture of *Candida lipolytica* and *C. intermedia* on five representative gas oil samples and paraffin wax and found that the organisms utilized *n*-paraffins ranging from dodecane through dotriacontane with the maximum efficiency of alkane removal in the nonadecane to tetracosane range. Dostálek and coworkers (3) observed different rates of degradation of individual *n*-alkanes present in gas oil by *C. lipolytica*. Wagner et al. (21) cultivated a *Nocardia* species on a mixture of *n*-alkanes ranging from decane to eicosane and found that all *n*-alkanes offered were almost completely utilized. Tanaka and Fukui (17) reported substrate specificities for yeasts grown on *n*-alkane mixtures.

In studies of selected individual branched paraffins, it has been reported that isoparaffins, in general, are utilized poorly or not at all (4, 18, 19). Very little is known about the nature of degradation of isoparaffins present in gas oil by yeast. There are conflicting reports in the literature on the paraffin utilization by *Trichosporon* species. Markovetz and Kallio (9) found that *T. capitatum* assimilated *n*-tetradecone and 1-tetradecene but not *n*-decane, *n*-dodecane, *n*-hexadecane, and *n*-octadecane. Iizuka and co-workers (6) observed that this organism grew well on kerosene after 3 days of culture, but *T. cutaneum* did not. Inability of the latter to grow on kerosene was also reported by Komagata et al. (8). More recently, Itoh and Doi (7) reported the characterization of a *Trichosporon* species which showed good utilization of *n*-alkanes ranging from decane to octadecane, and we described the isolation, characterization, and growth studies of a gas oil utilizing a *Trichosporon* strain (15, 20).

This communication reports the comparative utilization of individual *n*-paraffins and iso-paraffins by *T. pullulans*.

**MATERIALS AND METHODS**

**Organism.** *T. pullulans* RAY-13 (20) was used in this study. It was maintained on agar slants of a medium containing peptone, malt extract, yeast extract, and glucose.

**Culture medium.** Composition of the mineral salt medium was described in an earlier paper (15). Normal alkanes (98 to 99% purity) were obtained from British Drug Houses; Schuchardt München, West Germany; and Koch-Light Laboratories, Colnbrook, England. A paraffin mixture containing *n*-alkanes from decane to pentadecane was supplied by Texaco Inc. After adding suitable amounts of missing *n*-alkanes to this mixture, a working paraffin mixture was prepared,
having the composition (wt. %): n-decane, 2.9; n-undecane, 12.1; n-dodecane, 16.2; n-tridecane, 24.4; n-tetradecane, 21.7; n-pentadecane, 0.98; n-hexadecane, 13.0; n-octadecane, 4.3; and n-eicosane, 4.1.

A composite paraffin mixture containing normal and isoparaffins was obtained from a gas oil fraction (bp, 250 to 350°C) supplied by Assam Oil Co., Digboi, Assam, by the urea adduct procedure (5).

Cultivation methods. (i) Utilization of individual n-alkanes by the organism was determined in a 500-ml shaken conical flask containing 50 ml of mineral salt medium and 0.5 ml of the n-alkane to be tested. Yeast cells from a slant culture which had been incubated at 30°C for 36 hr were transferred to each flask. Incubation was carried out on a rotary shaker for 48 hr at 30°C. Nitrogen consumption, dry-cell yield, and pH change were measured in each flask.

(ii) Batch cultivation of the organism on composite paraffin mixture isolated from gas oil was carried out in an air-lift fermentor (15) of 3-liter capacity in the presence of mineral salt medium containing 2.5% of the paraffin mixture. Air-flow rate was controlled at 15 liters/min at 30°C and maintained at pH 4.5 by 2 N NH₄OH.

(iii) For the cultivation of the organism on pure alkane mixture, a conventional stainless-steel fermentor equipped with a pair of multiple blade impellers and air sparger was used. The fermentor was charged with 3 liters of culture medium containing 1% of n-alkane mixture. About 500 mg (dry wt) of growing cells from shaken flasks was used as inoculum. The medium was incubated at 550 rev/min; air-flow rate (5 liters/min) was maintained at 30°C (pH 4.5) by the addition of 2 N NH₄OH. Samples were taken out at regular intervals for analysis.

Analytical methods. Residual paraffins from samples were extracted thrice in a separatory funnel by shaking with 1 volume of acid-washed and distilled n-hexane. Recovery experiments showed that quantitative extraction of paraffins from the sample could be achieved by this procedure. The hexane extract was concentrated under reduced pressure, brought to a definite volume with purified and distilled hexane, and analyzed for individual paraffins. Purity of hexane used for extraction was adequate, and gas chromatographic analysis showed that it did not contain any compound that would interfere with the resolution of the paraffins used in these studies. Separation of individual paraffins in the extract by gas chromatography was achieved through 10% RYSORB BLK column in a Chrom-2 gas chromatograph with flame ionization detector and isothermal programming. Two chromatograms were run, one for lower paraffins and the other for higher paraffins eluted at two different temperatures (145 and 180°C). Quantitative estimations of paraffin components were done with reference to pure compounds.

To determine the dry weight, cells were washed with the solvent and dried to constant weight (15). Nitrogen in the ammonium salt was estimated by semimicro Kjeldahl distillation and titration.

RESULTS AND DISCUSSION

Utilization of individual n-alkanes ranging from n-decane to n-eicosane for growth by T. pullulans RAY-13 in the shaken flask is shown in Table 1. With the exception of n-pentadecane, the organism utilized all alkanes comparatively well, as indicated by the dry-cell yield, by the nitrogen consumption, and by the pH decrease. The experiment with n-pentadecane was repeated several times and gave essentially the same results. Because of its high melting point (36.5°C), normal eicosane was added to the flask as fine powder. Its dispersion in the medium was not as good as other liquid n-alkanes which may be the reason for its very poor utilization.

Paraffins isolated from gas oil by urea adduct method contained about 93.7% (wt/wt) of n-paraffins and 6.3% (wt/wt) of isoparaffins. In the chromatogram, isoparaffins appeared in regular sequences as smaller peaks between high peaks of n-paraffins (5). After cultivation of the organism on this paraffin mixture for 17 hr, the n-paraffin concentration of the residual hydrocarbon was decreased to 70% and, correspondingly, isoparaffin content was increased to 30% (Table 2). This indicates that n-paraffins were utilized by the organism but that isoparaffins were utilized poorly or not at all. The analysis of individual n-paraffins showed that the feed contained n-alkanes ranging from dodecane to heneicosane with trace amounts of undecane (Fig. 1A). Although the concentration of shorter-

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**Table 1. Growth of Trichosporon pullulans RAY-13 on various n-alkanes in the shaken flask**

| n-alkane      | pH of the medium | Nitrogen consumption (mg/50 ml of broth) | Dry-cell yield (mg/50 ml of broth) |
|---------------|------------------|----------------------------------------|-----------------------------------|
|               | Initial          | Final                                  |                                   |
| n-Decane      | 5.8              | 2.5                                    | 7.25                              | 164                               |
| n-Undecane    | 5.8              | 2.0                                    | 24.05                             | 209                               |
| n-Dodecane    | 5.8              | 2.0                                    | 17.30                             | 192                               |
| n-Tridecane   | 5.8              | 2.0                                    | 19.75                             | 231                               |
| n-Tetradecane | 5.8              | 2.0                                    | 12.35                             | 183                               |
| n-Pentadecane | 5.8              | 2.5                                    | 5.45                              | 86                                |
| n-Hexadecane  | 5.8              | 2.0                                    | 18.75                             | 208                               |
| n-Octadecane  | 5.8              | 2.0                                    | 17.30                             | 196                               |
| n-Eicosane    | 5.8              | 5.5                                    | 2.20                              | 29                                |

**Table 2. Relative degradation of normal and isoparaffins from a mixture by Trichosporon pullulans RAY-13**

| Paraffin          | Before cultivation | After cultivation |
|-------------------|--------------------|-------------------|
| Normal paraffin   | 93.7               | 70.0              |
| Isoparaffin       | 6.3                | 30.0              |

*Paraffin mixture isolated from gas oil by urea adduct procedure.
chain alkanes showed a decrease, longer-chain alkanes (n-nonadecane, n-eicosane, and n-
eheicosenes) showed an increased concentration in the residual hydrocarbon. This possibly indicates different utilization rates of the alkanes by the organism. The utilization of isomeric alkanes showed similar patterns (Fig. 1B).

The data obtained in an experiment designed to determine rates of alkane utilization showed that the substrate was rapidly consumed (Table 3). After 15 hr of cultivation, 73% was consumed and after 20.5 hr, 91% of the initial alkane was consumed; at the end of the fermentation (32 hr) only 23% of the initial alkane remained un-consumed and the dry-cel yield amounted to 6 g/liter.

The organism can utilize all the alkanes quite well since they were found in negligible amounts at the end of the fermentation. The pattern of consumption, however, was not uniform. This can be seen when the concentration of individual alkanes is expressed as per cent of the total residual alkanes at each interval (Table 3). The concentrations of n-decane, n-undecane, and n-dodecane showed regular decrease until the 15th hr of cultivation and then sharply increased. The concentrations of n-tridecane, n-tetradecane, n-hexadecane, n-octadecane, and n-eicosane, however, showed gradual increase as late as the 15th hr of cultivation and subsequently decreased. The concentration of n-pentadecane was never lower than the initial value at any time during the course of fermentation. These results indicate that the organism utilizes shorter-chain n-alkanes faster than the longer-chain ones. This fact is brought out more clearly in Fig. 2. The curves of n-decane, n-undecane, and n-dodecane show a steep decline until a low value has been reached

![Figure 1](image)

**Fig. 1. Distribution of normal and iso-paraffins in the paraffin mixture derived from gas oil before and after cultivation with Trichosporon pullulans RAY-13.**

Individual paraffin concentration is expressed as per cent of total n-paraffins (A) or iso-paraffins (B). Open bars represent initial and shaded bars, final paraffin concentrations, respectively. (a, b, c) Each bar represents different isomers of the corresponding paraffin.

**Table 3. Relative consumption of n-alkanes by Trichosporon pullulans RAY-13**

| n-Alkanes | 0 hr | 8.5 hr | 15 hr | 20.5 hr | 32 hr |
|-----------|------|--------|-------|---------|-------|
|           | G/liter | Per cent | G/liter | Per cent | G/liter | Per cent | G/liter | Per cent | G/liter | Per cent |
| n-Decane  | 0.33  | 2.91   | 0.16  | 2.14   | 0.03  | 1.02   | 0.07  | 9.72   | 0.05  | 17.85   |
| n-Undecane| 1.37  | 12.11  | 0.51  | 6.82   | 0.09  | 3.07   | 0.10  | 13.88  | 0.05  | 17.85   |
| n-Dodecane| 1.84  | 16.26  | 1.09  | 14.59  | 0.35  | 11.98  | 0.17  | 23.61  | 0.05  | 17.85   |
| n-Tridecane| 2.77 | 24.49  | 1.95  | 26.10  | 0.72  | 24.65  | 0.17  | 23.61  | 0.05  | 17.85   |
| n-Tetradecane| 2.46 | 21.75  | 1.83  | 24.49  | 0.77  | 26.37  | 0.17  | 23.61  | 0.05  | 17.85   |
| n-Pentadecane| 0.11 | 0.97   | 0.11  | 1.47   | 0.04  | 1.37   | 0.03  | 4.16   | 0.03  | 10.71   |
| n-Hexadecane| 1.47 | 12.98  | 1.10  | 14.72  | 0.52  | 17.80  | 0.10  | 13.88  | 0.02  | 7.14    |
| n-Heptadecane| 0.49 | 4.33   | 0.35  | 4.68   | 0.24  | 8.21   | 0.03  | 4.16   | 0.008 | 2.85    |
| n-Octadecane| 0.46 | 4.06   | 0.37  | 4.95   | 0.16  | 5.47   | 0.03  | 4.16   | 0.008 | 2.85    |
| Total alkanes| 11.30| —      | 7.47  |       | 2.92  |       | 0.70  |       | 0.266 |         |

* a Organism was cultivated on a mixture of pure n-alkanes in an impeller-agitated fermentor.
* b Grams of alkane per liter of broth.
* c Per cent (wt/wt) of total residual n-alkanes obtained at each period of cultivation.
* d Resolution in the chromatogram was unsatisfactory.
attained (after 15 hr of cultivation), whereas the curves of tridecane to eicosane (with the exception of n-pentadecane) show a slow decline in the early period of cultivation. They all reach a low and comparable value after 20 hr of cultivation. n-Pentadecane shows a lag period and does not reach the low value attained by other alkanes.

*T. pullulans* RAY-13 shows good growth on n-undecane, n-dodecane, n-tridecane, n-tetradecane, n-hexadecane, and n-octadecane and poor growth on n-pentadecane. Takeda and co-workers (18) did not find any difference between odd- and even-number alkanes for cell production of *Candida* and *Torulopsis* spp. On the other hand, Miller, Lie, and Johnson (12) observed lower cell yield of *C. intermedia* on n-heptadecane than either on n-hexadecane or n-octadecane. Incomplete oxidation of n-heptadecane was ruled out by them. Aiba and co-workers (1) reported lower cell production with *C. guilliermondii* on n-tridecane than on n-dodecane and n-tetradecane. n-Tridecane was also observed by Ratledge (14) to be least preferentially utilized by a *Candida* species but n-pentadecane was well utilized. It seems, therefore, that there can be no generalization on the utilization pattern of odd-number alkanes, but it is possible that various microorganisms have species specificity to odd-number alkanes of certain chain length.

Studies on alkane mixtures showed that shorter-chain alkanes (decane, undecane, and dodecane) are consumed by *T. pullulans* RAY-13 faster and at a more uniform rate than longer-chain ones. Ratledge (14) observed substantial loss of shorter-chain n-alkanes (as far as tridecane) by evaporation after 168 hr of agitation (1,000 rev/min) and aeration (3 liters/min) which can affect the consumption data on shorter-chain alkanes. However, because of the short cultivation period (32 hr) and low agitation rate (550 rev/min) and also because of the fact that most of the alkanes are consumed in 15 to 20 hr of cultivation, we feel that the evaporation loss of shorter-chain alkanes may be negligible in our experiment. Longer-chain alkanes (n-tridecane to n-eicosane) with the exception of n-pentadecane have a slower rate of degradation initially followed by a faster rate (Fig. 2). Inducible enzymes appear to be operative in the utilization of these alkanes. Dostilek and co-workers (3) cultivated a strain of *C. lipolytica* on gas oil and observed that shorter-chain n-alkanes (nonane to tetradecane) were more rapidly utilized than other alkanes in the early period of cultivation. In this case, the rate of degradation of pentadecane, hexadecane, and heptadecane was the same during the entire period of cultivation but rapid utilization of longer-chain alkanes, octadecane to pentacosane, did not occur before the concentration of other alkanes decreased. This pattern of consumption of n-alkanes was, however, not observed with a *Nocardia* sp. studied by Wagner et al. (21).

*T. pullulans* RAY-13 shows poor or no utilization of isoparaffins present in gas oil. This is in agreement with general findings on isoalkane utilization by microorganisms (4, 19). The extent of individual isoparaffin degradation cannot be obtained from the data in Fig. 1, as only relative concentration is shown. Isoparaffins with 14 to 18 carbon atoms, however, seem to be degraded by the organism to some extent as shown by the decreased relative concentration after cultivation. It has been reported that microorganisms attack isoalkanes containing sufficiently long unbranched chain and only one methyl side chain but not alkanes with a branched alkyl group longer than methyl or multiple methyl groups (4, 19). It is likely that isoalkanes with 14 to 18 carbon atoms may have only one methyl branch. As the number
of carbon atoms increases, it is quite possible that branching may be more complex and thus less utilizable by the organism.

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