A Bacillus Species Growing on the Lipopolysaccharide Fraction of *Salmonella*

Salmonella minnesota R-2050 (a streptomycin sensitive strain) was grown in a 80 l batch on the following medium: beef extract 1%; peptone 1%; NaCl 0.5%. The pH of the medium was adjusted to 7.2 before sterilization. After a growth period of 24 h at 37 °C the cells were harvested by centrifugation and dried with acetone. From the acetone-dried cell material the lipopolysaccharides were extracted according to the method of GALANOS, LÜDERITZ and WESTPHAL[1].

A synthetic medium was composed containing: $(NH_4)_2SO_4$ 0.1%; K_2HPO_4 0.1%; NaH_2PO_4 0.125%; $MgSO_4.7H_2O$ 0.05%; $CaCl_2$ 0.001%; $FeSO_4.7H_2O$ 0.001%; yeast extract 0.3%; lipopolysaccharides 0.2%; agar 1.5%. The pH of the medium was adjusted to 7.2 before sterilization. The sterile medium was distributed in petri dishes. Soil particles were inoculated on the surface of this agar medium. After an incubation period of 48 h at 28 °C, microbial growth was observed around the soil particles. The colonies were purified on the same agar medium but without yeast extract. After several purification transfers colonies were obtained consisting of gram-positive rods. This gram-positive rod culture was purified by heating a suspension during 10 min at 80 °C and streaking on the lipopolysaccharide agar. Luxurious growth of the purified culture was obtained on the lipopolysaccharide agar medium (without yeast extract).

The microorganism occurs singly, in pairs and short chains. The cells are gram-positive and stain uniformly. Grown on the lipopolysaccharide medium they are 0.9 by 4 microns. Spores are 0.9 by 1.4 microns, ellipsoidal to subterminal. The sporangia are only slightly swollen if at all. The cells are motile. Acid but no gas is produced (with peptone as source of nitrogen) from xylose, glucose, fructose, galactose, mannose, lactose, sucrose, mannitol, sorbitol, dulcitol and dextrine. No acid or gas is produced on arabinose, rhamnose or sorbitol. Starch is not hydrolyzed. Ammonium salts are not utilized as source of nitrogen. Nitrites are produced from nitrates. Urease not produced. Indole is not formed. No growth occurs in 10% NaCl. Citrates not utilized. On potato a weak yellowish pigment is formed. Gelatine stab: slow crateriform liquefaction. H_2S is not formed. On broth a light uniform turbidity. Acetylmethylcarbinol is not produced. The MR-test is positive. Milk is coagulated.

According to Bergey's Manual of Determinative Bacteriology, the microorganism should be classified as *Bacillus firmus*.

Experiments are now being undertaken to study the breakdown of *Salmonella*-lipopolysaccharides by this microorganism.

Résumé. Un bacille gram-positif, capable de se développer sur des lipopolysaccharides isolées de *Salmonella*, comme seule source de carbone, fut isolé de son milieu et fut identifié comme *Bacillus firmus*.

J. P. VOETS and G. BEYAERT

Department of General and Industrial Microbiology, Faculty of Agricultural Sciences, University of Gent, 9000 Gent (Belgium), 11 February 1970.

[1] C. GALANOS, O. LÜDERITZ and O. WESTPHAL, European J. Biochem. *9*, 245 (1969).

Microbial Degradation of Aromatic Hydrocarbons Used as Reactor Coolants

The organic heat-carriers used as coolants in nuclear reactors are usually mixtures of aromatic hydrocarbons: diphenyl, naphthalene and its alkylderivatives, terphenyls and higher aromatics. These compounds create serious difficulties in decontamination procedures when present or combined with radioactive contaminants, as they are chemically stable and insoluble in aqueous solutions. Consequently about 50 solvents have been examined for their solvent power and a choice was made on the basis of their chemical and physical properties, toxicity and cost for decontamination purposes[1].

Since the use of pure organic solvents is not possible at all in decontamination procedures, we successfully tried the use of solvent emulsions and two-phase systems[2]. However, the waste waters from this decontamination process could not easily be treated by usual coprecipitation method[3]. We therefore tried a bacteriological pretreatment of the waste waters, since some aromatic hydrocarbons are known to be metabolized by a large number of microorganisms[4].

The waste waters have approximately the following composition: 0.33‰ *m*-terphenyl; 0.17‰ *o*-terphenyl; traces of *p*-terphenyl and high-boilers; 1.5‰ thermip. Thermip is a heat-exchanger supplied by ESSO Standard Oil Co. with an approximative composition of 7% naphthalene, 70% methylnaphthalene, 23% diphenyl and alkylderivatives of naphthalene[5].

In the present paper the organisms able to grow on diphenyl, *m*-terphenyl and on the thermip-mixture are described, together with the biological decontamination procedure of the waste waters.

Experimental. The microbial degradation of the aromatic hydrocarbons which are present in the waste waters has been obtained in mineral medium cultures by 3 different organisms. The first one, *Pseudomonas desmolyticum*, able to utilize naphthalene and its alkylderivatives, had previously been isolated[6], while the other 2 organisms were obtained by enrichment cultures with diphenyl and *m*-terphenyl as sole carbon and energy source. Ispra soil was used as inoculum. The bacterium

[1] G. MOSSELMANS and J. NIENHAUS, EUR-Rapport 4228e, Ispra (1969).
[2] G. MOSSELMANS and J. NIENHAUS, EUR-Rapport 1360, Ispra (1969).
[3] S. J. B. KRAWCZYNSKI, *Radioaktive Abfälle* (Thiemig KG, München 1969).
[4] V. TRECCANI, *Progress Industrial Microbiology* (Heywood and Co. Ltd., London 1963), vol. 4, p. 1.
[5] B. VERSINO, F. GEISS, J. A. M. POELMAN and H. VISSERS, EUR-Rapport 3292i, Ispra (1967).
[6] V. TRECCANI, N. WALKER and G. H. WILTSHIRE, J. gen. Microbiol. *11*, 341 (1954).

able to grow on diphenyl is a motile, Gram-negative short rod which produces a fluorescent green pigment on King B medium, but not phenazine pigment on King A medium[7]; it does not produce gelatinase and by the criterion proposed by STANIER et al.[8] may be considered a strain of *Pseudomonas putida*. The other bacterium able to utilize *m*-terphenyl is a nonmotile, Gram-negative short rod, which does not produce fluorescent and phenazine pigment and gas from carbohydrates; it may be tentatively assigned to the genus *Acinetobacter*. At present no organisms have been isolated which are able to utilize *o*- and *p*-terphenyl.

The degradation experiments have been performed in two groups: a) aromatic hydrocarbons separately tested and b) organic mixture corresponding to waste water composition. Experiments of group a) have been carried out in 3000 ml glass jars containing 2000 ml of TAUSSON medium[9], supplied with the required organic compound at the rates described in Table I. Otherwise the degradation experiments of the organic mixture normally present in the waste waters (group b) were carried out in 200 l stainless steel tanks (150 cm height and 50 cm width) containing 150 l of TAUSSON medium to which *m*-terphenyl (0.33‰), *o*-terphenyl (0.17‰) and thermip (1.5‰) were added. The addition of POE-nonylphenol as surfactant did not significantly change the degradation conditions. Results are reported in Table II.

48 h pure cultures of *Ps. desmolyticum*, *Ps. putida* and *Acinetobacter sp.* grown on thermip, diphenyl and *m*-terphenyl respectively were used as inoculum separately or in mixture according to the organic compounds present in the culture fluid. The ratio of inoculum vs. culture fluid was 6%. The aerobic conditions were always effected by vortex aeration system[10]. After incubation the culture fluid was made alkaline and then extracted with trichloroethylene. The extract was dried and the solvent evaporated to a volume of 100 ml. The sample was examined by GLC-gaschromatography as previously described[11,12]. Data were referred to a 'blank' obtained by performing the solvent extraction immediately after inoculation.

Conclusions. From the above results it may be concluded that bacteriological pretreatment can be successfully employed for the decontamination processes of waste waters containing aromatic hydrocarbons used as reactor coolants.

The fermentation experiments, performed with 3 different organisms able to grow on diphenyl, *o*-terphenyl, naphthalene and alkylnaphthalenes, are reported in Table I and II. Sterile controls show that about 20% of thermip evaporates during incubation in 24 h. The degradation of *m*-terphenyl starts after the complete oxidation of thermip. Naphthalene and methylnaphthalenes are degraded more quickly than diphenyl. When thermip and *m*-terphenyl are both present in the cultures, their degradation is slower than when they are separately supplied as only carbon source.

Research is in progress on the degradative pathway of diphenyl and *m*-terphenyl.

Table I. 2000 ml of TAUSSON medium with thermip 1.5‰, or *m*-terphenyl 1‰, or thermip 1.5‰ and *m*-terphenyl 0.075‰

Hydrocarbons recovered	Time (h)			
	0	24	48	96
Thermip	100	2	2	1
m-terphenyl	100	10	5	3
Thermip[b]	100	18[a]	8	1
m-terphenyl[b]	100	100	100	20

Values are expressed as percent relative to quantities present at t_0. [a] Naphthalenes no more present. [b] Simultaneously supplied to the same culture.

Table II. 150 l of TAUSSON medium with *m*-terphenyl 0.33‰, *o*-terphenyl 0.17‰ and thermip 1.5‰

Hydrocarbons recovered	Time (h)			
	0	24	72	130
Thermip	100	30–40	10	5
m-terphenyl	100	100	80	0
o-terphenyl	100	100	100	100

Values are expressed as percent relative to quantities present at t_0.

Riassunto. Alcuni reattori nucleari impiegano come scambiatori di calore miscele di idrocarburi aromatici (bifenile, terfenile, naftalina e alcuni dei suoi alchilderivati). La decontaminazione delle acque di lavaggio dalle scorie radioattive presuppone l'eliminazione di tali composti organici. Le difficoltà incontrate nell'applicazione di metodi chimici hanno suggerito di tentare un trattamento microbiologico. La degradazione microbica degli idrocarburi aromatici, presenti nelle acque di lavaggio, è stata ottenuta mediante fermentazione con tre differenti microrganismi, due dei quali, capaci di crescere in presenza di bifenile e *m*-terfenile, sono stati isolati da colture di arricchimento, seminate con terra di Ispra.

D. CATELANI, G. MOSSELMANS, J. NIENHAUS[13], C. SORLINI and V. TRECCANI

Istituto di Microbiologia Agraria e Tecnica, Università di Milano (Italy), and Centro Euratom Ispra Servizio Protezione, I-21020 Ispra (Varese, Italy), 16 February 1970.

[7] E. O. KING, M. R. WAND and D. E. RANEY, J. Lab. clin. Med. *44*, 301 (1954).
[8] R. Y. STANIER, N. J. PALLERONI and M. DOUDOROFF, J. gen. Microbiol. *43*, 159 (1966).
[9] W. O. TAUSSON, Planta *4*, 214 (1927).
[10] E. B. CHAIN, S. PALADINO, F. UGOLINI, S. S. CALLOW and J. VAN DER SLUIS, Rend. Ist. Sup. Sanità *17* (Engl. Edit.), 61 (1954).
[11] B. VERSINO, F. GEISS and G. BARBERO, Z. analyt. Chem. *201*, 20 (1964).
[12] B. VERSINO, F. GEISS and H. SCHLITT, Z. analyt. Chem. *236*, 136 (1968).
[13] New adress: Studiengruppe fur Biologie und Umwelt, Thalkirchner Strasse 68, D-8 München 15 (Germany).