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Trends in Food Science & Technology 38 (2014) 149–157

TRENDS IN FOOD SCIENCE & TECHNOLOGY

Viewpoint

Microbial toxins and low level of foodborne exposure

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The effect of toxins on human health is diverse. In many cases, the relationship between toxins as the causative agent of disease in humans and a host response is difficult to determine. Acute effects of gastroenteritis may be easily identified; however, chronic effects resulting from ingestion of low to moderate levels of toxins can be difficult to recognize. Moreover, the repeated exposure to these sub(acute) doses can lead to their accumulation and (sub)chronic harms. This aspect is of high relevance in the case of lyophilic and stable toxins, such as microbial depsipeptides, cereulide and beauvericin. The risk assessment based approaches, based on in-depth toxicological studies, using *in vivo*, *in vitro* and in silico approaches, but also though modification of principles of threshold of toxicological concern (TTC) can be adapted to microbial (bacterial) toxins allowing the definition of levels of no-safety concern. Also the multiexposure, including both multi-toxin and multi-source, and repeated exposure phenomena need to be taken into account and put into perspective with prevalence of low toxin concentrations, total amount of food/toxin ingested, body weight, extended exposure time, and absorption, distribution, metabolism and excretion of the toxins. The aim of this article is to highlight some of the pressing research needs in this domain.

Introduction

Some of the most hazardous foodborne pathogens are characterized by the ability to produce toxins. Among the bacterial pathogens the most often incriminated ones are those of gram positive bacteria *Bacillus cereus*, *Clostridium botulinum*, *Clostridium perfringens* and *Staphylococcus aureus*, as exemplified in Table 1. These toxins cause annually about ten percent of all foodborne outbreaks reported in EU, as shown in Table 2 (EFSA, 2012; EFSA, 2011; EFSA, 2009). Their production may occur at almost any stage of the food chain and they can remain present and biologically active, even when the respective microorganism is inactivated, depending on their stability (Table 3). Namely, toxigenic bacteria are differentiated on the basis that they cause disease by producing toxins in foods prior to its ingestion (causing intoxication) or in the intestines of the host (causing toxico-infection). Our knowledge of the toxicity and levels of these toxins that cause (in) visible symptoms and damages is not at the desired level. Especially, for the low doses that do not cause expected visible symptoms. The aim of this manuscript is discuss research approaches for future investigation of exposure to subacute doses of microbial toxins and suggest there with related risk assessment strategies. Moreover, the manuscript addressed use of biological tools for toxin monitoring and toxicity testing.

Subacute dose, exposure and effects of foodborne microbial toxins

The effect of microbial toxins on human health is diverse. In many cases, the relationship between toxins as the causative agent of disease in humans is difficult to determine. The complexity of dose–response relationship is dictated by usual factors, but additionally troubled by gene-to-gut path that microbial toxins needs to travel before they reach the host in certain dose, and in certain environment. With some toxigenic bacteria (e.g. *B. cereus* emetic strains – strains producing cereulide), it is usual scenario that food containing preformed toxin causes a disease and dose–response relations are in this case essentially that for a chemical toxin. While, acute toxicity of gastroenteritis may be easily identified, and even fatalities occur (Naranjo *et al.*, 2011; Posfay-Barbe *et al.*, 2008) the chronic toxicity can be difficult to recognize. Moreover, toxicity resulting from subacute, subchronic and chronic exposure (more often repeated than continuous) has not been actually studied for the microbial toxins, other than for some of the well-established mycotoxins. Knowledge of mechanisms of toxicity, host receptors and reversibility are to the best of

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0924-2244/$ - see front matter Published by Elsevier Ltd.
http://dx.doi.org/10.1016/j.tifs.2014.04.006

Table 1. Example and properties of some of the most relevant foodborne bacterial toxins.

Bacteria/ syndrome/ toxin	*B. cereus*		*S. aureus*	*C. perfringens*	*C. botulinum* (foodborne botulism sensu stricto)
	Diarrheal syndrome (HBL, NHE, bceT, cytK)	Emetic syndrome (cereulide)	Enterotoxins	CPE	
Type Symptoms	Toxico-infection Abdominal pain, cramps, watery diarrhea (secretory type) and occasionally nausea	Intoxication Nausea, vomiting, malaise and ultimately a fatal liver failure	Intoxication Nausea, vomiting, sometimes diarrhea	Toxico-infection Intense abdominal cramps, diarrhea and flatulence	Intoxication Fatigue, weakness, and vertigo, blurred vision, dry mouth, and difficulty in swallowing and speaking. Vomiting, diarrhea, constipation and abdominal swelling may occur. The disease can progress to weakness in the neck and arms, respiratory muscles and muscles of the lower body are affected.
Mode of action	Receptor unknown, however HBL and NHE specific receptors are suggested; causes hemolysis and/or cytolysis	Binds to 5-HT3 cells; causes emesis by action on nervus vagus	Binds to TCRVb cells or to T cells causing emetic or potent superantigen responses, respectively	Binds to 22 kDa proteins in intestinal cells and causes pore formation. Binds to TCRVb	Binds to gangliosides and putative protein receptor; enters nerve cells by endocytosis and cleaves neuronal proteins involved in vesicular trafficking and neurotransmitter release
Incubation time (h)	8–24 (or longer)	0.5–5	1–5	6–24	12–36 (reported min 2, max 180)
Resolution time (h)	12–24 (up to several days)	6–24	6–24	Within 24	Several weeks, gradually
Intoxication/ Infection dose	Ingestion of more than 10^5 CFU of diarrheal toxin producing *B. cereus* strains	ca. 10 μg kg^{-1} bw, 0.01 μg g^{-1} of food (*B. cereus* of more than 10^5 CFU g^{-1} food, depending on the strain, food and conditions)	100 ng of ingested toxin, 0.05 ng ml^{-1} of food (produced when *S. aureus* counts reach ca. 10^5 CFU ml^{-1} (g^{-1})	10^6–10^7 CFU g^{-1} of food (ingested vegetative cells produce CPE during intestinal sporulation)	Extrapolated 1 μg/kg b.w. orally, for 70 kg man 0.09–0.15 μg intravenously or intramuscularly, 0.70–0.90 μg inhalationally
Toxin production required for acute effects	In the small intestine of the host	Preformed in the food	Preformed in the food	In the small intestine of the host	Preformed in the food

Information has been summarized from different sources (Akhtara, Paredes-Sabja, Torres, & Sarker, 2009; Cai, Singh, & Sharma, 2007; Ceuppens *et al.*, 2012a; Ceuppens *et al.*, 2011; Ceuppens *et al.*, 2012b; Danielsen, Hansen, & Karlsdottir, 2013; Hennekinne, De Buyser, & Dragacci, 2012; Hyeon *et al.*, 2013; ICMSF, 1996a, 1996b; Jessberger, Dietrich, Bock, Didier, & Maertlbauer, 2014; Larkin, Carman, Krakauer, & Stiles, 2009; Lindstrom & Korkeala, 2006; Miyakawa, Creydt, Uzal, McClane, & Ibarra, 2005; Peck, 2010; Pinchuk, Beswick, & Reyes, 2010; Rajkovic, Kljajic, Smigic, Devlieghere, & Uyttendaele, 2013, 2012; Smedley, Fisher, Sayeed, Chakrabarti, & McClane, 2005; Solano *et al.*, 2013; Stewart, 2005; Tallent, DeGrasse, Wang, Mattis, & Kranz, 2013; Thanongsaksrikul & Chaicumpa, 2011).

author's knowledge greatly an unknown issue. In such cases, when symptoms are not visible, a response to measure and the method for measuring that response are yet to be described for many of the microbial toxins. While these toxin doses do not cause immediately visible symptoms, they may have important effect on different health aspects. These effects remain an unknown factor in food safety and public health protection.

For example, the acute intoxication with emetic symptoms by *B. cereus* emetic toxin, cereulide, occurs at relatively high doses of 8 μg/kg body weight based on the dose that induces emesis in *Suncus murinus* (Agata, Ohta, Mori, & Isobe, 1995). If humans are similarly sensitive (assumption not fully appropriate), and epidemiological data is assumed reliable, food containing cereulide at levels of 0.01–1.28 μg/g would be able to cause acute emetic intoxication (Agata, Ohta, & Yokoyama, 2002; Jaaskelainen *et al.*, 2003). Nevertheless, recent research demonstrated a wide prevalence of low concentrations of cereulide in rice and pasta dishes (Delbrassinne *et al.*, 2012). The cereulide concentrations found in samples were approximately 0.004 μg/g of food. Analysis of samples originating from patients suffering from diagnosed emetic food poisoning revealed cereulide in different concentrations in gastric fluid (0.004 μg/mL), blood serum (0.004 μg/mL), urine (0.008 μg/mL) and specially stool (0.16–0.80 μg/g) (Shiota *et al.*, 2010). Therefore, it seems appropriate to suggest that the novel approach in studying effects of cereulide should include: