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In their cell walls, plants contain structural chemical constituents that are difficult for animal enzymes to digest. These constituents, therefore, are of great protective value against predation. Many plants also devote energy to the production of compounds apparently not essential to the functioning of the living plant cell, but that have antinutritional effects on animals. The chemical nature of these compounds is extremely varied (hydrocarbons; alcohols; phenols; ethers; aldehydes; ketones; quinones; acids; lactones; esters; higher terpenoids; oxygen heterocyclic compounds such as furans, pyrones, coumarines, flavonoids, xanthones; amino compounds such as amines, nonprotein amino acids; nitriles such as cyanogenetic glycosides; alkaloid derivatives of pyrrolidine, piperidine. pyridine, indole, pyrrolizidine, imidazole, quinolizidine; purines; sulphur compounds).

For over 15 years, the biological significance of these «secondary» plant compounds has been thought to be a defense against predation. However, evolutionary events in herbivores were surely driven by the selective pressure made by these toxins, among other factors. Evolution has actually lead to multiple mechanisms of great efficiency to overcome these plant defenses. These range from behavioral adaptations that would limit the intake of a particular toxin, to more complex physiological adaptations in the herbivore, particularly in its digestive system.

Detoxification mechanisms are regulated processes that are very diverse physiologically and chemically. They can be found in many of the organs exposed to the toxins contained in ingested plants:

-in the mouth, the modification of the saliva composition in herbivores may play a role in the clearance of some antinutritional compounds (e.g. binding of tannins by saliva rich in proline-containing proteins; Austin et al, 1989);

-in the foregut, one of the most remarkable adaptations is the development of a microbial fermentation chamber anterior to the secretory («true» glandular) stomach, containing a sort of primordial soup (with ingredients such as carbon dioxide, hydrogen, methane, formate, ammonia) where microorganisms have found a suitable anaerobic niche. These microorganisms then provide the host with reduced foodstuffs (short chain fatty acids) from otherwise undegradable compounds, as well as providing the benefits of a wide range of biochemical reactions, including metabolism (modification or degradation) of toxins;

-in the small intestine epithelium, liver and kidneys, cell metabolism of dietary toxins can also take place. Biotransformation of many compounds usually occurs in two steps. Phase I consists of oxidative-reductive reactions that make the molecule more reactive, and phase II involves conjugation. These reactions increase the water solubility of the compound making it excretable. The liver can be an important site for the detoxification of pyrrolizidine alkaloids in ruminants (Cheeke, 1994).

This paper will mainly discuss bacterial detoxification in the gut, possible ecological and genetic regulatory mechanisms of detoxification in the rumen, and the biotechnological implications of bacterial detoxification activities.

The rumen as a detoxification chamber

Most wild ruminants and other foregut fermenters consume a variable diet throughout the year, subjected to the availability of plant material. This suggests that for dietary toxins that are seasonally consumed, the evolutionary pressure would not be enough to drive animal system adaptations. Microbes have more versatile systems and adapt more quickly, since they have a short generation time and a wide range of biochemical activities. The location of foregut fermentation chambers

(anterior to the absorptive intestine) inhabited by microbes, is strategic for microbial detoxification purposes. One obvious advantage of microbial detoxification in the rumen, is the economy of the host animal's biochemical investment.

There are well known examples where the rumen results in an efficient detoxification chamber. Ruminants therefore, tolerate poisonous plants better than non ruminants. The transformation of toxins generally results in less toxic compounds. However there are few cases where more toxic compounds are produced, e.g. HCN may be produced from cyanogenic glycosides; pyrogallol is a hepatotoxin and nefrotoxin is produced in the degradation of hydrolyzable tannins in the rumen (Reed, 1995). Examples of digestive microbial detoxification, (including rumen) of plant toxins are given in table I. Since the nature of intestinal reactions (mainly hydrolysis, reductions and fissions) resemble that of the rumen, reference to the degradation of toxins by intestinal microbes have also been included in table I. Sometimes, ruminants can tolerate a slow increase in the intake level of some plants which contain toxins (omitted in table I). This suggests that microbial detoxification may occur (for example, 2,4 diaminobutyric acid from flaptea; Rasmussen et al, 1993).

It has been shown that a gradual increase in the intake of a toxic plant by the ruminant, increases the animal's tolerance to the plant. This means that if the animal interrupts the intake of the toxic plant, then its detoxification capacity will be diminished. Tropical wild generalist herbivores graze or browse on different plants throughout the year, according to plant availability (deciduous plants loose their leaves in the dry period). Therefore the detoxification capacity for a specific toxin is not permanently required. It may be that these animals naturally allow themselves a period of physiological adaptation. The events during this period remain unknown, but they are surely related to microbial changes that increase toxin-degrading activity. One could expect that :

-the numbers of the degrader strains significantly decrease when the toxin is absent but recover gradually as the animal reingests the toxin-containing plant;

-the bacterial expression of the degrading enzyme is slowly induced by substrate;

-the activity is plasmid-coded, and bacteria loose and regain activity according to the absence or presence of the substrate;

-chromosomal mutations in the gene coding for the degrading enzyme occur, so that active degraders lose their degrading ability in the absence of the substrate, and when the animal reingests the toxin, revertant mutants dominate;

-the bacterial expression of the degrading enzyme is repressed by a more readily fermentable substrate (catabolite repression), so that degradation occurs only when the repressor substrate has disappeared.

Nothing is known about which of these events could explain the gradual adaptation of a ruminant to a given toxin, and the observation of the disappearence of the degrading activity in degrader strains. DHP degrading activity in vitro has been lost by freezing degrader strains for a long period, or by maintaining them in the absence of the DHP. In some cases, the activity has reappeared, but the genetic nature of the phenomenom remains unknown.

Other natural detoxification systems

Detoxifying capabilities are not restricted to the rumen or intestinal bacteria. Other wild herbivores, mammal or non mammal foregut fermenters, also have detoxifying bacterial symbionts. The bird Hoatzin (*Opisthocomus hoazin*) consumes toxin-containing leaves (Dominguez-Bello et al, unpublished results), probably detoxified by the fermentative symbionts in its crop.

Insect bacterial symbionts are known to play a role in the survival of their host by detoxification processes (Dowd, 1991). In some cases, the bacterial-borne toxin resistance has not been proved: eg. metabolism of canavanine by *Caryedes brasiliensis* (Rosenthal, 1982); resistance of *Psyllidae* to *Leucaena* toxins (Beardsley, 1986).

Legume plants contain high concentrations of the most diverse toxins, and they all depend on their *Rhizobium* and fungal symbionts to survive. It is likely that these bacteria have biochemical mechanisms that overcome the toxicity of the antibacterial compounds of the plants. *Rhizobium* strains from *Leucaena* roots have recently been shown to catabolize

Table I. Plant toxins metabolized by digestive bacteria.

COMPOUNDS	TRANSFORMATION	REFERENCE
Alcohols		
Benzyl alcohol	Reduction to toluene derivatives by intestinal microflora.	Scheline, 1972
Phenols		
Arbutin (monoglucoside of hydroquinone)	Hydrolyzed by enterobacteria.	Drasar and Hill, 1985
Stilbene	Reduction of double band by intestinal bacteria and biliary excretion of glucuronide conjugates.	Tay and Sinsheimer, 1975
Aromatic aldehydes		
Salicylaldehyde	Metabolized by rat cecal microorganisms	Scheline, 1972
Acids		
Oxalate	Metabolized by rumen flora with production of methane, and by other intestinal bacteria.	Shirley and Schmidt-Nielsen, 1967
Quinic acid	Aromatisation by intestinal and rumen microorganisms.	Allison, 1985 Martin, 1982
Shikimic acid	Reduced by rumen and intestinal bacteria.	Scheline, 1968
Phenolic acid	Reduced by rumen and intestinal bacteria.	Balba and Evans, 1977 Scheline, 1968 Krumholz and Bryant, 1986a
Esters		
Hydrolyzable tannins	Degraded by rumen and other digestive bacteria.	D'Mello, 1989 Dobkin et al, 1983
Terpenoids		
Steviol	Hydrolyzed by rat cecal flora.	Wingard et al, 1980
Glycyrrhizic acid	Hydrolyzed by human cecal bacteria.	Hattori et al, 1983
Saponins	Metabolized by mouse intestinal flora.	Shimitzu et al, 1985
Cardia steroids (glycosides, digoxin)	Reduced by human intestinal flora.	Dobkin et al, 1983
Heterocyclic compounds		
Coumarins	Metabolized by intestinal flora in rats and humans.	Drasar and Hill, 1985
Flavonoids		
Apigenin	Degraded by intestinal flora.	Griffiths and Smith, 1972
Rutin, quercetin	Degraded by rat intestinal flora, rumen bacteria.	Booth and Williams, 1963 Krumholt and Bryant, 1986b
Naringin	Degraded by rat caecal and rumen bacteria.	Cheng et al, 1971
Catechins	Metabolized by intestinal flora in rats.	Groenewoud and Hundt, 1986
Biochanin A	Demethylated by rumen flora.	Nilsson et al, 1967
Organic cyanides		
Amygdaline	Hydrolyzed by rumen and intestinal microbes.	Majak and Cheng, 1987
Nonprotein amino acids		
Canavanine	Degraded by rumen bacteria.	Dominguez-Bello and Stewart, 1990
Mimosine	Degraded by rumen bacteria.	Dominguez-Bello and Stewart, 1991 Tan et al, 1994 Allison et al, 1992
SMCO	Fermented to toxic compounds by rumen bacteria.	Barry et al, 1985
Nitro compounds		
Miserotoxin, nitropropionic, nitropropanol	Metabolized by rumen fluid.	Anderson et al, 1993
Nitrogen heterocyclic compounds		
Pyrrolizidine alkaloids, heliotrine	Reductive fission by rumen microbes of sheep, goat and to a lesser extent, cow.	Cheeke, 1994 Craig et al, 1992 Majak, 1992
Retrosine-N-oxide	Reduced by sheep rumen fluid and rat intestinal microbes.	Mattocks and Driver, 1987
Sulfur compounds		
Glucosinolates (progoitrin)	Converted to the active goitrin by sheep rumen bacteria.	Lanzani and Piana, 1974
Sulfoxides	Reduced by goat rumen contents and intestinal microflora.	Renwick et al, 1986

mimosine and 3,4DHP (Muchard et al, 1994). Fungal strains may also be a source of plant toxin-degrading enzymes. A fungus grown on a water solution of mimosine left accidentally on the bench for over a week, was able to completely degrade mimosine (Dominguez-Bello, 1989).

Detoxification of plant toxins by biotechnological techniques would allow the nutritional exploitation of numerous tropical plants, such as legumes, as a source of proteins for feeding monogastric animals and humans. This approach has to consider all the biological sources of degrading enzymes. Clearly, two of the most adapted microbial ecosystems to deal with plant toxins are the foregut of herbivores (anaerobes) and the roots of legume plants (aerobes). The latter system has probably the advantage of being more easily adapted to biotechnological applications, although genetic manipulation could surely allow the transfer of activities from anaerobic systems to well known aerobic bacteria.

Literature cited

Allison MJ (1985) Anaerobic oxalate-degrading bacteria of the gastrointestinal tract. *In: Plant Toxicology Proceedings of the Australia- USA Poisonous plants symposium Brisbane* (Seawright et al, eds) Australia, 1984, Queenlands Poisonous Plants Committee, Yeerongpilly, 120

Allison MJ, Mayberry WR, McSweeney CS, Stahl DA (1992) *Synergistes jonesii*, gen-Nov, sp. nov.: A rumen bacterium that degrades toxic pyridinediols. *Syst Appl Microbiol* 15, 522-529

Anderson RC, Rasmussen MA, Allison MJ (1993) Metabolism of the plant toxins nitropropionic acid and nitropropanol by ruminal microorganisms. *Appl Environ Microbiol* 59, 3056-3061

Austin PJ, Suchar LA, Robbins CT, Hagerman AE (1989) Tannin-binding proteins in saliva of deer and their absence in saliva of sheep and cattle. *J Chem Ecol* 15, 1335-1349

Balba MT, Evans WC (1977) the origin of hexahydrohippurate (cyclohexanoylglycine) in the urine of herbovors. *Biochem Soc Trans* 5, 300-302

Barry TN, Manley TR, Redekopp C, Allsop TF (1985) Endocrine regulation of metabolism in sheep given kale (*Brassica oleracea*) and ryegrass (*Lolium perenne*)-clover (trifolium repens) fresh forage diets. *B J Nutr* 54, 165-173

Beardsley JW (1986) Psyllidae or jumping plant lice: notes on biology and control. *Leuc Res Rep* 7, 2-5

Booth AN, Williams RT (1963) Dehydroxilation of catechol acids by intestinal contents. *Biochem J* 88, 66-71

Cheeke PR (1994) A review of the functional and evolutionary roles of the liver in the detoxification of poissonous plants, with special reference to pyrrolizidine alkaloids. *Vet Hum Toxicol* 36, 240-247

Cheng KJ, Krishnamurty HG, Jones GA, Simpson FJ (1971) Identification of products produced by the anaerobic degradation of naringin by *Butyribivrio* sp. C3. *Can J Microbiol* 17, 129-134

Craig AM, Latham CJ, Blythe LL, Schmotzer WB, O'Connor OA (1992) Metabolism of toxic pyrrolizidine alkaloids from tansi ragwort (*Senecio jacobaea*) in ovine ruminal fluid under anaerobic conditions. *Appl Environ Microbiol* 58, 2730-2736

D'Mello (1989) *Asp Appl Microbiol* 19, 29

Dobkin JJ, Saha JR, Bulter VP, Neu HC, Lindenbaum J (1983) Interethnic variation in metabolic inactivation of digoxin by the gut flora. *Science* 220, 25-27

Domínguez-Bello MG (1989) Microbial ecophysiology in the rumen of sheep fed tropical forages. *PhD Thesis*, Univ Aberdeen, Scotland

Domínguez-Bello MG, Stewart CS (1990) Effects of feeding *Canavalia ensiformis* on the rumen flora of sheep, and of the toxic amino acid canavanine on rumen bacteria. *Syst Appl Microbiol* 13, 388-393

Domínguez-Bello MG, Stewart CS (1991) Characyteristics of a rumen *Clostridium* capable of degrading mimosine, 3(OH)-4-(1H)-pyridone and 2,3 dihydroxypyridine. *Syst Appl Microbiol* 14, 67-71

Dowd (1991) Symbiont-mediated detoxification in insect herbivores. *In: Microbial mediation of plant-herbivore interactions* (Barbosa P, Krischik VA, Jones CC, eds) John Wiley and Sons, New York, 411-40

Drasar BS, Hill MJ (1985) *Human Intestinal Flora*. Academic Press, London, 63

Griffiths LA, Smith GE (1972) Metabolism of myricetin and related compounds in the rat Metabolite formation in vivo and by the intestinal microflora in vitro. *Biochem J* 130, 14-16

Groenewoud G, Hundt HKL (1986) The microbial metabolism of condensed (+) catechins by rat-cecal microflora. *Xenobiotica* 16, 99-104

Hattori M, Sakamoto T, Kobashi K, Namba T (1983) Metabolism of glycyrrhicin by human intestinal flora. *Planta Med* 48, 38-43

Krumholtz LR, Bryant MP (1986a) *Syntrophococcus sucromutans sp. nov.* uses carbohydrates as electron donors and formate, methoxy-monobenzenoids or *Methanobrevibacter* as electron acceptor systems. *Arch Microbiol* 144, 8-14

Krumholtz LR, Bryant MP (1986b) *Eubacterium oxidoreducens sp. nov.* requiring H_2 or formate to degrade gallate, pyrogallol, phloroglucinol and quercetin. *Arch Microbiol* 143, 313-319

Lanzani A, Piana G (1974) Changes in *Brassica napus* progoitrin induced by sheep rumen fluid. *J Anim Oil Chem Soc* 51, 517-521

Majak W (1992) Further enhancement of 3-nitropropanol detoxification by ruminal bacteria in cattle. *Can J Anim Sci* 72, 863-870

Majak W, Cheng KJ (1987) Hydrolysis of the cyanogenic glycosides amygdalin, prunasin and linamarin by ruminal microorganisms. *Can J Anim Sci* 67, 1133-1138

Majak W, Cheng KJ, Hall JW (1986) Enhanced degradation of nitropropanol by ruminal microorganisms. *J Anim Sci* 62, 1072-1076

Martin AK (1982) The origin of urinary aromatic compounds excreted by ruminants III The metabolism of phenolic compounds to simple phenols. *Proc Nutr Soc* 34, 69-74

Mattocks AR, Driver HE (1987) Metabolism and toxicity of anacrotine, a pyrrolizidine alkaloid, in rats. *Chem Biol Interact* 63, 91-94

Muchard S, Hemscheidt TK, Borthakur D (1994) Mimosine, a toxin present in trees (*Leucaena* spp.), induces a mimosine-degrading enzyme activity in some rhizobium strains. *Appl Environ Microbiol* 60, 4268

Nilsson A, Hill JL, Lloyd Davies H (1967) An in vitro study of formononetin and biochanin A metabolism in rumen fluid from sheep. *Biochim Biophys Acta* 148, 92-97

Rasmussen MA, Allison MJ, Foster JG (1993) Flaptea intoxication in sheep and indications of ruminal adaptation. *Vet Hum Toxicol* 35, 123-127

Reed JD (1995) Nutritional toxicology of tannins and related polyphenols in forage legumes. *J Anim Sci* 73, 1516-28

Renwick AG, Strong HA, George CF (1986) The role of the gut flora in the reduction of sulphoxide containing drugs. *Biochem Pharmacol* 35, 64-69

Rosenthal (1982) *Plant Nonprotein Amino Anids.* Acad Press, New York, 273 p

Scheline RR (1968) The metabolism of drugs and other organic compounds by the intestinal microflora. *Acta Pharmacol Toxicol* 26, 332-338

Scheline RR (1972) The metabolism of some aromatic aldehydes and alcohols by the rat intestinal microflora. *Xenobiotica* 2, 227-231

Shimizu K, Amagaya S, Ogihara Y (1985) Structural transformation of saikosaponins by gastric juice and intestinal microflora. *J Pharmacobio Dyn* 8, 718-722

Shirley EK, Smith-Nielsen K (1967) Oxalate metabolism in the pack rat, sand rat, hamster and white rat. *J Nutr* 91, 496-499

Tan P, Wang X, Wang J (1994) Rumen bacteria degrading toxic mimosine and dihydroxypyridine compounds in China. *Wei Sheng Wu Hsueh Pao* 34, 379-384

Tay LK, Sinsheimer JE (1975) Intestinal bacterial reduction of 4-4'-dihydroxystilbene to 4-4'-dihydroxybenzyl. *J Pharm Sci* 64, 471-476

Wingard RE, Brown JP, Enderlin FE, Dale JA, Hale RL, Seitz CT (1980) Intestinal degradation and absorption of the glycosidic sweeteners stecioside and rebaudoside. *Experientia* 36, 519-524