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The Biological Degradation of Nicotine by Nicotinophilic Microorganisms

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SUMMARY

Various microorganisms are capable of breaking down tobacco alkaloids by different biochemical processes and possess characteristic enzymatic systems for the catabolism of nicotine. Bacteria of the genus *Pseudomonas* and the fungus *Cunninghamella echinulata* degrade nicotine via N-methylmyosmine and pseudooxynicotine which is linked to the opening of the pyrrolidine ring (pyrrolidine pathway), whereas *Arthrobacter oxidans* hydroxylates the pyridine ring in the 6-position. 6-hydroxynicotine is produced as a primary product (pyridine pathway). Tobacco plants, and some fungi (e.g. *Pellicularia filamentosa*) degrade nicotine via demethylation to nornicotine (methyl pathway).
As a result of the microbial degradation of nicotine and other tobacco alkaloids, carbon and nitrogen are made bioavailable. Following metabolic conversion to carboxylic acids, the reaction products are used by unicellular organisms as primary nutrients and a source of energy for the synthesis of new cell compounds.

ZUSAMMENFASSUNG

Bestimmte Mikroorganismen können Tabakalkaloide über unterschiedliche biochemische Prozesse abbauen und besitzen charakteristische Enzymsysteme zur Katabolisierung von Nikotin. Bakterien der Gattung *Pseudomonas* und der Pilz *Cunninghamella echinulata* führen die Nikotinverstoffwechselung via N-Methylmyosmin und Pseudooxynikotin durch, wobei es zur Öffnung des Pyrrolidin-Fünfrings kommt. (Pyrrolidin-Stoffwechselweg). Im Gegensatz hierzu bevorzugt *Arthrobacter oxidans* eine Hydroxylierung in Position 6 des Pyridinringes. Es entsteht als primäres Intermediat 6-Hydroxynikotin (Pyridin-Stoffwechselweg). Die Tabakpflanzen und auch einige Pilze (z.B. *Pellicularia filamentosa*) verfügen über die Fähigkeit, Nikotin unter Abspaltung der Methylgruppe in Nornikotin umzuwandeln (Methyl-Stoffwechselweg).
Durch den biologischen Abbau des Nikotins und weiterer Tabakalkaloide werden neue Kohlenstoff- und Stickstoffquellen von den Mikroorganismen erschlossen und nach der Metabolisierung zu Carbonsäuren als energiereiche Substrate dem Primärstoffwechsel zur Synthese neuer Zellbestandteile und zur Energiegewinnung zur Verfügung gestellt.

RESUME

Ils existent des micro-organismes qui peuvent décomposer les alcaloïdes de tabac à l'aide de différents processus biochémiques et qui possèdent des systèmes enzymatiques caractéristiques pour rendre possible la catabolisation de la

nicotine. Les bactéries du type *Pseudomonas* de même que le champignon *Cunninghamella echinulata* dégradent la nicotine au moyen du N-methylmyosmine et du pseudooxynicotine qui ouvrent le cycle de pyrrolidine (métabolisme de pyrrolidine). L'*Arthrobacter* oxidans, au contraire, hydroxyle le cycle de pyridine à la position 6. Ainsi l'hydRoxynicotine est formée comme substance intermédiare (métabolisme de pyridine). Les plantes de tabacs et aussi quelques champignons dégradent la nicotine (la *pellicularia filamentosa*, par example) à travers la démethylisation du nornicotine (métabolisme de méthyle).
La décomposition biologique de la nicotine et d'autres alcaloïdes dégage le carbone et l'azote. Après la transformation métabolique en acide carboxylique, les produits de réactions sont à nouveau disponible aux organismes unicellulaire comme nutriment primaire et comme source énergétique pour permettre la cytogénie.

INTRODUCTION

Various methods of waste management based on the biological degradation of otherwise toxic and hazardous substances are of great interest to all branches of industry and to society as a whole.
Microorganisms with specific enzyme systems are proving increasingly important in waste disposal (33). Some strains of bacteria, which can catabolize tobacco alkaloids can be employed for the degradation of nicotine in tobacco after harvesting (13) and therefore may find potential use in the composting of nicotine and tobacco waste (40), too.
Microorganisms are often associated by the lay with disease and in connection with pathogens, however they also have a useful role and are essential in the natural cycle of organic life. In common with all living organisms they require nutrients for cell synthesis and need hydrogen, carbon, nitrogen, mineral salts and some trace elements. To sustain growth and development of the cell, proteins, nucleic acids, sugars and lipids are required. In order to obtain the nutrient requirements many microbes need only one source of carbon and nitrogen.
Nicotine is of major importance as a natural compound of tobacco products. The nicotine content of most cultivars in the *Nicotiana tabacum* species, which represent the majority of the commercially-utilized tobaccos, varies considerably. In addition to the main alkaloid nicotine, the plant also produces several related alkaloids, such as nornicotine and anabasine which account for 1.1 % and 0,4 % of the total alkaloid content, respectively.
In 1993, German customs officials confiscated 625 million smuggled cigarettes. Generally, no taxes have been paid for these products and they may not be resold. This poses the problem of how they can be safely disposed of. At present, several investigations are under way to study the biodegradation of nicotine in waste tobacco products using nicotinophilic bacteria (32).
The following review describes the degradation of tobacco alkaloids to intermediates and end products by microbes which use nicotine as a sole nutrient. The current state of scientific knowledge on the enzymatic and chemical reactions involved is presented.

NICOTINOPHILIC MICROORGANISMS IN THE ENVIRONMENT

Nicotinophilic microorganisms and their close association with the tobacco plant have long been discussed. In 1955 Frankenburg revealed that even tobacco seeds are colonized by bacteria with nicotine-metabolizing properties (21). The fully developed tobacco plant is densely populated by species which are non-pathogenic. Under favourable conditions (humidity, temperature), tobacco leaves can host up to 16,000 bacteria per cm^2 (13). About half of these bacteria belong to the genus *Pseudomonas* and *Arthrobacter* and several strains of these genera are capable of nicotine catabolism, preventing the accumulation of tobacco alkaloids in cultivated soils.
It is assumed that certain species of bacteria excrete substances very similar to plant growth regulators and thus also influence the nicotine metabolism of tobacco. Approximately 30 - 40 % of the alkaloid content can be reduced by the bacteria hosted by the plant (13). Even after the tobacco leaf has been harvested, during processing and up to the manufacture of the finished product, bacteria on the leaf surface perform an important function. During fermentation, for example, the nicotinophilic microorganisms contribute substantially to a reduction in alkaloid content (38). Therefore, the idea of using the catabolic and modulating properties of these microorganisms for composting tobacco and tobacco waste seems highly plausible.

Indeed, there is nothing new about the idea of supplying tobacco alkaloids to microorganisms as the sole source of carbon and nitrogen. As early as 1942, Bucherer (12) carried out the first metabolic studies into the degradation of nicotine in nutritive media inoculated with bacteria. The purpose of the investigation was to obtain specific substances and enzymes from the nicotine catabolizing microorganisms and use them as an antidote in nicotine poisoning in animals and humans. Bucherer isolated three nicotinophilic strains of bacteria which he called *Bacterium nicotinovorum*, *Bacillus nicotinobacter* and *Bacterium nicotinophagum*. In the fifties, Tabuchi discovered 50 aerobic strains of bacteria with nicotinophilic properties. These included the genera *Pseudomonas*, *Alicaligenes*, *Achromobacter*, *Bacterium* and *Bacillus* (36). Almost at the same time, Sguros described

three strains of *Arthrobacter* which utilize nicotine as a nutritive substrate (34). Shortly before, Wada and Yamasaki had identified the first intermediates of bacterial nicotine catabolism (43). On the basis of their investigations using cultures of *Pseudomonas* they concluded that an oxidative degradation of the alkaloid occurred via the intermediates pseudooxynicotine and 3-succinoylpyridine.

Most of the earlier investigations were limited to isolating and identifying excretion products after administration of nicotine. However, Frankenburg and Vaitekunas discovered different catabolic pathways in nicotinophilic bacteria found on the surface of tobacco seeds (21). Bacteria of the genera *Arthrobacter* and *Pseudomonas* vary in particular with regard to their metabolic intermediates (8) and were both widely used in these investigations. Both can be found in the soil but react differently when using the gram method (*Arthrobacter oxidans*: gram positive; *Pseudomonas*: gram negative). In addition, it is noteworthy that the property of nicotine degradation is not a characteristic of the species but depends on the strain. Hochstein and Rittenberg (24), Decker *et al.* (16), Hylin (25) and Wada (42) have made important contributions to our understanding of the processes of nicotine degradation with these bacteria.

In the early eighties, it became apparent that some other microorganisms in addition to nicotinophilic bacteria, also possess nicotine metabolizing enzymes (39). Analogous to the nicotinophilic bacteria, some fungi have developed nicotine catabolizing properties enabling them to exploit tobacco alkaloids as a source of carbon and nitrogen or converting nicotine to nornicotine as in the tobacco plant (1, 35, 39). Nicotinophilic properties have been detected in the saprophytic fungus *Cunninghamella echinulata*. Other fungi prefer the demethylation of nicotine to nornicotine (e.g. *Pellicularia filamentosa*).

We now know that numerous microorganisms exhibit enzyme systems required for the degradation of nicotine. Several strains of *Alcaligenes paradoxus*, *Enterobacter cloacae* and *Achromobacter nicotinophagum*, for example, possess nicotinophilic properties. Some researchers have succeeded in isolating and characterizing bacterial enzymes and genes which contribute to the process of degradation (4, 7, 10). Recent studies suggest that the genetic information for the catabolizing enzymes is not only located on the bacterial chromosome but is also to be found on the plasmids (8, 15, 37). This indicates that in future it should be possible to develop microorganisms modified by modern methods of biotechnology and capable of directly eliminating undesirable tobacco alkaloids. This objective should form the basis of further investigations.

BIOCHEMICAL MECHANISMS OF NICOTINE DEGRADATION IN NICOTINOPHILIC MICROORGANISMS

Nicotinophilic microorganisms are distinguished by their ability to catabolize tobacco alkaloids such as nicotine, nornicotine and anabasine. Nevertheless, tobacco alkaloids are often not the sole primary substrate required for these bacteria to grow and multiply. Several strains of *Pseudomonas putida* have been found to possess the ability to subsist on industrial chemicals such as octane, naphthalene and polyhalogenated hydrocarbons (17, 41).

Usually, the enzyme system required for nicotine catabolism is only induced if the supply of readily available subtrates (e.g. glucose, ammonia) is exhausted (14). In *Arthrobacter oxidans*, some enzymes required for nicotine metabolism are encoded by 160 kb plasmid DNA, (8, 15). Likewise, the genes of *Pseudomonas convexa* seem to be located outside the bacterial chomosome (37). However, there are marked differences in nicotine metabolism between the two genera *Arthrobacter* and *Pseudomonas* in the manner of metabolizing the offered nicotine. *Pseudomonas putida* reacts extremely fastidiously to racemic mixtures of DL-(±)-nicotine and degrades only the L-(-)-isomer due to a stereo-selectively acting enzyme (20). *Arthrobacter oxidans* is less specialized in this respect and oxidizes both isomeric compounds, the naturally occurring L-(-)-nicotine to the intermediate L-6-hydroxynicotine and D-(+)-nicotine to D-6-hydroxynicotine (18, 23), which can be further metabolized. The special properties of *Pseudomonas putida* in degrading the natural L-(-)-isomer can be applied in the production of extremely pure D-(+)-nicotine or the manufacture of milder smoking tobaccos (18). The unnatural D-(+)-isomer which is not affected by the bacterium accumulates to a purity of 99,6 % (20) and can be recovered by extraction.

Nicotine can be catabolized by *Pseudomonas putida* and *Arthrobacter oxidans* to the oxidative state of carboxylic acids. However, both organisms exhibit different metabolic pathways in order to utilize the alkaloid to make energy available. *Arthrobacter oxidans* prefers the so-called pyridine pathway which starts with the hydroxylation in the 6-position of the pyridine ring and secondarily splits the pyrrolidine ring. By contrast, *Pseudomonas putida* degrades nicotine via N-methylmyosmine, then opens the pyrrolidine ring to form pseudooxynicotine and subsequently hydroxylates the pyridine ring in the 6-position (pyrrolidine pathway). The latter process of metabolic conversion of the alkaloid seems also to be practised by the fungus *Cunninghamella echinulata*. After transfer to a nicotine-containing medium, metabolites of the pyrrolidine pathway are synthesized by the fungus (39). In other fungi, (e.g. *Pelli-*

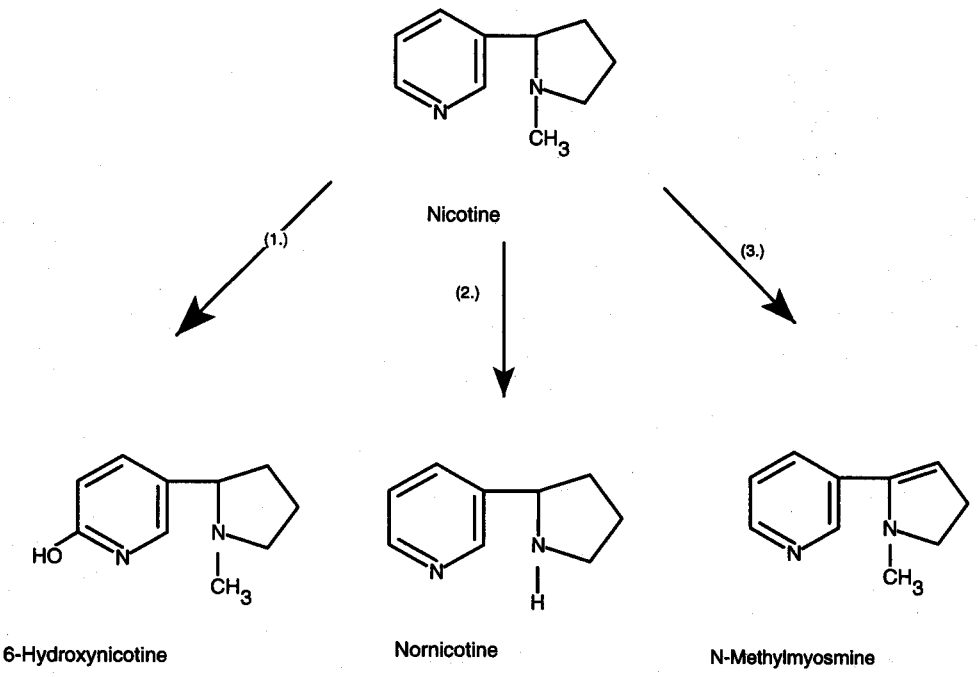

Figure 1.
Scheme of nicotine degradation pathway in microorganisms and tobacco.
1. Pyridine pathway; followed by bacteria
2. Methyl pathway, followed by tobacco plants and fungi
3. Pyrrolidine pathway, followed by bacteria and fungi

cularia filamentosa) a demethylative degradation pathway of nicotine via nornicotine has been identified (methyl pathway). This demethylation of nicotine also occurs in tobacco plants (1). A summary of the various pathways of nicotine metabolism by microorganisms and *Nicotiana tabacum* will be presented in Figure 1.

The nicotine catabolism of Arthrobacter oxidans

One of the most extensively investigated nicotinophilic species is *Arthrobacter oxidans*. After the isolation and identification of numerous metabolic intermediates and the fundamental elucidation of the reaction sequence during the fifties (21, 24), recent scientific publications describe the properties of some enzymes involved in this specific pathway (11, 14, 22 and 23). Nicotine catabolism by *Arthrobacter oxidans* is outlined in Figure 2. As already mentioned, the natural L-(-)-nicotine, as well as the naturally not occuring D-(+)-nicotine can be utilised as a source of energy by the bacterium. The first step of the inducible pyridine pathway is catalysed by an unspecific acting nicotine dehydrogenase, converting L-(-)-nicotine and D-(+)-nicotine into L-6-hydroxynicotine and D-6-hydroxynicotine, respectively. The enzyme is described as a molybdenum-containing hydroxylase, consisting of three subunits with a molecular weight of M_r 120000 for the native enzyme (2, 22). In addition, spectroscopic data reveal, that the activated form of the enzyme contains 4 iron, at least 2 acid labile sulfide groups, 1 pterin and 1 flavine which is identified as non-covalently bound FAD (30).

Both optical antipodes of 6-hydroxynicotine are metabolized by a specific oxidase (6-hydroxy-L-nicotine oxidase and 6-hydroxy-D-nicotine oxidase, respectively) into 6-hydroxy-N-methylmyosmine. No relationship exists between the amino acid sequence and peptide patterns of both enzymes which show different evolutionary pathways (11). 6-hydroxy-L-nicotine oxidase consists of two identical subunits, whereas 6-hydroxy-D-nicotine oxidase is formed by a single protein chain. Further investigations on the induction of both stereospecific hydroxy nicotine oxidases indicated that the L-(-)-specific enzyme can be activated by the two nicotine isomers with similar intensity. By contrast, 6-hydroxy-D-nicotine oxidase is selectively induced by D-(+)-nicotine (23). The two enantiozymes contain FAD as a cofactor. In 6-hydroxy-L-nicotine oxidase FAD is connected non-covalently to the polypeptide chain. In 6-hydroxy-D-nicotine oxidase however, FAD is bound covalently to a histidine residue (11). The terminal oxidation product of these reactions, 6-hydroxy-N-methylmyosmine, is spon-

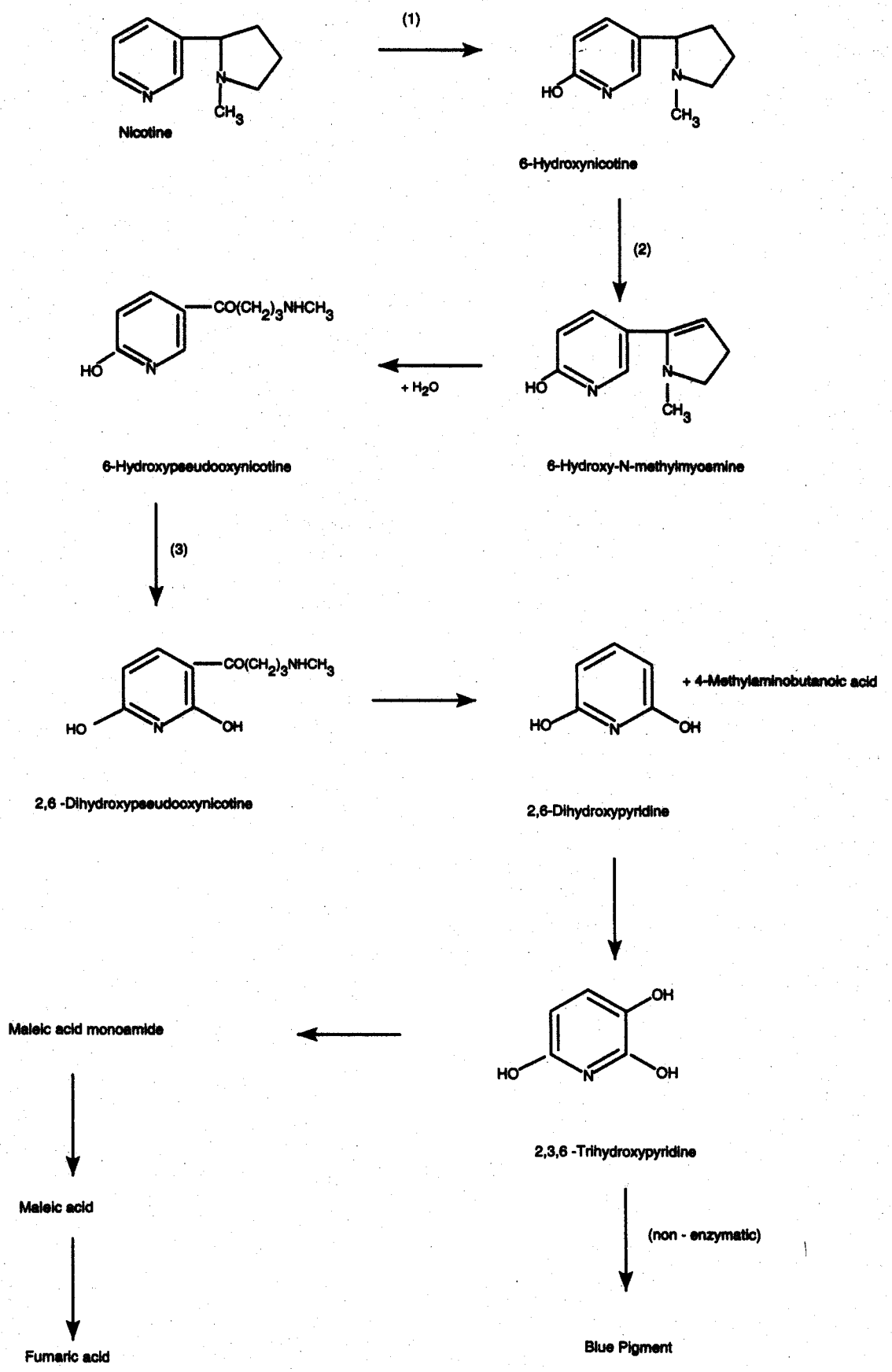

Figure 2.
Nicotine metabolism of Arthrobacter oxidans.
1. Nicotine dehydrogenase
2. 6-Hydroxynicotine oxidase
3. Ketone oxidase

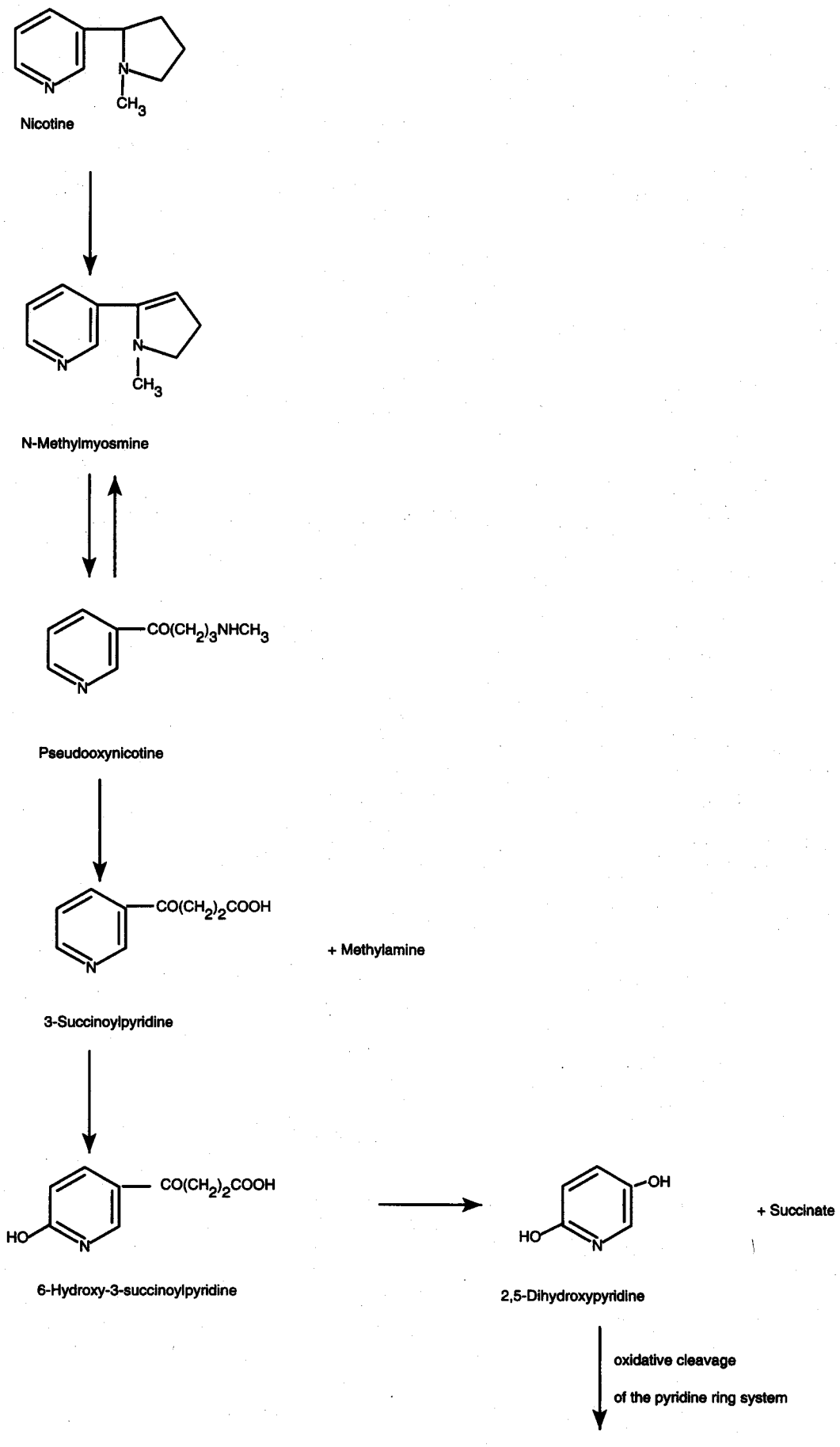

Figure 3.
Nicotine degradation scheme of Pseudomonas.

spontaneously hydrated to 6-hydroxypseudooxynicotine. Only at this stage of the nicotine degradation process in *Arthrobacter oxidans* does the non-enzymatic opening of the pyrrolidine ring occur. In the following hydroxylation step, catalysed by ketone oxidase, 6-hydroxypseudooxynicotine is converted to 2,6-dihydroxypseudooxynicotine.
As with nicotine dehydrogenase and the L-(-)-specific enzyme of the hydroxynicotine oxidases, ketone oxidase is induced by both optical antipodes of nicotine. In a further reaction, the side chain of 2,6-dihydroxypseudooxynicotine is removed as 4-methylaminobutanoic acid yielding 2,6 - dihydroxypyridine. After 3-hydroxylation in the pyridine ring the unstable intermediate 2,3,6-trihydroxypyridine is formed, which is non-enzymatically broken down to the characteristic blue pigment of *Arthrobacter oxidans* (19) or transfered by opening of the pyridine ring into maleic acid monoamide.
The carboxylic acid derivate maleic acid monoamide is degraded to maleic acid and fumaric acid and channelled into the primary metabolism of the bacterium for the generation of energy or the production of new cell compounds. The blue pigment formed during this process is closely related to indigoidine and other blue bacterial pigments (26).

The nicotine catabolism of Pseudomonas

By contrast to *Arthrobacter oxidans*, the genus *Pseudomonas* selects another degradation pathway which, as mentioned above, is known as the pyrrolidine pathway. The enzymes capable of catabolizing nicotine have been less extensively investigated in *Pseudomonas* than in *Arthrobacter oxidans*. Consequently, the description of the pyrrolidine pathway is limited to some general aspects (Figure 3). The inital step in the metabolism of nicotine by *Pseudomonas* is the production of N-methylmyosmine followed by the opening of the pyrrolidine ring to form pseudooxynicotine.
N-methylmyosmine exists in an equilibrium with pseudooxynicotine at physiological pH (27). The conversion to pseudooxynicotine has been observed between pH 2 and 9,5 and proceeds in a reversible change via nicotine-1',2'-iminium ion. By oxidation of pseudooxynicotine and removal of methylamine, 3-succinoylpyridine is formed. 6-Hydroxylation of the pyridine ring by Pseudomonas results in the synthesis of 6-hydroxy-3-succinoylpyridine. In contrast to Arthrobacter oxidans, 6-hydroxylation of the pyridine ring by Pseudomonas occurs at a later catabolic stage in nicotine degradation. After removal of the side chain as a succinate, the intermediate 2,5-dihydroxypyridine is formed. The reaction sequence ends with the oxidative cleavage of the pyridine ring system yielding maleamic acid and fumaric acid which can be used together with succinate as sources of energy in the primary metabolism of the bacterium.
Some nicotinophilic bacteria (e.g. *Achromobacter nicotinophagum*) catabolize 3-succinoylpyridine formed in the pyrrolidine pathway, by successive degradation of the side chain via 3-pyridyl propyl ketone and 3-pyridyl methyl ketone to nicotinic acid. Further catabolism of nicotinic acid results in the hydroxylation in the 6-position the oxidative opening of the pyridine ring. Malonic acid and oxalic acid are yielded as final products of this alternative pyrrolidine pathway (26).

The catabolism of anabasine and nornicotine

The ability of microorganisms such as *Pseudomonas* and *Arthrobacter oxidans* to degrade tobacco alkaloids is not restricted to nicotine. Nornicotine, myosmine and anabasine are also a source of energy for these bacteria (26). From one of these strains (*Pseudomonas* 4, group B) intermediates of the metabolism have been isolated. The reaction sequence is presented in Figure 4.

Hydroxylation of the pyridine ring in the 6-position and dehydrogenation of the pyrrolidine ring of nornicotine and the piperidinyl residue of anabasine form 6-hydroxymyosmine and 1', 6'-dehydro-6-hydroxyanabasine, respectively. In the case of nornicotine the intermediate is converted to 6-hydroxy-3-succinoylpyridine by oxidative cleavage of the pyrrolidine ring system. Dehydrogenated anabasine is further catabolized to 3-(4-carboxybutanoyl)-6-hydroxypyridine.

Induction and regulation of the microbial nicotine metabolism

In the last few years considerable efforts have been made to define the biochemical mechanisms involved in the induction of nicotine degrading enzymes. In particular, *Arthrobacter oxidans* has been subject to detailed studies on the regulation of catabolic nicotine pathways. Whereas naturally occurring tobacco alkaloids induce a large number of specific L-nicotine degrading enzymes, the addition of naturally not occuring compounds such as D-nicotine or 6-hydroxy-D-nicotine activates a special 6-hydroxy-D-nicotine oxidase with the ability to convert the D-isomer, as mentioned above (23).
The genes for the enzymes which facilitate nicotine degradation are localised on the plasmid pA01 of *Arthrobacter oxidans*. 6-hydroxy-L-nicotine oxidase is part of a regulon comprising several of the nicotine catabolizing enzymes, while the D-specific 6-hydroxynicotine oxidase forms a separate induction unit on pA01

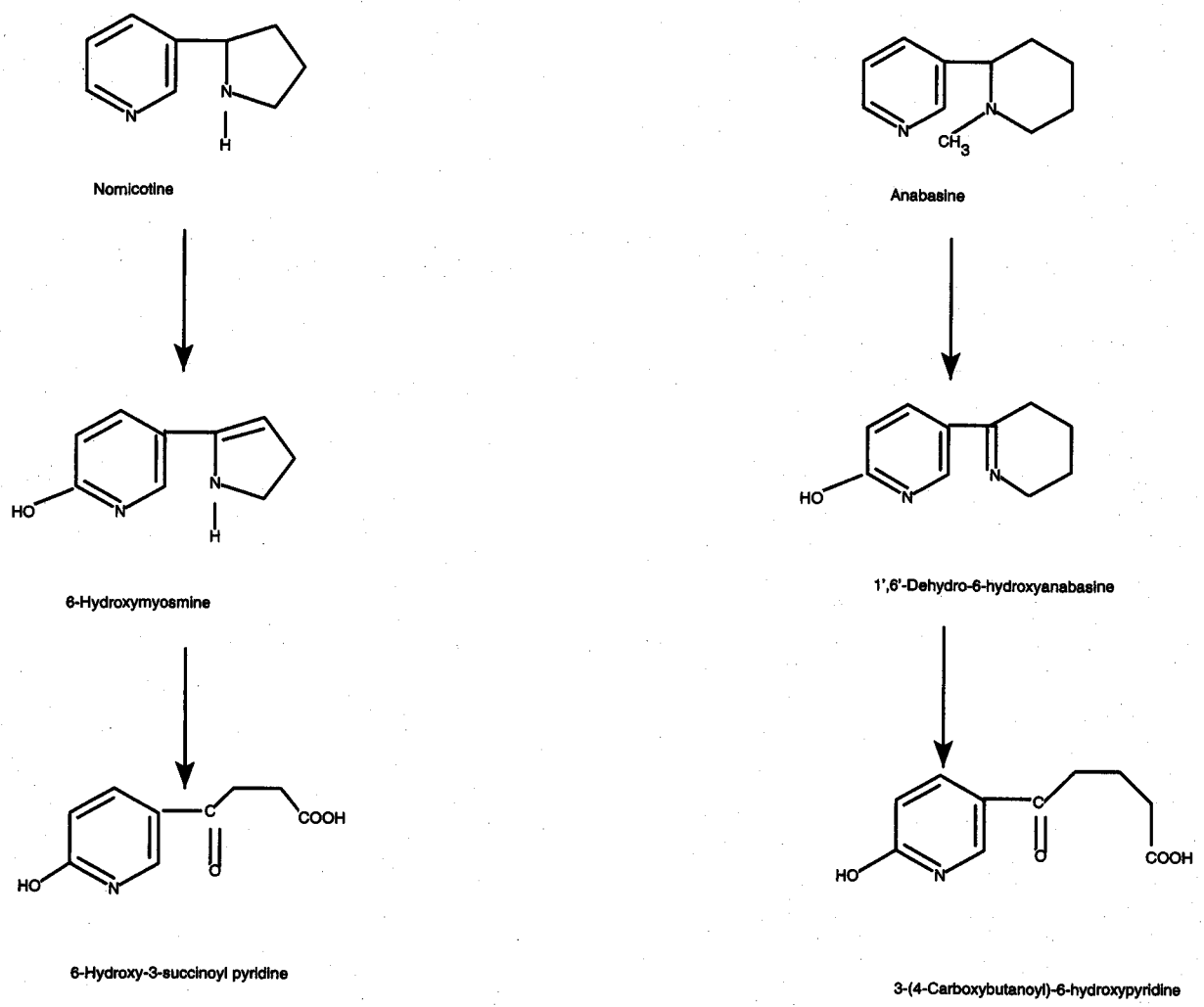

Figure 4.
Degradation of nornicotine and anabasine by Pseudomonas.

(15). Cells of *Arthrobacter oxidans* lacking the pA01-plasmid do not grow on a nicotine substrate and exhibit no nicotine catabolizing properties. However, the capacity for nicotine degradation can be reestablished by transfer of pA01 into the plasmid-deficient strains (2).

In the mid-1980s Brandsch *et al.* succeeded in incorporating the 6-hydroxy-D-nicotine oxidase gene into the genome of *Escherichia coli* and expressing the genetic information in a cell-free coupled transcription-translation assay (5, 9). The 6-hydroxy-D-nicotine oxidase derived gene from a 2,8 kb pA01 DNA fragment was already established by Brandsch et al. in 1987 (11). The promoter region of the gene exhibits two homologous palindromic sequences (IR 1 and IR 2) carrying the binding site for the DNA dependent RNA polymerase (2, 28).

A regulatory protein (Nic R1) has recently been identified, which prevents binding of RNA-polymerase to the 6-hydroxy-D-nicotine oxidase promoter region *in vitro* (2). Earlier experiments had established that glucose acts as an inhibitor of the D-specific nicotine oxidase (28). Moreover, further studies have shown that a riboflavin-dependent co-regulation of the nicotine regulon genes occurs at the level of transcription (3, 6). It is interesting that enzyme activity of 6-hydroxy-D-nicotine oxidase is induced in the stationary phase of cell growth, whereas all the other known enzymes in nicotine metabolism in *Arthrobacter oxidans* are coordinately activated in the logarithmic phase (29). As previously stated, the ability of different strains of *Arthrobacter oxidans* to degrade nicotine seems to depend on the presence of the pA01 plasmid in bacteria. The successful transfer and expression of such a gene into pA01 deficient cells by electroporation (2) offers a method for directly incorporating nicotine-metabolizing enzymes into suitable microorganisms. The results given here underline the fact that our understanding of the mechanisms at work in the induction and regulation of microbial nicotine metabolizing enzymes has advanced considerably in the last few years. In the case of *Arthrobacter oxidans*, in particular, a lot of research has gone into sections of the pathway, e.g. the two stereospecific hydroxynicotine oxidases.

DISCUSSION

Scientific research carried out during the last decades suggest that several genera of bacteria have nicotinophilic properties and can metabolize tobacco alkaloids into organic acids. Formost among these bacteria are the genera *Arthrobacter* and *Pseudomonas*. Both genera are present in the natural bacterial flora of the tobacco plant. For this reason direct effects on the alkaloid content of the plant are evident even during the plant's growth. Enzymatic control of the alkaloid content of tobacco leaves during fermentation has also been widely documented. New advances and processes in biotechnology could lead to the development of specific and more efficient nicotinophilic microorganisms. Elimination of tobacco alkaloids could possibly be intensified by a combination of the different catabolic pathways into one bacterium. Tobacco alkaloids could thus be degraded and re-entered into the metabolic cycle without leaving undesired by-products. The characteristic of the nicotinophilic microorganisms to catabolize these alkaloids efficiently suggest their possible use in the disposal of tobacco and tobacco waste (32), and elimination of air contaminants.

Weidner, M. and his co-workers at the Botanical Institute of Cologne University experimented with nicotine metabolizing strains of *Arthrobacter oxidans* in order to identify systems for the removal of chemical compounds in indoor air. Indoor plants in cooperation with plant bacteria could contribute to a reduction in concentrations of such pollutants. Preliminary results indicate that the synergism of *Arthrobacter oxidans* and indoor plants could be of use in the degradation of nicotine from tobacco smoke in indoor air (44).

The largest company engaged in the disposal of confiscated contraband cigarettes in Germany is the Parac Recycling GmbH in Brandenburg. In the past, the company used a process whereby a mixture of cellulose comprising biomass, cigarettes, pasterboard, paper and wood were decomposed using a "bacterial cocktail" of various species, including strains of *Pseudomonas nicotinofaga* and *Pseudomonas putida*. In prospective experiments the company intend to intensify its engagement in breeding and utilizing special strains of bacteria to breakdown nicotine and efficiently convert the alkaloid constituents of tobacco (31).

In 1993 the CORESTA task force on the "Disposal of Tobacco Waste" was formed to summarize the relevant means for the disposal of tobacco waste and outline the conditions for the handling and disposal of tobacco waste. Microbial methods are one of the recommended technologies for the rapid decomposition of tobacco waste.

These examples show that nicotinophilic microorganisms are already being introduced in industrial processes. Obviously, this is still at an early stage but their industrial utilization appears to hold great potential. By using these organisms it might be possible to remove air contaminants easily and at little expense. The waste products of the tobacco industry could also be disposed of more efficiently and in a more environment-friendly manner. Moreover, the by-products of degradation can easily be channelled back into the metabolic pathways of living organisms.

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