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EMULSIFIER PRODUCTION BY *PSEUDOMONAS FLUORESCENS* DURING THE GROWTH ON HYDROCARBONS

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THE growth of micro-organisms on hydrocarbons is often accompanied by emulsification of the insoluble carbon source in the culture medium[1–3]. This is generally attributed to the production of extracellular emulsifier during the growth on hydrocarbons[3–5]. Recently the surface active molecules of microbial origin have attracted considerable interest due to their potential application in food processing, pharmacology and petroleum industries[6–9].

During preliminary investigation on the screening of microbes capable of growing on hydrocarbons, we isolated six bacterial strains from various soil samples. Among them *Pseudomonas fluorescens* was the most potential hydrocarbon degrader. We had earlier reported production of amino acids by submerged cultivation of *P. fluorescens* on gasoline[10]. In this note we report the production of bioemulsifier by *P. fluorescens*.

P. fluorescens biotype C was isolated from soil samples and identified by the Marine Research Institute, Scotland (UK). The maintenance and culture conditions were earlier described[10]. Basal salt medium[11], with slight modification and consisting of the following was used for emulsifier production: NH_4NaHPO_4, 10; K_2HPO_4, 0.5; KH_2PO_4, 0.5; $MgSO_4 \cdot 7H_2O$, 0.5; $MnCl_2 \cdot 4H_2O$, 0.2; $CaCl_2$, 0.2; $FeCl_3$, 0.03 and $ZnSO_4$, $7H_2O$, 0.2. The pH was adjusted to 7.4. Sixty ml of the medium was placed in 250 ml Erlenmeyer flasks and autoclaved at 103 KPa for 15 min. Hydrocarbons were filter-sterilized and added as required. The emulsifier was extracted in hexane and the activity was estimated[12]. A 0.1 ml mixture of hexadecane and 2-methyl naphthalene (1 : 1) was added to 7.5 ml of tris-(hydroxy methyl) aminomethane (tris) magnesium buffer (0.02 M tris-HCl, pH 7.2 containing 10 mM $MgSO_4 \cdot 7H_2O$) containing 100 μg emulsifier in a 100 ml flask. The content was shaken on a rotary shaker (200 rpm) for 1 h at 25°C and the optical density at 540 nm was measured. OD was converted into Klett units. The activity of emulsifier is expressed in unit. A unit of emulsifier was defined as the amount of emulsifier which causes an increase in 13.3 Klett units in the assay conditions. Carbohydrates and lipids were determined by well-known methods[13,14]. The protein content of emulsifier was estimated by the modified method of Lowry *et al*[15] as described by Hartree[16]. The results reported are the average values of at least three independent experiments.

Table 1 summarizes the production of emulsifier by *P. fluorescens* during the growth on various hydrocarbons. The maximum yield of emulsifier was obtained with gasoline as a substrate. The yeild is comparable to that reported earlier[17,18]. The emulsifier(s) produced by *P. fluorescens* grown on different hydrocarbons exhibited different levels of emulsification activity against gasoline or respective carbon source. Higher emulsifying activity was observed against the hydrocarbon which is used as the growth substrate. When aliphatic hydrocarbons were used as substrate, the growth rate of organism reduced significantly and took 8 days to complete the growth cycle and the emulsifier yield was low. The growth and production of emulsifier by *P. fluorescens* was further reduced when toluene was used as a carbon source compared to aliphatic hydrocarbons. Interestingly, when the organism was supplemented with a mixture of toluene

Table 1 *Production and activity of emulsifiers during growth of P. fluorescens on various hydrocarbons*

Hydrocarbon substrate	Emulsifier production (μg/ml)	Emulsifier activity (U/100 μg emulsifier) With gasoline	Emulsifier activity (U/100 μg emulsifier) With growh substrate
Gasoline	233	135.6	135.6
n-Paraffin C_{11}–C_{14}	63	69.6	80.0
n-Dodecane	80	82.6	106.8
n-Tetradecane	110	91.6	106.8
n-Paraffin	86	91.6	106.8
n-Paraffin + Toluene	114	97.6	104.4
Diesel	140	41.6	44.0
Glucose	164	41.0	4.0

Experimental conditions were the same as described in the text, except indicated, hydrocarbon as 4% was used as a growth substrate. Mixed substrates were used as 2% each.

and *n*-paraffin as the sole source of carbon, the growth and emulsifier production enhanced substantially giving the maximum emulsifier production in 3 days as compared to 8 days with *n*-paraffin. The data agree with those of Rosenberg *et al*[12], dealing with growth associated emulsifier production in *Arthrobacter* sp. RAG 1. We have not detected emulsifier activity during any stage of growth from the cells suggesting that emulsifier was released as soon as it was formed in the cell.

The emulsifier produced on different hydrocarbon substrates by *P. fluorescens* differ quantitatively in the carbohydrate and protein content (table 2). This indicates that various types of emulsifiers are produced during the growth of *P. fluorescens* on different hydrocarbons. The maximum yield of emulsifier was obtained when *P. fluorescens* was grown on gasoline. Apart from carbohydrate (58%), protein (19.58%) and ash, it contains 10% lipid. The lipid extracted from the emulsifier showed emulsification of gasoline as 33 units against 135.6 units/100 μg of the polymer. Decrease in the activity might be due to the absence of copolymer(s), protein and carbohydrate required for activity or due to the poor stability of lipid fraction as shown by Zajic *et al*[19]. This finding also suggests that a lipid fraction of the polymer is not totally responsible for emulsification of hydrocarbon. Thin layer chromatographic study of lipid from emulsifier showed the presence of lipid-*o*-dialkyl-monoglycerides and wax esters. The data correlate with those of Cooper *et al*[20]. Trehalose was one of the major components of the carbohydrate moiety. The presence of trehalose as a major carbohydrate in many emulsifiers has been well-established[2–5].

The present results lead us to suggest that *P. fluorescens* possesses a remarkable hydrocarbon degrading ability, associated with the production of different types of extracellular emulsifiers. If exploited further, this organism may become a potential source of bioemulsifier with the application in petroleum industries.

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Table 2 *Protein and carbohydrate content of emulsifiers produced on various carbon substrates*

Carbon substrate	Carbohydrate (%)	Protein (%)
Gasoline	58.4	19.6
Diesel	42.7	11.7
n-Paraffin C_{11}–C_{14} (P)	23.7	17.3
Dodecane	42.3	14.7
Tetradecane	32.6	15.8
P + Benzene	21.2	13.8
P + Toluene	46.8	13.9
Glucose	40.4	12.5

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