Biosafety Issues Of Unintended Horizontal Transfer Of Recombinant DNA

Evolution of Herbicide Resistance Weeds

On a large geographical scale, many independent evolutionary events could simultaneously interplay for the emergence of herbicide resistance (Bonny, 2016). Regular use of glyphosate on a considerable proportion of GM crop fields makes the assumption of glyphosate resistance development a reasonable hypothesis. It is not mandatory for weeds to be a poorer competitor than susceptible weeds as no fitness differential was detectable between susceptible and resistant biotypes (Busi et al., 2013). In tolerance development, various mechanisms could help the plant such as target site over production, modification in intracellular herbicide compartmentation, minimal herbicide absorbance and translocation, herbicide detoxification and insensitivity to target site (Brower et al., 2012).

Resistance to Insecticide and Pesticide

Controlling pests through conventional and chemical techniques have been proven to be challenging as evolution of insecticide and pesticide resistance has been witnessed in many cases (Dale et al., 2002). More specifically, the possibility of evolution of Bt-resistant insect pests can't be negated because of constitutive expression of Bt toxins in all plant tissue imparts higher selection pressure on target species (Yu et al., 2011). Use of Bt bio-pesticides by organic farmers lead to resistant diamondback moth populations in Central America, Florida, Japan, Philippines, Hawai, and China (Tabashnik et al., 2013).

HGT of Recombinant DNA to Eukaryotic Cells

The uptake of food derived DNA into host intestinal cells or tissues has been raised as a potential concern related to the introduction of GMO based food sources. Such exposure must be seen in relation to the broad variety of DNA naturally present in food and hence, whether specific qualitative or quantitative genetic changes are present in the GMO that would create a higher risk/impact of DNA exposure from this source (Nawaz et al., 2019; Rizzi et al., 2012; Nordgard et al., 2007).

The fate of dietary DNA in the gastrointestinal tract (GIT) of animals has gained renewed interest after the commercial introduction of genetically modified organisms (GMO). Among the concerns regarding GM food, are the possible consequences of horizontal gene transfer (HGT) of recombinant dietary DNA to bacteria or animal cells (Rizzi et al., 2012). The exposure of the GIT to dietary DNA is related to the extent of food processing, food composition, and to the level of intake. Animal feeding studies have demonstrated that a minor amount of fragmented dietary DNA may resist the digestive process (Rizzi et al., 2012; Nordgard et al., 2007).

Feed derived DNA taken up from the gastrointestinal tract and detection in leucocytes, spleen, liver, and kidneys in mice, in the brain, eyes, liver, and heart of the offspring of mice (plasmid DNA), detection in the liver and spleen of mice following feeding with soybean leaves (Hohlweg

and Doerfler, 2001), and detection of fragments of plant DNA in muscle, liver, spleen, and kidneys in chicken and cattle (Einspanier et al., 2001). It has been estimated that approximately 0.1% to 1% of dietary DNA is absorbed from the gastrointestinal tract (Nielsen et al., 2005a; 2006). A precise measurement of this process is complicated because absorption from the gastrointestinal tract takes place over several hours and absorbed DNA undergoes continuous transport, degradation and elimination. Nevertheless it is clear that DNA in food may reach the bloodstream and be exposed to and localized to various host cells and tissues (Rizzi et al., 2012).

Biological risk assessment of food containing recombinant DNA has exposed knowledge gaps related to the general fate of DNA in the gastrointestinal tract (GIT). DNA macromolecules are continually introduced into the gastrointestinal tract (GIT) as a natural part of food.

Whereas the majority of feed-derived DNA is broken down during digestion (Palka-Santini et al., 2003; Tony et al., 2003), several studies have now shown that minor proportions of feed-derived DNA survive immediate degradation and reach the bloodstream in various animals (Deaville and Maddison, 2005; Einspanier et al., 2001; Jennings et al., 2002) or are detectable as minor fragments in faeces (Chowdhury et al., 2004; Wilcks et al., 2004). The fate of chromosomal DNA in the gastrointestinal tract (GIT) of humans and animals has recently received increased attention due to the introduction of novel ingredients derived from genetically modified organisms (GMOs) in the food chain (Sharma et al., 2006). Biological risk assessment of GMOs has exposed knowledge gaps related to how DNA is degraded, or survive degradation in various compartments of the GIT (Nordgard et al., 2007).

The Gastrointestinal Tract of Human: A Hotspot for Horizontal Gene Transfer

The human body is generally studied as a single organism, although it functions more as a complex ecosystem since it hosts trillions of bacteria in different body habitats. The GIT alone is inhabited by 1013-1014 bacteria (Sender et al., 2016). There is a gradient in bacterial concentration along the GIT from low concentrations in the stomach and the duodenum (103-104 bacteria/g), increasing in the ileum (108 bacteria/g) with the highest bacterial concentrations found in the colon and stools where ?1011 bacteria/g are present. Dysbiosis of the gut microbiota is implicated in a wide range of diseases such as inflammatory bowel disease, diabetes, cardiovascular disease, or even autism spectrum disorders (Cho and Blaser, 2012). The dynamics of these bacterial communities is complex. However, one hallmark of these communities is that bacteria can share different phenotypic traits through a transfer of genetic material. This was first described in 1928 by Fred Griffith, when DNA from a virulent bacterial strain (Streptococcus pneumonia) was isolated and mixed with an avirulent form of the bacterium (Griffith, 1928). This was subsequently found to be caused by a mechanism known as horizontal gene transfer (HGT), by which bacteria can share different traits such as antibiotic resistance (van Schaik, 2015).

Cross-section of the gut showing the absorption of antibiotic following enteral administration followed by antimicrobial resistance (AMR) development in the large intestine. (A) Antibiotic absorption to the systemic circulation through the walls of the small intestine. (B) Selective propagation of resistant gut bacteria following exposure to sub-lethal antibiotic concentrations in the slower moving large intestine. (C) Excretion and spread of resistant bacteria in the feces

along with associated antimicrobial resistance genes (ARGs) into the surrounding environment.

Human Exposure to Foreign DNA

Humans are continually exposed to foreign DNA (GM and/or non-GM) from a broad range of food and feed sources including inhaled organisms (e.g. bacteria, viruses, pollen etc.), from a broad variety of food sources including the microorganisms present in food, via microorganisms normally present in and on humans, and infectious agents entering the body. The study conducted by Rizzi et al. (2012) indicated that a few years ago it was assumed that ingested DNA is completely degraded in the digestive tract of humans and animals.

However, with the global commercialization of GM food and feed, there has been a renewed interest in the fate and effects of GM derived extracellular DNA in the body of the consumer. Thus the human body has mechanisms to protect host cells and utilize and degrade or remove foreign DNA molecules. For instance, free bacterial DNA in the blood triggers immune system reactions (Cohen 2002). It is estimated that humans ingest 0.1 g to 1 g of DNA per day (Doerfler 2000). The quantity of any recombinant DNA ingested will be a minor fraction of the total DNA consumed per human per day. Transgenes are considered chemically equivalent to any other gene present in food (Jonas et al., 2001).

DNA in food

DNA molecules of broad size ranges are present in large numbers in all raw and unprocessed food sources. Depending on the extent of processing various fractions of DNA molecules of a reduced size may be present in the consumed product. The broad application of sensitive PCR technology has thus exemplified the widespread occurrence and persistence of DNA molecules in various food sources, including processed food such as corn chips and chocolate (Rizzi et al., 2004).Thus the overall concentration and distribution of DNA of a size that enables entire protein coding genes to be horizontally acquired from various food sources by host cells or bacteria remains largely undetermined. Studies conducted by Duggan et al. (2003) have demonstrated that the persistence of DNA in food and by Van and Young (2014); Gryson (2010); Kharazmi et al. (2003) revealed that processing often decreases the size of DNA, and such molecules can be undetectable in extensively processed food.

DNA stability in the digestive tract

Most free DNA molecules entering the digestive system undergo substantial degradation by enzymes attacking DNA (nucleases, DNases), released from the pancreas and by bacteria present in the intestine (Wilcks et al., 2004). In addition, the low pH of the stomach may chemically modify the DNA molecules. Remaining DNA fragments are excreted in the faeces with variation in the degradation efficiency between mammals. For instance, Chowdhury et al. (2003a; 2003b) reported that maize DNA could be detected in pig faeces. Study by Netherwood et al. (2004) reported that whereas some DNA fragments survived passage through the small bowel, transgenes could not be detected in the faeces of human volunteer’s feed GM soy products.

Most studies on DNA stability in the digestive systems of mammals have used purified DNA and may therefore not capture the impact of various food components, treatments and locations on

DNA degradation and stability (Martin-Orue et al., 2002). Although deoxyribonuclease I (DNase I) is detected in saliva, it is believed that DNA digestion starts in the stomach (Liu et al., 2015) where histones are separated from DNA by the action of pepsin (the primary enzyme in the stomach) and the acidity of the environment. DNA is further broken down by gastric acid and DNA nucleases along the GIT and thus only small fragments are presented to intestinal epithelial cells.

Two possible processes involved in extracellular DNA uptake into the cells. (i) Transcytosis of dsDNA: Uptake of DNA fragments across the intestinal epithelia mediated by vesicular transport. (ii) Endocytosis of dsDNA: Naked dsDNA can be spontaneously internalized by sequence dependent mechanism by which genetic information can enter living cells at significant amounts in a bioactive form. The process is also cell type dependent (Nawaz et al., 2019).

HGT of Recombinant DNA to Prokaryotic Cells

HGT of transgenes into pathogenic beneficial or environmental microorganisms resulting in potential unanticipated fitness effects has been voiced as a potential biosafety issue. A broad range of DNA compositions is continually released from decaying organic matter. Microorganisms are responsible for the majority of organic matter decomposition and therefore also DNA degradation. Thus, microorganisms present in the human gastrointestinal tract and in agricultural environments experience continual exposure to DNA released from themselves and the organisms in their immediate surroundings. DNA fragments exposed to bacteria will most often be utilized as a nutrient source (Nielsen et al., 2007). However, in rare circumstances foreign DNA may also be integrated into the bacterial genome. Experimental studies do not suggest bacteria integrate foreign unrelated chromosomal DNA at measurable frequencies over the limited time span (hours to days) (De Vries et al., 2001; Nielsen et al., 2005).

A high uptake frequency is also unlikely because bacteria are continually exposed to a high diversity of DNA compositions in their environments and unchecked uptake of DNA would quickly reduce the fitness of the bacterium and soon become lethal. Thus, microbial communities are in some cases already exposed to naturally occurring counterparts to these protein encoding genes (Nielsen 2003a; Nielsen et al., 2005). The introduction of similar protein coding genes from recombinant sources to soil is therefore often inferred in biological risk assessments to cause little additional environmental impact, if a HGT event occurred (Nielsen 2003a).

The novelty of the transgenes inserted into GMOs is likely to increase in the future due to development of novel gene constructs (synthetic and artificial bifunctional and multifunctional proteins) obtained through gene fusions, reshuffling and de novo construction of novel protein encoding domains (Nielsen, 2003b).

Concluding Remarks

HGT is defined as the transfer of genetic material from one organism to another independent of reproduction. HGT results in unidirectional gene flow, usually of one to several genes from a donor organism to the genome of a recipient organism. Ku and Martin have (Ku and Martin, 2016) indicated that eukaryotes do not acquire genes through continual HGT like prokaryotes.

From the current scientific evidence, HGT from GMOs to other organisms presents negligible risks to human health and safety or the environment due to the rarity of such events relative to those HGT events that occur in nature and the limited chance of providing a selective advantage to the recipient organism. The risk assessment of a transgenic bacterium must consider the potential for transfer of introduced genes to other microorganisms in the environment. (Ku et al., 2015) suggested the risks of gene transfer from GM crops currently commercialized as being negligible and the function, characteristics, and potential health impact of the introduction of different transgenes of microbial origin into commercial GM plants and finally concluded that unintended horizontal gene transfer to bacteria was unlikely to raise health concerns. Transfer of antibiotic resistance marker genes from GM plant to the gut microflora of humans and animals and their expression is most probably a rare event, given the low amounts ingested and degradative conditions in the gastrointestinal tract.