Utilization of catechin by microorganisms

N. Vasudevan and A. Mahadevan
Centre for Advanced Studies in Botany, University of Madras, Madras 600 025, India

Decomposition of condensed tannin by microorganisms has been a major environmental problem because of its recalcitrant nature. Recently evidence has accumulated that indicates degradation of tannins by microorganisms. The degradation of the condensed tannin catechin by bacteria and fungi and its enzymology reveal the existence of diverse pathways of tannin degradation in microorganisms.

THE problem of tannery effluent is a serious environmental threat especially in the developing countries[1]. In addition to inhibiting aquatic organisms[2] and higher plants[3], it poses a health hazard to higher animals[4]. The major component in the effluent is condensed tannin, composed primarily of catechin (I). Despite the gravity of the problem, biological degradation of condensed tannins has not been completely understood[5]. Only in recent years have a few microorganisms been screened for their capacity to decompose catechin. The findings indicate the existence of diverse pathways of tannin utilization in microorganisms.

Degradation of catechin

Aspergillus fumigatus, *A. niger*, *Penicillium freguentans*, *P. janthinellum*, *Fusarium* sp.[6]; *A. flavus*, *A. fumigatus*, *A. terreus*, *Penicillium*[7]; *C. cupreum*[8], *Pseudomonas solanacearum*[9], *Rhizobium* sp.[10]; *Bradyrhizobium japonicum*[11]; and *Endothia parasitica*[12] have been shown to utilize catechin. Utilization of catechin by fungi was slow; as many as 6 to 8 days were needed for part utilization. But bacterial degradation was comparatively rapid (2 to 3 days). However, *B. japonicum*, a slow-growing bacterium, needed as many as 12 days to cleave catechin.

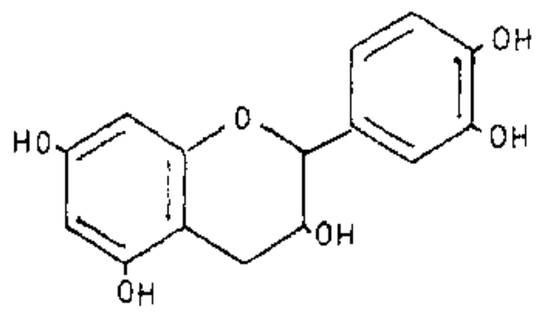
The concentration of catechin utilized by *Rhizobium* sp. and *B. japonicum* was about 2 to 5 mM[10,11]. Chandra *et al.*[7] reported that catechin at 10 mM was optimum for *A. flavus*, *A. niger*, *A. terreus* and *Penicillium* sp. However, *Pseudomonas solanacearum* utilized 2 to 10 mM of substance[13], and *C. cupreum*, up to 10 mM.

Degradation products

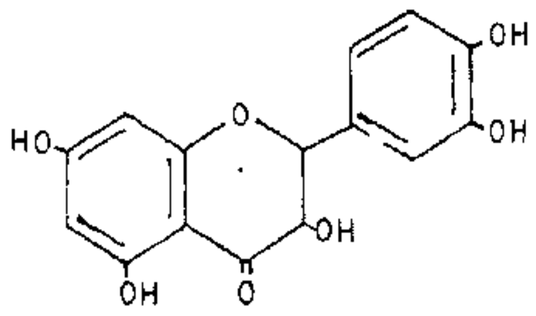
We have no information on the mechanism of uptake of catechin by microorganisms. Nor can we draw any analogy from the available literature, which is limited to uptake of benzoate by *Pseudomonas putida*[14].

Catechin was degraded by *A. flavus* to protocatechuic acid, phloroglucinolcarboxylic acid and an unidentified substance[7] (Figure 1). Catechol was identified in addition to protocatechuic acid and phloroglucinolcarboxylic acid in *C. cupreum* cultures[8]. Jeffrey *et al.*[15] showed the formation of taxifolin (II) from (+) catechin by an aerobic strain isolated from rat faeces. Taxifolin was further oxidized to protocatechuic acid, arising from the B ring of the flavononol. *Pseudomonas* sp. isolated from rat feaces oxidized catechin in presence of NAD(P)H and molecular oxygen to taxifolin, which was converted to dihydrogossypetin (III). Dihydrogossypetin was oxidized by cleavage of the A ring to form oxaloacetic acid from C-5, C-6, C-7 and C-8 together with 5-(3,4-dihydroxyphenyl)-4-(hydroxy-3-oxovalero-γ-lactone by catechin-grown cells[16] (Figure 2).

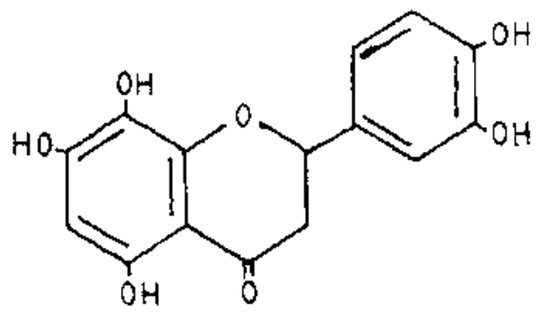
Rhizobium sp., *P. solanacearum* and *B. japonicum* utilized catechin by different routes (Figure 1). The complete pathway was worked out by Boominathan[13]. Phloroglucinol and protocatechuic acid appeared from A and B rings respectively. Further degradation of phloroglucinol proceeded via phloroglucinolcarboxylic



(I) Catechin



(II) Taxifolin



(III) Dihydrogossypetin

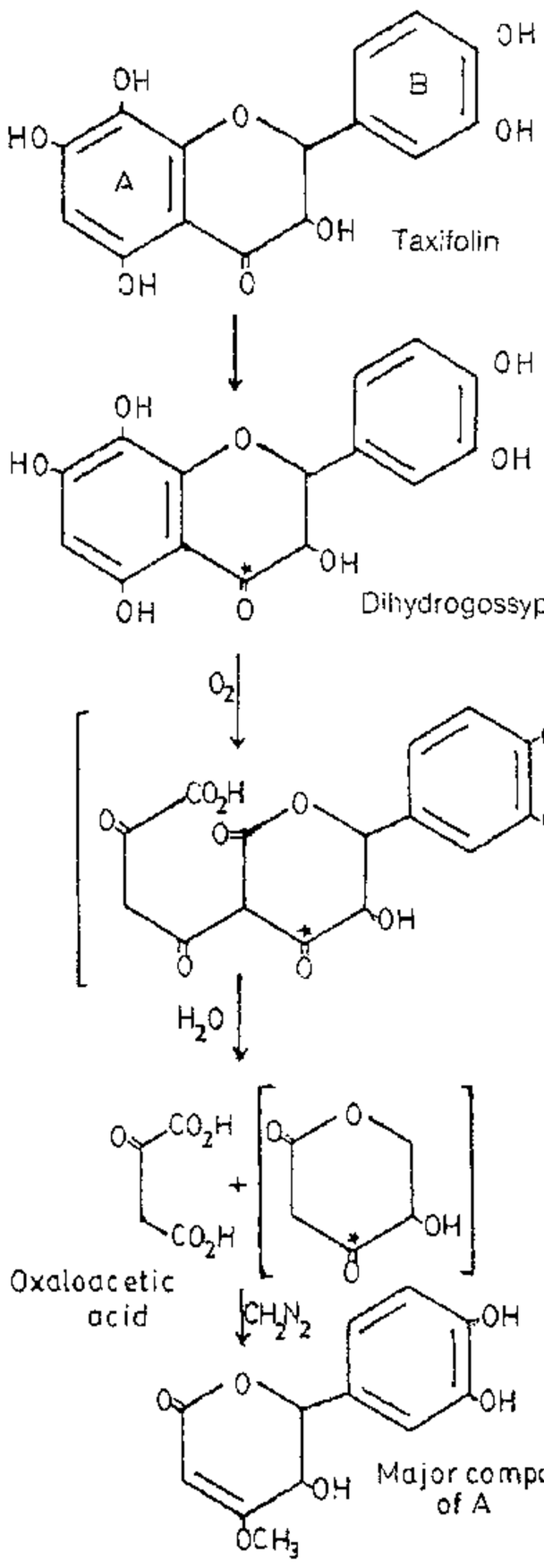


Figure 1. Degradation of taxifolin by *Pseudomonas* sp.

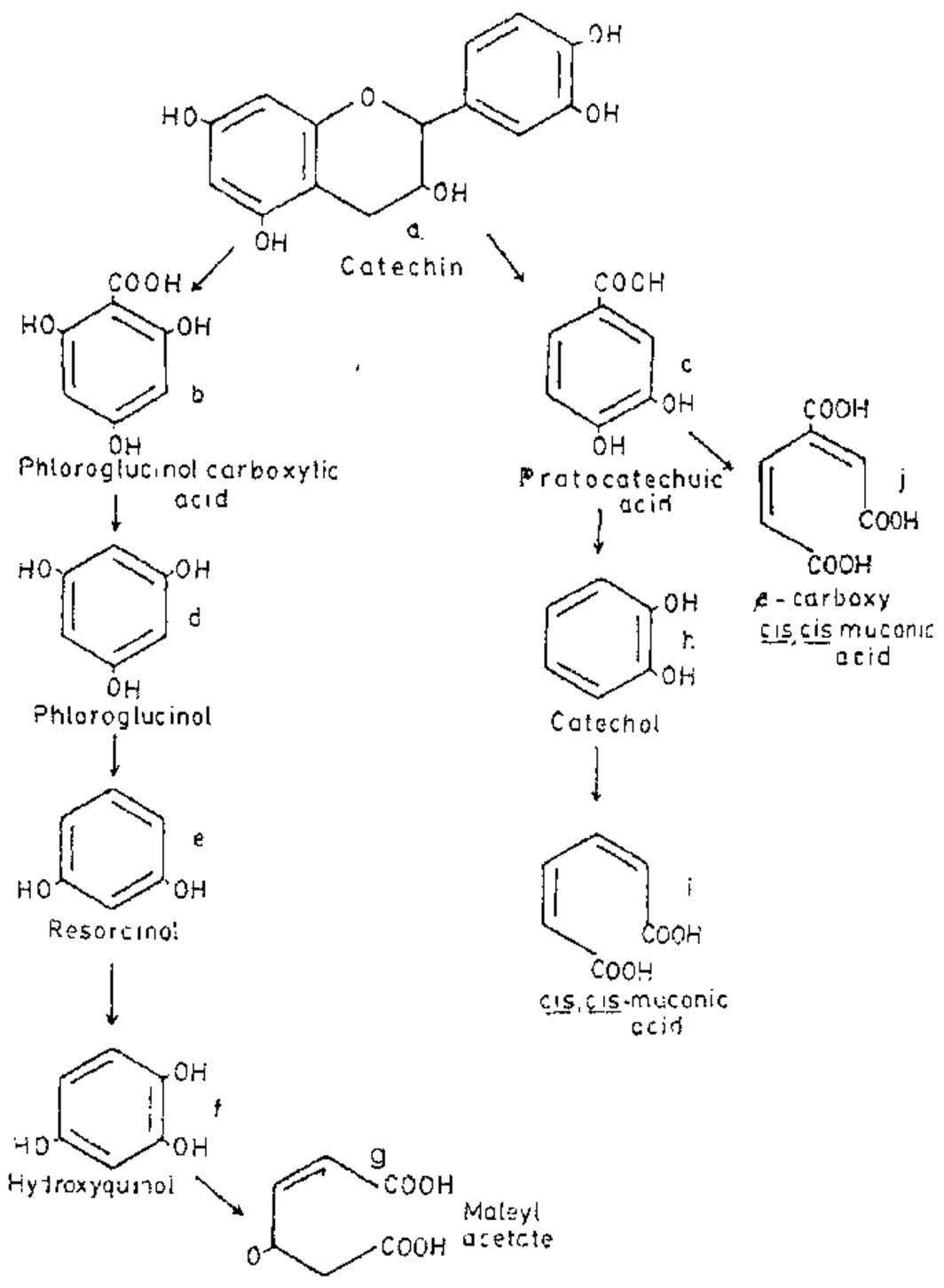


Figure 2. Degradation of catechins *Rhizobium* sp. (a to i); *B. japonicum* (a to g, j); *P. solanacearum* (a to j); *C. cupreum* (b, c, h); *A. flavus* (b, c).

acid, resorcinol and hydroxyquinol. Protocatechuic acid was converted to catechol. The pathway is similar to that in *Rhizobium* sp.[10] However, *B. japonicum* differed slightly, with cleavage of protocatechuic acid directly without the formation of catechol. Hydroxyquinol was the last aromatic-ring structure in the pathway, which was cleaved via *ortho* fission, forming maleyl acetate. Protocatechuic acid was cleaved directly via *ortho* pathway. However, when accumulated, it was decarboxylated to form catechol, which was ring-opened through *ortho* cleavage.

Catechin was used as carbon source by the fast-growing *R. leguminosarum*, *R. phaseoli*, *B. trifolli* and *Rhizobium* sp.[6] Crude enzyme from *B. japonicum* contained catechin oxygenase, protocatechuate 3,4-dioxygenase and hydroxyquinol 1,2-dioxygenase[17]. After cleavage of catechin, the degradation of phloroglucinol-carboxylic acid was through phloroglucinol by *B. japonicum*[17].

Phloroglucinol was converted to resorcinol by *B. japonicum*. *Pseudomonas* sp. converted phloroglucinol to resorcinol and then to hydroxyquinol, which was ring-opened to maleyl acetate[18]. Jamieson *et al.*[19] showed the formation of dihydrophloroglucinol during phloroglucinol metabolism by *Pseudomonas* sp. Anaerobically phloroglucinol was degraded by *Coprococcus* sp.[19] and *Eubacterium oxidoreducens*[21] to form acetate and butyrate. Walker and Taylor[22] reported that *Fusarium solani* converted phloroglucinol to pyrogallol, which was further metabolized via *meta* pathway to pyruvate.

Resorcinol was converted to hydroxyquinol through resorcinol hydroxylase in *B. japonicum*[17]. Resorcinol was hydroxylated to hydroxyquinol by resorcinol hydroxylase in *B. japonicum*. *Azotobacter vinelandii* converted resorcinol to pyrogallol[22].

Enzymology of catechin degradation

Despite these few studies, the enzymology of condensed-tannin degradation by microorganisms has surprisingly been ignored. However, enzymology of catechin degradation has been studied in two laboratories.

Sambandam[8] purified catechin oxygenase from *C. cupreum*. The enzyme acts at lower and higher pH (2.8 and 7.0 respectively). Its molecular weight is around 40,000. It has a K_m value of 4×10^{-7} M. Galiotou–Panayotou and Macris[24] reported that *Calvatia gigantea* produced an enzyme that degraded catechin. The enzyme exists in two forms, which were purified to 114- and 90-fold respectively[25]. Each of these forms is composed of two components, of molecular weight 50,500 (I_1 and II_1) and 49,500 (I_2 and II_2). A K_m value of 3×10^{-1} M was shown by both the forms. Optimum pH was 8.0 and optimum temperature 35°C.

Catechin oxygenase was detected in cultures of *P. solanacearum*[13] and *B. japonicum*[17]. Jeffrey *et al.*[15] detected taxifolin hydroxylase during the oxidation of taxifolin formed from catechin by *Pseudomonas* sp.

Molecular biology of catechin degradation

Information on the molecular biology of catechin degradation is confined to *P. solanacearum*[9]. The authors have implicated the involvement of a mega-plasmid of 450×10^6 Da in the complete degradation of catechin. Age of the culture also influenced copy number of the plasmid in *P. solanacearum*[26]. Cultures from the stationary phase showed high copy number. In fungi, however, we do not know the mechanism. Much research is yet to be done to identify the genes involved in the synthesis of enzymes that participate in the cleavage of catechin. This information would help us formulate strategies to evolve clones that would rapidly cleave wattle and condensed tannins present in tannery effluents.

1. William, F., Boominathan, K., Vasudevan, N., Gurujeyalakshmi, G. and Mahadevan, A., *J. Sci. Ind. Res.*, 1986, **45**, 232.
2. Muthukumar, G. and Mahadevan, A., *Hydrobiologia*, 1980, **72**, 73.
3. Muthukumar, G., Sivaramakrishnan, R. and Mahadevan, A., *Proc. Indian Natl. Sci. Acad.*, 1985, **B51**, 270.
4. Sastry, C. A., *Environmental Biotechnology*, Nagpur, India, 1988, p. 28.
5. Boominathan, K. and Mahadevan, A., 53rd Annual Meeting, Soc. Biol. Chem., New Delhi, India, 1985.
6. Lewis, J. A. and Starkey, R. L., *Soil Sci.*, 1969, **107**, 235.
7. Chandra, T., Madhavakrishna, W. and Nayudamma, Y., *Can. J. Microbiol.*, 1969, **15**, 303.
8. Sambandam, T., Biochemical studies on microbial degradation of tannins, Doctoral thesis, Univ. of Madras, Madras, 1983, p. 141.
9. Boominathan, K. and Mahadevan, A., *FEBS Microbiol. Lett.*, 1987, **40**, 147.
10. Gajendiran, N. and Mahadevan, A., *Plant Soil*, 1988, **130**, 383.
11. Waheeta, A., William, F. and Mahadevan, A., 25th Annual Meeting of the Association Microbiologists, Pantnagar, India, 1985.
12. Elkins, J. R., Pate, W., Lewis, J. and Poterfield, C., III International Congress on Plant Pathology, Munchen, 1978.
13. Boominathan, K., Study of catechin degradation and CAT plasmid in *Pseudomonas solanacearum*, Doctoral thesis, University of Madras, Madras, 1987.
14. Thayer, J. R. and Wheelis, M. L., *J. Gen. Microbiol.*, 1982, **128**. 1749.
15. Jeffrey, A. M., Knight, M. and Evans, W. C., *Biochem. J.*, 1972, **130**, 373.
16. Jeffrey, A. M., Jerina, D. M., Self, R. and Evans, W. C., *Biochem. J.*, **130**, 383.
17. Waheeta, A., Degradation of catechin by *Bradyrhizobium japonicum*, Doctoral thesis, Univ. of Madras, Madras, 1987.
18. Blackwood, A. C., Hang, Y. S., Robern, H. and Mathur, D. K., *Bacteriol. Proc.*, 1970, **70**, 124.
19. Jamieson, W. D., Taylor, A., Mathur, D. K. and Blackwood, A. C., *Can. J. Biochem.*, 1970, **48**, 215.
20. Patel, T. R., Jure, K. G. and Jones, G. A., *Appl. Environ. Microbiol.*, 1981, **42**, 1010.
21. Tsai, C. G., Gates, D. M., Ingledew, W. W. and Jones, G. A., *Can. J. Microbiol.*, 1976, **22**, 159.
22. Walker, R. L. and Taylor, G. L., *Arch. Microbiol.*, 1983, **134**, 123.
23. Groseclose, E. E. and Ribbons, D. W., *J. Bacteriol.*, 1981, **146**, 460.
24. Galiotou-Panayotou, M. and Macris, B. J., *Appl. Microbiol. Biotechnol.*, 1980, **23**, 502.
25. Galiotou-Panayotou, M., Rodis, P., Macris, B. J. and Stathakos, D., *Appl. Microbiol. Biotechnol.*, 1988, **28**, 543.
26. Rajini Rani, D. B., Boominathan, K. and Mahadevan, A., *Physiological Plant Pathology*, Today and Tomorrow Publishers, New Delhi, India, 1988, pp. 164–171.

ACKNOWLEDGEMENT. We thank the Department of Environment, New Delhi, for financial assistance.

30 March 1990