Proc. Indian Acad. Sci. (Chem. Sci.), Vol. 93, No. 7, September 1984, pp. 1143–1153.

Microbial transformation of pyridine compounds

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Abstract. Studies on biotransformation of pyridine derivatives have revealed two alternative patterns of biodegradation. Pyridine and α-picoline are not degraded *via* the hydroxylated derivatives. A reductive pathway involving succinic semialdehyde (SSA) or glutaric semialdehyde is involved in degradation of pyridine, α-picoline and possibly other alkyl pyridines. α-Picolinate, nicotinate, isonicotinate and hydroxypyridines are degraded *via* hydroxypyridine derivatives. Studies on a *Nocardia* strain capable of growth on pyridine-N-oxide (PNO), 2-hydroxypyridine and pyridine rule out the involvement of PNO or 2-hydroxypyridine in pyridine metabolism. PNO is metabolized *via* 2-hydroxypyridine while pyridine is metabolized *via* SSA. Biodegradation of isoniazid and isonicotinate (INA) appears to be more complex. INA is degraded in several organisms *via* 2-hydroxy INA and citrazinic acid. However, a strain of *Bacillus brevis* isolated in our laboratory metabolises isonicotinate *via* SSA, and a pathway similar to that of N-methyl-INA may be operative. Several organisms degrading quinoline and isoquinoline have been isolated. Formation of characteristic pigments suggests the involvement of hydroxylated compounds as intermediates.

Keywords. Microbial transformation; biodegradation; bioconversion; pyridines; isonicotinate; alkylpyridines.

1. Introduction

Pyridine and its derivatives enter the biosphere through burning of coal, in the form of alkaloids, as industrial solvents and intermediates, and from insecticide and herbicide formulations (Klingsberg 1961; Martin and Worthing 1979). Nicotinic acid and pyridoxine serve as vitamins; nicotine and anabasine have been used in insecticide formulations. Pyridine derivatives are also used as detergents and antitubercular drugs (isoniazid and ethionamide). Pyridine and quinoline derivatives have been detected in tobacco smoke, and the aroma of tea, coffee, rice and corn; these compounds are, therefore, regularly ingested in the body. Quinoline and its derivatives have been found to cause hepatocarcinogenicity and have also shown mutagenic action in Ame's test (Nagao *et al* 1977). In animal systems, pyridine is chiefly metabolised *via* N-methylation and N-oxidation, while the quinoline ring is hydroxylated in the 2-, 3-, 5-, 6- positions and excreted as glucuronide and sulphate esters (Williams 1959). Biodegradation of pyridine and quinoline in soil is known since the turn of this century (Funchess 1917) and some organisms degrading pyridine have been isolated. However, the major pathways for pyridine degradation have been deciphered in the last decade, and the information is summarised in this article.

2. Pathways of pyridine metabolism

The biodegradation of benzenoid compounds generally proceeds *via* the introduction of at least two hydroxyl groups ortho- or para- to each other, and dioxygenative cleavage

CDRI communication No. 3551

to yield cis-cis-muconic acid or muconic semialdehyde derivative as intermediates (Dagley 1975). This general pattern of ring cleavage is observed in pyridine derivatives to a limited extent only for degradation of hydroxypyridines and pyridine carboxylic acids. Pyridine and alkyl-pyridines are not degraded *via* this mechanism. The pyridine ring differs from the benzene ring in having a nitrogen with a lone pair of electrons. The pyridine ring is less susceptible to electrophilic attack than to nucleophilic attack. The pyridine ring is, however, susceptible to reduction as evidenced by the role of nicotinamide nucleotides as universal redox cofactors in biological systems. These characteristic properties of the pyridine ring have been exploited by microorganisms for its degradation.

3. Hydroxypyridine

The pathways for the degradation of 2-, 3- and 4-hydroxypyridines have been elucidated, and they follow a pattern of successive hydroxylation of the molecules followed by ring cleavage. Ensign and Rittenberg (1963) isolated an *Arthrobacter crystallopoites* growing on 2-hydroxypyridine; it also utilized pyridine as growth substrate. Growth of this organism on 2-hydroxypyridine produced copious amounts of a blue pigment of azaquinone nature formed by autooxidation of di-/tri-hydroxypyridine intermediates. Three strains of *Achromobacter* isolated by enrichment on 2- and 3-hydroxypyridine were found to convert the respective substrates to pyridine 2,5-diol (Houghton and Cain 1972). Pyridine 2,5-diol was further degraded by 2,5-dihydroxypyridine oxidase to yield maleamate as intermediate (Cain *et al* 1974). The maleamate pathway is also utilized by an *Arthrobacter* sp. for degrading 2-hydroxypyridine (Gupta and Shukla 1975) and by a gram-negative rod for degrading 3-hydroxypyridine (Khanna and Shukla 1977).

The metabolism of 4-hydroxypyridine by *Agrobacterium* sp. is initiated by hydroxylation *via* FAD-dependent 4-hydroxypyridine-3-hydroxylase. The resulting pyridine-3,4-diol is cleaved by a labile dioxygenase between C_2 and C_3 of the ring yielding 3-formimino-pyruvate, 3-formylpyruvate and finally pyruvate, formate and ammonia as products (Watson *et al* 1974).

4. Pyridine carboxylic acids

4.1 *Nicotinic acid*

The pathways for the degradation of nicotinic acid have been elucidated in several microorganisms, and three different routes are discernible. The first step in the metabolism of all the organisms is the formation of 6-hydroxynicotinate. The hydroxyl group is derived from water, and hydroxylation can occur anaerobically in the presence of suitable electron acceptors. Further metabolism of nicotinic acid in *Pseudomonas fluorescens* proceeds *via* the maleamate pathway involving 2,5-dihydroxypyridine, maleamate, maleate and fumarate as intermediates (Behrman and Stanier 1957). The 2,5-dihydroxypyridine-oxidase has been purified and characterised (Gauthier and Rittenberg 1971). It cleaves pyridine 2,5-diol to maleamic acid and formate; free-N-formylmaleamic acid is not an intermediate. This pathway of nicotinate metabolism is also utilized by a *Sarcina* sp (Gupta and Shukla 1978a) as well as a gram-negative *Coccus* (Shukla *et al* 1977).

In *Bacillus* sp nicotinate is metabolized *via* a slightly different pathway involving 2,6-dihydroxynicotinic acid and 2,3,6-trihydroxypyridine as intermediates (Ensign and Rittenberg 1964). The hydroxylases have been purified and characterised as flavoproteins. They are coordinately induced by 6-hydroxynicotinate (Hirschberg and Ensign 1971, 1972) (Figure 1).

Clostridium sp metabolises nicotinic acid *via* a reductive pathway yielding propionate, acetate, CO_2 and NH_3 as the final products (Paston *et al* 1964). 6-Hydroxynicotinate is converted by a ferrodoxin-dependent enzyme to 1,4,5,6-tetrahydro-6-oxonicotinic acid. α-Methyleneglutaric acid, methylitaconic acid and dimethylmaleic acid have been characterised as other intermediates (Stadtman *et al* 1972).

4.2 *α-Picolinic acid*

α-Picolinic acid has been reported to be phytotoxic, and herbicides like picloram are its derivatives. Picolinamide and picolinic acids are also obtained by photodegradation of diaquat; α-picolinate also acts as a selective inhibitor of sporulation. Some organisms degrading picolinic acid and picolinamide have been isolated and they may participate in the final disposal of these compounds. An *Aerococcus*, a *Rhodotorula* (Dagley and Johnson 1963) and *Arthrobacter* sp (Tate and Ensign 1974) have been reported to

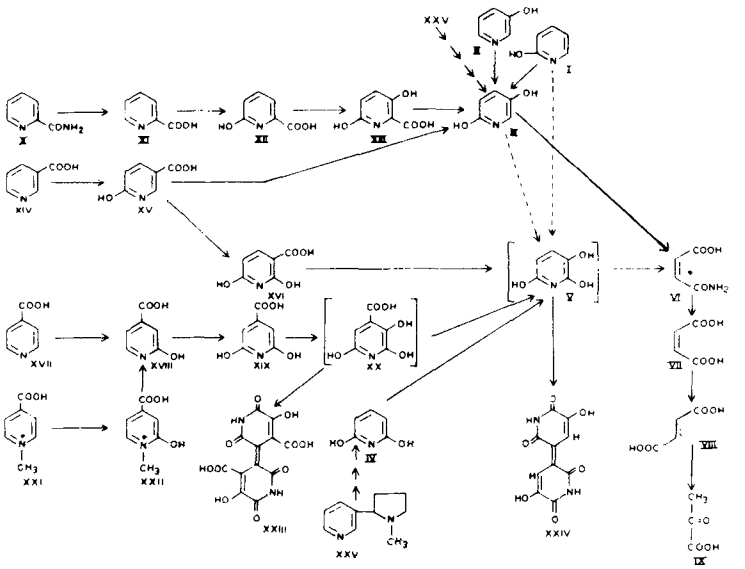

Figure 1. Pathways for the microbial transformation of hydroxypyridines and pyridine carboxylic acids: I: 2-hydroxypyridine; II: 3-hydroxypyridine; III: 2,5-dihydroxypyridine; IV: 2,6-dihydroxypyridine; V: 2,3,6-trihydroxypyridine; VI: maleamic acid; VII: maleic acid; VIII: fumaric acid; IX: pyruvic acid; X: picolinamide; XI: α-Picolinic acid; XII: 6-hydroxypicolinic acid; XIII: 3,6-dihydroxypicolinic acid; XIV: nicotinic acid; XV: 6-hydroxynicotinic acid; XVI: 2,6-dihydroxynicotinic acid; XVII: isonicotinic acid; XVIII: 2-hydroxyisonicotinic acid; XIX: 2,6-dihydroxyisonicotinic acid (citrazinic acid); XX: 2,3,6-trihydroxyisonicotinic acid; XXI: N-methylisonicotinic acid; XXII: 2-Hydroxy-N-methylisonicotinic acid; XXIII: Azaquinone pigment-1; XXIV: Azaquinone pigment-2; XXV: Nicotine.

convert α-picolinate to 6-hydroxypicolinate, but further catabolic pathway is not known in these organisms. A gram-negative rod (Orpin *et al* 1972a), a strain of *Bacillus brevis* (Shukla and Kaul 1973) and a gram-negative coccus (Shukla *et al* 1977), have been demonstrated to catalyse the formation of 6-hydroxypicolinate and 2,5-dihydroxypyridine from α-picolinate; formation of 3,6-dihydroxypicolinate was demonstrated in *Bacillus* sp only. 6-Hydroxypicolinate is oxidised by all the organisms while 2,5-dihydroxypyridine is oxidised by the gram-negative rod and *Coccus* but not by *B. brevis*; maleamate is also degraded by these organisms. Maleamate pathway thus seems to be involved in α-picolinate degradation in these organisms (figure 1).

4.3 *Isonicotinic acid, its hydrazide, and N-methylisonicotinate*

Isonicotinic acid (INA) and N-methylisonicotinic acid are produced by photolytic degradation of herbicides paraquat. Isonicotinic acid hydrazide (INH) is a powerful antitubercular compound and INA can be derived from it. Metabolism of INH by mycobacteria has been reported to generate isonicotinic acid and 4-pyridylmethanol, and metabolism of INH by mycobacteria has been linked to its selective antitubercular action. Further metabolism of INA in mycobacteria is, however, not known (Krishna Murti 1974). In a *Sarcina* sp, INA, 2-hydroxy INA, citrazinic acid and 2-oxoglutarate were characterised as probable intermediates of INH (Gupta and Shukla 1978a and 1979a). Nicotinate and maleate were, however, metabolised to yield pyruvate (Gupta and Shukla 1978b). A novel pathway involving hydrolysis of citrazinic acid to yield 2-oxoglutarate has been suggested. The two enzymes hydroxylating INA molecule in α positions, using H_2O as source of oxygen for the hydroxyls have been characterized as high molecular weight flavoproteins (Gupta and Shukla 1979b) (figure 1).

The metabolism of INA in a *Pseudomonas* sp yielded citrazinic acid as the principal metabolite which was oxidized by cells. A blue pigment originating from abiogenic oxidation of citrazinic acid was also characterized (Ensign and Rittenberg 1965).

Organisms capable of degrading N-methylisonicotinate (NMI) have been isolated by Orpin *et al* 1972b; Wright and Cain 1972. A gram-positive rod (bacteria 4 C_1) did not oxidise isonicotinate or hydroxypyridines but oxidised NMI and 2-hydroxy-NMI. The carboxyl group of the molecule was liberated as CO_2 and N-CH_3 as formaldehyde. Cell-free extracts converted 2-hydroxy INA into citrazinic acid, and maleamate was also metabolised. A pathway involving hydroxylated compounds was therefore, proposed for NMI degradation by this organism (Orpin *et al* 1972b) (figure 1). The *Achromobacter* sp of Wright and Cain and $4C_2$ bacteria of Orpin *et al* did not metabolise INA, 2-hydroxy INA, mono- & dihydroxypyridines or their N-methyl derivatives. A novel pathway involving partial reduction of the pyridine ring followed by oxygenative cleavage of C_2–C_3 bond, and rearrangements yielding succinic semialdehyde and methylamine has been proposed for NMI catabolism in these organisms (Wright and Cain 1972) (figure 2).

4.4 *Dipicolinic acid*

Degradation of dipicolinic acid has been reported in *Achromobacter* sp (Arima and Kobayashi 1962; Kobayashi and Arima 1962) and *Bacillus brevis* (Singh 1981; Singh 1982); 3-hydroxydipicolinic acid and 2-oxoglutarate are the probable intermediates.

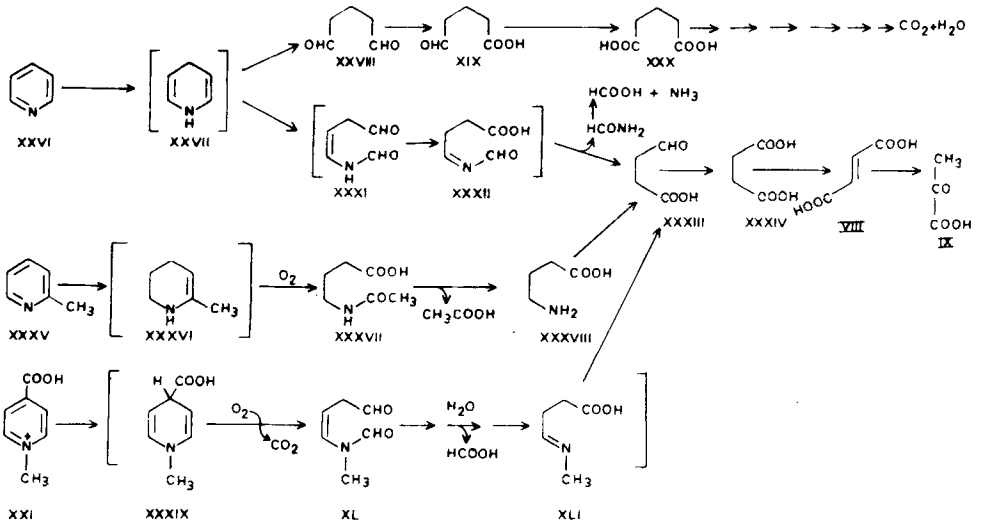

Figure 2. Pathways for microbial transformation of pyridine, α-picoline and N-methylisonicotinic acid: XXVI: pyridine; XXVII: 1,4-dihydroxypyridine; XXVIII: glutaric dialdehyde; XXIX: glutaric semialdehyde; XXX: glutaric acid; XXXI: N-formylaminovinylacetaldehyde; XXXII: N-formylaminovinylacetic acid; XXXIII: Succinic semialdehyde: XXXIV succinic acid; XXXV: α-picoline; XXXVI: 1,4,5,6-tetrahydro-α-picoline; XXXVII: N-acetyl-4-aminobutyric acid; XXXVIII: 4-aminobutyric acid; XXXIX: N-methyl-1,4-dihydroisonicotinic acid; XL: γ-(N-formyl-N-methylamino)vinylacetaldehyde; XLI: 2-(N-methylaminovinyl)acetate.

5. Nicotine

A *Pseudomonas* sp and an *Arthrobacter* sp degrading nicotine have been isolated and pathways for nicotine degradation in these organisms have been elucidated. In *Pseudomonas* sp, the pyrrolidine ring of nicotine is attacked giving pseudooxy-nicotine which yields 3-succinoylpyridine. The pyridine ring is then hydroxylated to 3-succinoyl-6-hydroxypyridine. The side chain is then cleaved and the resulting 2,5-dihydroxypyridine is metabolised *via* the maleamate pathway (Wada 1957; Thacker and Hedegaard 1972).

In *Arthrobacter* sp the hydroxylation of nicotine to 6-hydroxynicotine is the first step. The cleavage of the pyrrolidine ring and another hydroxylation yields 2,6-dihydroxy-pseudooxynicotine which is cleaved to 2,6-dihydroxypyridine. This compound is further metabolised *via* 2,3,6-trihydroxy-pyridine and maleamate as intermediates (Gherna *et al* 1965; Holmes and Rittenberg 1972; Holmes *et al* 1972).

6. Pyridoxine

Snell and his associates (Rodwell *et al* 1958; Ikawa *et al* 1959; Burg *et al* 1960) studied the metabolism of pyridoxine and pyridoxamine employing two strains of *Pseudomonas i.e.* strain IA and strain MA capable of degrading pyridoxine and pyridoxamine respectively. Pyridoxine was first oxidised to pyridoxic acid, the pyridine ring being then cleaved by a flavine containing oxygenase between C_2–C_3

yielding α-hydroxymethyl-α'-(N-acetylamino-methylene) succinic acid. In the case of pyridoxamine, substituents at C_3 and C_4 were oxidised to yield 2-methyl-3-hydroxypyridine-(4,5)-dicarboxylic acid, which was decarboxylated to yield 2-methyl-3-hydroxypyridine-5-carboxylic acid. This compound was cleaved by oxygenase to yield α-N-acetylaminomethylene-succinic acid which was finally degraded *via* succinic semialdehyde.

7. Pyridine

Biodegradation of pyridine, when added to soil, has been observed at the turn of this century (Funchess 1917). Several workers have reported the utilization of pyridine as a source of nitrogen by bacteria (Dooren-de-Jong 1926; Ostroff and Henry 1939; Moore 1949). Ensign and Rittenberg (1963) isolated *Arthrobacter* sp which utilized 2-hydroxypyridine and pyridine as the sole source of carbon, nitrogen and energy. Stafford and Callely (1970) reported the degradation of pyridine by a *Pseudomonad*. However, the pathway for pyridine degradation in the above organisms has not been elucidated.

The mode of pyridine degradation has been deciphered due to work on *Corynebacterium* sp, *Brevibacterium* sp (Shukla 1973; Shukla and Kaul 1974, 1975), *Bacillus* sp and *Nocardia* Z_1 (Watson and Cain 1972, 1975). These organisms do not utilize hydroxypyridines as growth substrates, and mono or dihydroxy-pyridines are not oxidised by pyridine-adapted cells. Only in the case of *Nocardia* Z_1, slow conversion of 3-hydroxypyridine to 2,3- and 3,4-pyridinediol has been reported but they are not further metabolised. Pyridine degrading organisms have not been found to oxidise piperidine, N-methylpyridine and pyridine-N-oxide ruling them out as intermediates. Pyridine is not oxidised by cell-free extracts of the organisms even when various cofactors, thiols etc are added. Metabolism of pyridine in presence of inhibitors did not result in accumulation of any mono or dihydroxypyridine, phenolic or other intermediates. However, pyridine metabolism in the presence of semicarbazide yielded characteristic oxo-acid intermediates. Succinic semialdehyde has been identified as the intermediate of pyridine metabolism in *Corynebacterium*, *Brevibacterium* and *Bacillus* sp, formamide and ammonia were other products of pyridine metabolism in these cases; glutaric semialdehyde has been detected in *Nocardia* Z_1. Inducible SSA-dehydrogenase has also been demonstrated in the first 3 organisms, while glutaric semialdehyde dehydrogenase has been found in *Nocardia* Z_1. Formation of these oxo-acids indicates reduction of the pyridine ring before or just after pyridine ring cleavage. If the ring cleavage occurred with an intact pyridine ring, maleic semialdehyde would be expected to be the product which would form SSA on reduction. Maleic semialdehyde was not found to be metabolized by pyridine-adapted *Corynebacterium*; γ-aminobutyrate (GABA) and N-formyl-γ amino butyrate, piperidine were not metabolized and reduction of the pyridine ring to tetrahydro- or hexahydropyridine stage also does not seem to be involved (Shukla and Kaul 1974).

From these studies it would appear that the first step in pyridine metabolism in these organisms involves the reduction to 1,4-dihydropyridine. In case of *Nocardia* Z_1 this putative intermediate undergoes a hydrolytic N-C_2 ring cleavage and subsequently is deaminated to yield glutaric dialdehyde and glutaric semialdehyde (Watson and Cain 1975). In other organisms, 1,4-dihydropyridine is probably cleaved by a dioxygenase at

C_2–C_3 bond forming N-formylaminovinyl-acetaldehyde which undergoes oxidation to corresponding N-formylaminovinyl acetate. Hydrolysis of the C_6–N bond yields succinic semialdehyde, formate and ammonia, probably *via* formamide (Shukla and Kaul 1974; Watson and Cain 1975). Formamide hydrolase activity has been detected in the pyridine degrading organisms. Work with cell-free systems is essential to detect the various intermediates of pyridine metabolism (figure 2).

8. Alkylpyridines

Bacteria degrading α-picoline (Shukla 1974), β-picoline γ-picoline, 2-ethylpyridine, 2,4-lutidine, 2,6-lutidine, and 2,4,6-collidine (Shukla 1975) have been isolated from soil by elective culture. These organisms exhibit high specificity and do not generally metabolize hydroxypyridine or pyridine carboxylic acids. The α-picoline-degrading *Arthrobacter* utilized 2-ethylpyridine and piperidine as growth substrates; cells grown on α-picoline oxidised 2-ethylpyridine and *vice versa*. Pyruvate and succinic semialdehyde were isolated and characterized as α-picoline metabolites accumulating in presence of semicarbazide as inhibitor. Acetamide was not metabolized, GABA and N-acetyl-GABA were oxidised by α-picoline-adapted cells, but 4-amino crotonate was not metabolized; N-formyl-GABA hydrolase has been detected in cell-free extracts. Pyridine ring of α-picoline is probably cleaved by dioxygenase after reduction of ring to tetrahydro stage yielding N-acetyl GABA as the intermediate which is the precursor of SSA in this organism (Shukla 1974).

9. Microbial decomposition of pyridine-N-oxide

Biological oxidation of pyridine to pyridine-N-oxide (PNO) occurs in animal systems as an important detoxication reaction, and conversion of PNO to 2-hydroxypyridine is also feasible chemically. A pathway involving PNO and 2-hydroxypyridine can, therefore, be proposed for degradation of pyridine in microbial systems. The failure of the pyridine-degrading organisms to oxidise these compounds may be ascribed to their impermeability. PNO also has two routes open for degradation; it can be metabolized *via* the pathway of 2-hydroxypyridine catabolism as discussed earlier. Alternatively, it can be reduced to pyridine and degraded. Reduction of pyridine-N-oxide is feasible and is catalyzed by *E. coli* and yeast (May 1957). An organism was isolated from soil by enrichment on pyridine-N-oxide as the sole source of carbon, nitrogen and energy (Shukla and Kaul 1977) and characterized as *Nocardia* sp. This organism utilizes pyridine and 2-hydroxypyridine as alternative growth substrates but not other pyridine derivatives. PNO-adapted cells readily grow on all the 3 substrates, but pyridine and 2-hydroxypyridine-grown cells grew on PNO medium only after prolonged incubation. Colonies of the organism spontaneously arise which have lost the PNO utilizing ability irreversibly; they however, readily grow on 2-hydroxypyridine and pyridine.

PNO-adapted cells oxidize PNO and 2-hydroxypyridine rapidly; pyridine is not oxidised. 2-Hydroxypyridine-grown cells do not oxidise PNO or pyridine. Similarly, pyridine-adapted cells oxidize only pyridine. Growth of *Nocardia* sp on PNO on 2-hydroxypyridine is accompanied by formation of a blue pigment (λ_{max} 615 nm); no such pigment is found during pyridine metabolism by this organism. Formation of mono or dihydroxypyridine or other UV-absorbing metabolites could not be detected during

normal fermentation of these substrates or incubation of resting cells with substrates in presence of inhibitors. However, incubation of PNO-adapted cells with PNO, under anaerobic conditions resulted in its conversion to 2-hydroxypyridine (Shukla and Kaul 1981). Formation of 2-hydroxypyridine was also observed when aeration was interrupted during fermentation of PNO, and this compound disappeared when aeration was resumed. Pyruvate was shown to accumulate as the major keto acid during PNO and 2-hydroxypyridine metabolism in presence of semicarbazide, while pyridine metabolism yielded succinic semialdehyde under the same conditions.

PNO-adapted cells oxidized 2-hydroxypyridine; other mono and dihydroxypyridines were not oxidized but maleamate, fumarate etc were oxidised at significant rates. 2-Hydroxy-pyridine-adapted cells did not oxidize PNO, but they oxidized 2,5-dihydroxy-pyridine and other intermediates of maleamate pathway. Pyridine-grown cells did not oxidize PNO, 2-hydroxypyridine or other intermediates of maleamate pathway, but readily oxidized succinic semialdehyde.

These studies indicate that PNO and 2-hydroxypyridine are metabolized *via* maleamate pathway. Pyridine is, however, degraded *via* a reductive pathway involving SSA as intermediate (Kaul 1983) (figure 3).

10. Microbial transformation of isonicotinic acid by *Bacillus* sp

An aerobic, gram-negative, spore-forming *Bacillus* sp degrading INA has been isolated from soil by elective culture technique using pasteurized soil samples. The organism has been identified as *B. brevis* on the basis of morphological, biochemical tests as well as base composition (43 % G + C). This organism also utilizes INH and 2-hydroxypyridine after a lag phase. Other pyridine derivatives are not utilized (Singh and Shukla 1981).

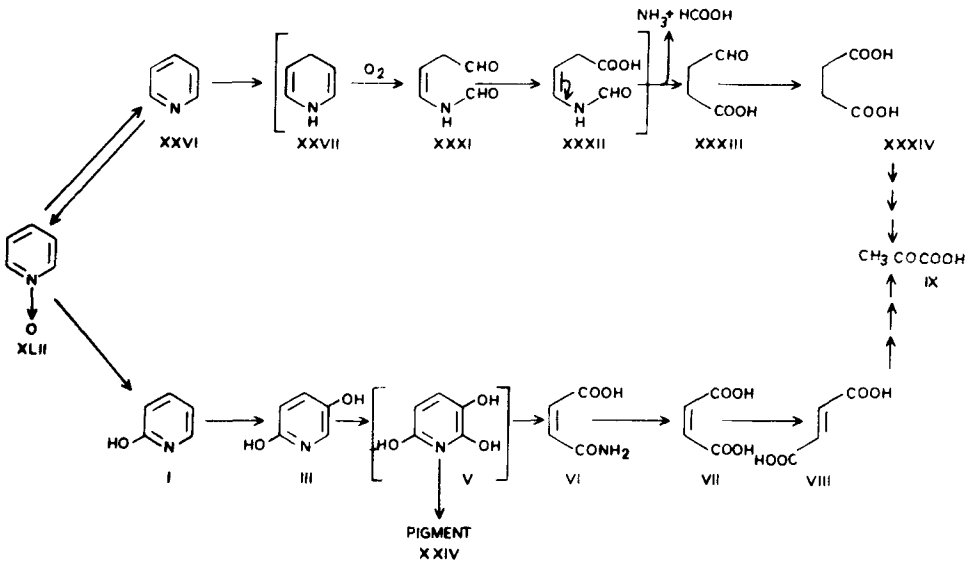

Figure 3. Pathways for microbial transformation of pyridine-N-oxide (XLII), (other legends as given in figures 1 and 2).

INA-adapted cells, rapidly oxidise INA, while succinate grown cells oxidize INA slowly. Repeated transfer of organism in succinate or glucose media completely abolished the capacity of INA oxidation. Mono and dihydroxypyridines, pyridine carboxylic acids other than INA, 2-hydroxy-INA and citrazinic acid were not metabolized. No ultraviolet-absorbing or phenolic intermediates could be detected during fermentation of INA by *B. brevis* or incubation of substrate with resting cells in the absence or presence of inhibitors. The nitrogen atom of INA was released as ammonia. Metabolism of INA by this organism in presence of semicarbazide resulted in accumulation of an oxoacid which has been isolated as dinitrophenyl-hydrazone and characterized as succinic semialdehyde. SSA is oxidized by INA-adapted cells, and repeated transfer of the organism on glucose and succinate media led to simultaneous loss of oxidation of both INA and SSA. A $NADP^+$ linked SSA dehydrogenase activity has been demonstrated in the cell-free extracts. γ-Aminobutyric is not utilized for growth, and not oxidized by INA-adapted cells; it cannot, therefore, be considered as a precursor of SSA in this organism. The INA degradation pattern in *B. brevis* is analogous to the reductive pattern of the pyridine ring degradation observed in several organisms (Singh 1983). Bacilli are known to metabolize α-picolinate as well as nicotinate *via* oxygenated intermediates. INA is also metabolized in *Pseudomonas* and *Sarcina* sp *via* hydroxylated intermediates. The utilization of INA by a reductive pathway in this organism may be due to chance acquisition of a plasmid coding for such a pathway during our isolation. The isolation and characterization of other bacilli degrading INA and elucidation of their pathways may resolve this paradox (figure 4).

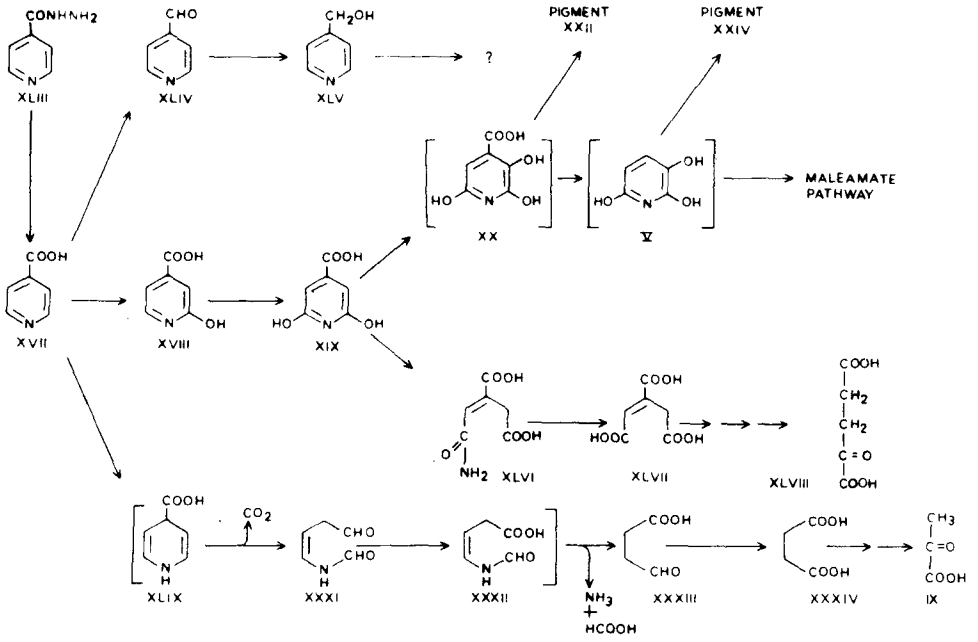

Figure 4. Pathways for microbial transformation of isonicotinic acid hydrazide and isonicotinic acid. XLIII: isonicotinic acid hydrazide; XLIV: isonicotinic aldehyde; XLIV: 4-pyridylmethanol XLVI: *cis*-aconitic acid amide; XLVII: *cis*-aconitic acid (other legends as given in figures 1 and 2).

11. Quinoline and isoquinoline

Only indirect evidence for biodegradation of quinoline was available earlier. The demonstration of mutagenic and carcinogenic potential in quinoline and its derivatives has focussed attention on their biodisposal. Quinoline has been observed to be readily degraded in aquatic systems, and several bacteria degrading quinoline have been isolated by enrichment (Grant and Al Najjar 1976; Shukla and Kaul 1981). These organisms exhibit specificity and do not attack isoquinoline, quinaldine, lepidine and other pyridine derivatives. Characteristic pigments have been detected during quinoline metabolism by microorganisms, suggesting that hydroxy quinolines are being formed as intermediates. 2-Hydroxyquinoline, 2,6-dihydroxyquinoline and a trihydroxyquinoline appear to be intermediates of quinoline metabolism in *Moraxella* sp (Grant and Al Najjar 1976). Strains of *Pseudomonas putida* also co-metabolize quinoline to *o*-aminophenyl-β-hydroxypropionic acid (Kucher *et al* 1980) suggesting a cleavage of the pyridine ring first.

Enrichment on isoquinoline, using sewage microbial community has yielded microbes which slowly degrade isoquinoline with the formation of an insoluble red pigment (λ_{max} 495 and 525 nm). The purified cultures from this community do not degrade isoquinoline; these organisms have, however, the capacity to degrade quinoline. It is likely that similar pathways are employed for the degradation of quinoline and isoquinoline in these organisms.

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