These enzymes include 6-phosphogluconate dehydrogenase [10], phosphoenolpyruvate carboxylase and pyruvate carboxylase [11]. Glucose metabolism in peripheral tissues can be stimulated by Mn to utilize the blood glucose. The suggested roles of Mn in physiological function are many. Excess or deficient amounts of Mn cause various dysfunctions. However, the real mechanisms of Mn in most biological events are not clear; e.g., the pancreas contains high levels of Mn but whether Mn plays a role for its endocrine function is not known. Recently a more potent hypoglycemic activity against the STZ-dependent diabetes has been found in vanadium [12]. This element may interact directly with insulin receptor sites in peripheral tissues. Similarity and dissimilarity between vanadium and Mn in their action sites needs to be clarified.

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1. Kawada, J., et al.: Endocrinol. Japon. *32,* 635 (1985)
2. Sakurai, H., et al.: Biochim. Biophys. Acta *841,* 208 (1985)
3. Rossini, A. A., et al.: Diabetes *26,* 1120 (1977)
4. Junod, A., et al.: Proc. Soc. Exp. Biol. Med. *126,* 201 (1967)
5. Failla, M. L., Kiser, R. A.: J. Nutr. *111,* 1900 (1981)
6. Bond, J. S., Failla, M. L., Unger, D. F.: J. Biol. Chem. *258,* 8004 (1983)
7. Kawada, J., Tanaka, N., Nozaki, Y.: Endocrinol. Japon. *28,* 235 (1981)
8. Lowry, O. M., et al.: J. Biol. Chem. *193,* 265 (1951)
9. Nishida, M., et al.: Experientia *44,* 756 (1988)
10. Glock, G. E., McLean, P.: Biochem. J. *55,* 400 (1953)
11. Baly, D. L., Keen, C. L., Hurley, L. S.: Biol. Trace Elem. Res. *11,* 201 (1986)
12. Meyerovitch, J., et al.: J. Biol. Chem. *262,* 6658 (1987)

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Cleavage of Dibenzofuran and Dibenzodioxin Ring Systems by a *Pseudomonas* Bacterium

P. Fortnagel, H. Harms, and R.-M. Wittich
Institut für Allgemeine Botanik der Universität, Abteilung für Mikrobiologie, D-2000 Hamburg

W. Francke, S. Krohn, and H. Meyer
Institut für Organische Chemie der Universität, D-2000 Hamburg

Halogenated derivatives of dibenzo-p-dioxin and dibenzofuran are of actual interest since they exhibit highest levels of toxicity. The potential danger created by the above mentioned substances as environmental contaminants with respect to their xenobiotic character and high persistence in soil [1] encouraged attempts of many microbiologists to screen for organisms possessing degradative and detoxificative potentials for these pollutants. Microbial decomposition of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) in a model aquatic environment revealed a half-life of this compound being greater than 500 days [2]. Further studies [3] demonstrated very slow formation of polar products from ^{14}C-labeled TCDD by the action of a bacterial population. Hydroxylated derivatives of the parent compound were assumed. Cooxidation of dibenzofuran, the non-chlorinated and monochlorodibenzo-p-dioxins by the action of an unspecific dioxygenase of naphthalene- and biphenyl-degrading bacteria led to the formation of cis-dihydrodiol derivatives of the parent compounds [4] which were not degraded further.

Here we report on products of the degradation of dibenzofuran and of the cleavage of two of the three rings of dibenzo-p-dioxin by the action of a *Pseudomonas* strain. Dibenzofuran and dibenzo-p-dioxin were chosen as model compounds to elucidate metabolic steps involved in the breakdown of these systems. This knowledge will be essential for future construction of bacteria utilizing haloaromatics [5] which may achieve the degradation of chlorinated dibenzofurans and dibenzo-p-dioxins.

Recently we isolated a bacterium – tentatively identified as a *Pseudomonas* sp. strain HH69 – from non-contaminated soil after enrichment with dibenzofuran as sole source of carbon and energy. The strain grows aerobically on dibenzofuran (Fig. 1), biphenyl, and a number of isomeric hydroxybenzoates and hydroxysalicylates. Dibenzofuran is completely degraded via salicylate and gentisate (Fig. 2). Enzymes responsible for dibenzofuran metabolism are constitutively formed during growth on glucose or complex media and are obviously coded chromosomally since plasmids were not detectable so far.

When washed *Pseudomonas* sp. HH69 cells, pregrown aerobically in peptone medium until late logarithmic growth

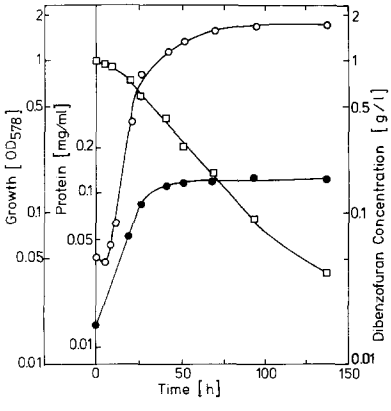

Fig. 1. Growth of *Pseudomonas* HH69 on dibenzofuran as the only source of carbon and energy. Cells were grown in minimal salt medium at 28 °C in a baffled flask on a rotary shaker. The initial dibenzofuran concentration was 1 g l^{-1}; ○ culture turbidity, ● protein content, □ dibenzofuran concentration

phase, were incubated with dibenzofuran or dibenzo-p-dioxin metabolic products accumulated in the medium. They were extracted, purified by HPLC, and identified by GC-MS and NMR analysis. The major products formed are shown in Fig. 2.

In contrast to the cleavage of the oxygen-containing heterocycles, dibenzothiophen was converted by *Pseudomonas* HH69 to the sulfoxide and the sulfone (Fig. 2). The elucidation of the initial reactions of the degradative pathways of dibenzofuran and dibenzo-p-dioxin is in progress.

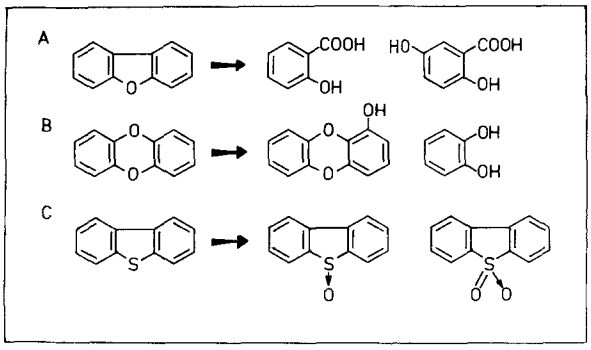

Fig. 2. Products formed from dibenzofuran (A), dibenzo-p-dioxin (B), and dibenzothiophen (C) by *Pseudomonas* HH69. Washed cells, pregrown in peptone medium, were incubated in phosphate buffer pH 7.2. Dibenzofuran, dibenzo-p-dioxin, or dibenzothiophen were directly added to the cell suspension which was incubated in an Erlenmeyer flask with baffles on a rotary shaker at 28 °C. Excretion of polar products was monitored by the use of reversed-phase HPLC. The incubation was terminated when the rate of product formation decreased. The cell-free medium was extracted with ethylacetate at neutral pH and reextracted after acidification at pH 2. After evaporation of the solvent the residue was dissolved in methanol and separated by preparative HPLC on a C-18 column using an acidified (pH 2) methanol/water mixture (60:40). Fractions obtained from this procedure were subjected to GC-MS and NMR studies. Gas chromatographic separations were carried out on a 30-m fused-silica column with OV-1 as a stationary phase using a temperature program (80 °C/5 °C). Identifications are based on comparison of 70-eV mass spectra obtained with a VG 70-250 SE GC/MS system. NMR data were obtained with a Bruker WM 400 at 400 MHz. Synthetic samples served as references

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1. Kearney, P. C., Woolsen, E. A., Isenee, A. R.: Environ. Health Perspect. *5,* 273 (1973); Crosby, D. G., Wong, A. E.: Science *195,* 1337 (1977); DiDomenico, A., et al.: Ecotoxicol. Environ. Safety *4,* 339 (1980)
2. Ward, C. T., Matsumura, F.: Arch. Environ. Contam. Toxicol. *7,* 349 (1978)
3. Philipp, M., et al., in: Microbial Degradation of Xenobiotics and Recalcitrant Compounds (FEMS Symp. No. 12, Leisinger, T., et al., eds.). London: Academic Press 1981; Quensen III, J. F., Matsumura, F.: Environ. Toxicol. Chem. *2,* 261 (1983)
4. Cerniglia, C. E., Morgan, J. C., Gibson, D. T.: Biochem. J. *180,* 175 (1979); Klecka, G. M., Gibson, D. T.: ibid. *180,* 639 (1979); Appl. Environ. Microbiol. *39,* 288 (1980)
5. Reineke, W., Knackmuss, H.-J.: Nature *277,* 385 (1979)

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GABA-like Immunoreactivity Suggests an Inhibitory Function of the Thoracic Low-frequency Neuron (TN1) in Acridid Grasshoppers

T. Sokoliuk, A. Stumpner, and B. Ronacher
Institut für Zoologie II der Universität Erlangen-Nürnberg, D-8520 Erlangen

In the auditory system of acridid grasshoppers about 40 auditory interneurons have been morphologically and physiologically identified [1 – 5]. Behavioral tests after surgical interferences on *Chorthippus biguttulus* have shown that the metathoracic ganglion plays an important role in preprocessing auditory information [6]. Our knowledge about the interconnections between metathoracic neurons, however, is scarce. Double recordings demonstrated an excitatory connection from the BSN1 to the AN1 and TN3 and a – not necessarily monosynaptic – inhibitory influence of SN1 on AN1 in *Locusta migratoria* [4]. Beyond this, only speculations about interactions between auditory interneurons are possible; such speculations are based on the comparison of threshold curves [7], or on the overlapping of dendritic areas in the frontal neuropil and a comparison of latencies [8] (see also [9]).

Many of the auditory interneurons ascending from the metathoracic ganglion show an intensity-dependent inhibition, starting at 10 – 20 dB above threshold, and, in several cases, converting tonic into phasic reactions [4, 5]. One question of interest is how this inhibition is achieved: Is it produced by other interneurons or – less likely – do the receptor cells themselves have an inhibitory influence on interneurons (cf. [4, 10])? An interneuron that would be especially suited to exert such inhibitory effects is the TN1 (in the locust: [3] synonymous with thoracic low-frequency neuron, [1]; in *Ch. biguttulus:* [5]). This cell reacts tonically, in a very similar way as low-frequency receptors, and its threshold curve is nearly identical to that of type-1 receptors in the locust [7]. The very same