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ACREMONIUM SALMONEUM GAMES & LODHA — A NEW HYPERPARASITE ON *PUCCINIA ARACHIDIS* SPEG.

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DURING August-September 1985–86, it was observed that rust pustules of *Puccinia arachidis* Speg. on groundnut leaves were at times covered with a whitish mycelial growth. This was isolated and identified as *Darluca filum* (Biv.) Cast. and *Acremonium salmoneum* W. Games & Lodha. Several hyperparasites viz. *Darluca filum* (Biv.) Cast.; *Eudarluca caricis* (Fr.) O. Ericks; *Tuberculina castaricana* Syd.; *Verticillium lacani* (Zimm.) Viegas; *Penicillium islandicum* Scopp. and *Acremonium persicinum* (Nicot.) W. Games have been reported to parasitize *Puccinia arachidis* by several workers[1–6] from India and abroad. *A. salmoneum* is recorded for the first time.

The fungus is characterized by floccose mycelial growth, pink to pale in colour. Vegetative hyphae is thin-walled with abundant sporulation. Conidiophores are usually branched a few times, 30–50 μm tall, phialids 15–35 μm long and tapering towards the apex. Conidia are borne in slimy heads, rather thick-walled, smooth, ovoidal with a minute truncate base, 4.2–5.6 × 2.5–3 μm in size. It differs from *A. persicinum*[1] where conidia are borne in chains and measure 4–6 × 2.6–3.5 μm.

The fungus *A. salmoneum* parasitized only the rust pustules on inoculation and no discernible changes could be observed on the host plant. The uredospores from the parasitized rust pustules failed to germinate and infect groundnut plants on artificial inoculations. While the uredospores from healthy pustules germinated almost 100% and produced good infection on inoculation on groundnut plants under identical conditions.

To study the relationship between *A. salmoneum* and uredospores, uredospores from infected rust pustules were mounted in glycerine after staining in cotton blue. It was observed that the mycelium of *A. salmoneum* grows between the uredospores and encircles the spores. Some branches of mycelium enter in the uredospores through germ pore. The intra-cellular mycelium is of various types, from single unbranched hyphae to branched mycelium; sometimes it occupies the whole cavity of the uredospores. The infected uredospores appear to be empty. From this it is concluded that *A. salmoneum* absorbs its food material from the uredospores by means of intra-cellular mycelium, which enters the uredospores through germ pores.

These studies clearly demonstrated the hyperparasitic nature of *A. salmoneum* on *P. arachidis*, the rust of groundnut. The fungus is able to parasitize the rust pustules only when humidity is high (above 80%).

The specimen is deposited in the Herbarium Cryptogamae Indiae Orientalis, Division of Mycology and Plant Pathology, IARI, New Delhi (HCIO 39327) and culture of the fungus is deposited in Indian Type Culture Collection as ITCC No. 3700.

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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.