Regulatory Toxicology and Pharmacology 73 (2015) 164–171

Contents lists available at ScienceDirect

Regulatory Toxicology and Pharmacology

journal homepage: www.elsevier.com/locate/yrtph

Determining the safety of microbial cultures for consumption by humans and animals

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ARTICLE INFO

Article history:
Received 24 June 2015
Received in revised form
3 July 2015
Accepted 4 July 2015
Available online 9 July 2015

Keywords:
Microbial culture safety
Probiotic safety
Safety evaluation
Safety decision tree
Fermented food
Fermented feed
Food safety

ABSTRACT

Fermented foods and feeds have been consumed for millennia, and microorganisms isolated from traditional fermentations have been used as probiotics. There is interest in developing new microbial cultures for these uses, but to date safety evaluation procedures have only been discussed in general terms. We propose a comprehensive approach for determining the safety of microbial cultures that lack an established history of safe use for their intended new applications. Three scenarios are considered: (1) substantially increased exposure to a culture that has an established record of safety in a more limited application; (2) a new strain without a history of safe use that was isolated from a food or feed that has a history of safe use; and (3) a new strain isolated from a non-food or non-feed source. Our safety evaluation process is based on scientific procedures and is in the form of a decision tree composed of 13 questions. Our decision tree for determining the safety of microbial cultures for consumption by humans or animals is modeled on previous decision trees that are used worldwide to evaluate the safety of microbial enzymes for use in human food or animal feed.

1. Introduction

It is estimated that the human body consists of about 37 trillion human cells and about 500 trillion microbial cells, most of which are bacteria residing in the colon (Bianconi et al., 2013; Berg, 1996; Savage, 1977). From this perspective humans appear to be 93% microbial, so we may forgive an imaginary extraterrestrial alien who visits Earth and concludes that humans are little more than mobile fermenters, intended for the propagation of microorganisms. Of course humans presume something quite the opposite, and strive to manage their relationships with the microbial world accordingly by reducing/eliminating exposure to pathogens that can cause illness and disease, while encouraging interaction with microorganisms that provide beneficial functions in foods and/or may benefit health.

Microbiologists recognize that the vast majority of microorganisms are harmless for healthy humans and other animals. While in theory just about any microbe can cause illness in a *compromised* host, to do so it must first gain access to tissue sites that are normally protected by host barriers, for example skin, mucous membranes, and the immune system. Accordingly, infections in deep wounds that are produced by otherwise harmless bacteria, or infections by normally harmless microorganisms in individuals with compromised immune systems, are referred to as "*opportunistic infections*". Otherwise harmless microorganisms that are associated with such infections in compromised hosts are referred to as "*opportunistic pathogens*".

In contrast to opportunistic pathogens, which cause illness only rarely in compromised hosts, a true pathogenic microorganism has an intrinsic capability to cross or evade *non-compromised* host barriers (e.g., intact skin and a normally functioning immune

Abbreviations: cGMP, current Good Manufacturing Practices; CO_2, carbon dioxide; DNA, deoxyribonucleic acid; EFFCA, European Food and Feed Cultures Association; EFSA, European Food Safety Authority; EU, European Union; FDA, U.S. Food and Drug Administration; GI, gastrointestinal; GRAS, generally recognized as safe; HTST, high temperature, short time; IDF, International Dairy Federation; MvirDb, microbial virulence database; QPS, Qualified Presumption of Safety; rDNA, ribosomal DNA.

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http://dx.doi.org/10.1016/j.yrtph.2015.07.003
0273-2300/

system) and thereby infect individuals who would otherwise be considered healthy (Falkow, 1997; Mims, 1991). True pathogens are able to do this because they possess and express genes for *virulence factors*, which are defined as pathogen-produced substances that promote the establishment and maintenance of disease (http://www.msu.edu/course/mmg/301/Lec32.pdf) Pathogenic microorganisms may also produce toxins that induce illness apart from infection *per se*.

1.1. Microorganisms and food

Food microbiologists classify microorganisms into three broad categories: microbes that cause food-borne illness through infection, the production of toxins that are active via the oral route, or both; microbes that may spoil food without causing illness; and microbes that are useful in food fermentations. Probiotic microorganisms comprise another microbial group and are often, but not always, derived from food fermentations.

1.1.1. Food poisoning

Microbes that cause food-borne illness through infection and/or the production of toxins that are active via the oral route represent a very small fraction of the microbial world (Fischetti, 2000; Doyle et al., 1997). Examples include *Salmonella* sp., which induce illness by infecting the GI tract; *Escherichia coli* 0157:H7, which is both infectious and toxigenic; certain strains of *Staphylococcus aureus* that produce an explosive food-borne illness, euphemistically referred to as "the two-bucket disease", via the *Staphylococcal* enterotoxins that inflame the GI tract by acting as "superantigens"; strains of *Clostridium botulinum* that produce the botulinal neurotoxins (the causes of botulism); and *Clostridium perfringens* which, following ingestion, produces an enterotoxin during sporulation in the gut that induces food poisoning symptoms typically including nausea and diarrhea (Aktories and Just, 2000; Alouf and Freer, 1999; Rappuoli and Montecucco, 1997; Betley et al., 1992).

In addition to the proteinaceous food poisoning toxins produced by these pathogenic bacteria, certain molds produce small molecular weight non-protein toxins (mycotoxins) that are active when ingested, for example strains of *Apsergillus flavus* strains that produce aflatoxin B_1, which is causally associated with liver cancer (Doyle et al., 1997). Some species of blue-green algae (*Cyanobacteria*) also produce small molecular weight non-protein toxins that are active when ingested, for example saxitoxin, the cause of paralytic shellfish poisoning (Schantz et al., 1975).

1.1.2. Food spoilage

In contrast to the relatively tiny food-borne pathogen group, many microbes are capable of spoiling food (Doyle et al., 1997). By definition spoilage organisms grow in a food, metabolize its nutrients (sugars, amino acids, etc.), and produce metabolites that humans find *undesirable* in terms of flavor, aroma, texture, etc. It should be emphasized that spoilage is not synonymous with the induction of illness. Microorganisms that spoil food often suffer an unfavorable reputation despite the fact that they do not induce illness, and may, to the contrary, produce metabolites that actually inhibit the growth of food-borne pathogens.

1.1.3. Food and feed fermentation

The third category is comprised of a large group of microorganisms that are useful in food and/or feed fermentations. Fermented foods and feeds have been consumed by humans and agricultural animals, respectively, for thousands of years (McGovern et al., 2004; Shawky et al., 1990), and the microorganisms that produce them grow in the food (or feed), metabolize nutrients (sugars, amino acids, etc.), and produce metabolites that humans (or animals) find *desirable* in terms of flavor, aroma, texture, etc. Some of the metabolites produced during food fermentations (e.g. organic acids, bacteriocins) also serve to preserve foods/feeds and inhibit the growth of spoilage organisms and food-borne pathogens (IDF, 2012).

There is a personal/cultural dimension to the perception of food spoilage versus food fermentation. For example, Limburger cheese, which is produced by fermenting milk with *Brevibacterium linens*, has a strong and distinctive aroma that some people find objectionable. Others, however, enjoy eating Limburger cheese. Those who object will likely refer to it as smelly spoiled milk, whereas those who enjoy Limburger cheese will likely say that it is an acceptable fermented milk product.

1.1.4. Probiotic microorganisms

The concept of "probiotics" was initiated more than 100 years ago when Metchnikov (1907) hypothesized that lactic acid bacteria in traditional fermented foods may have health-promoting properties, and since then the number of species used as probiotics has grown substantially. A comprehensive list of microorganisms used as probiotics is not currently available, but many are listed on standardized inventories of microorganisms with documented histories of safe use in fermented food products that are maintained by the International Dairy Federation (IDF, 2012) and the European Food Safety Authority (EFSA, 2013). These cultures have been isolated from traditional fermented foods and animal feeds such as silage, microbial food or animal feed starter cultures, or from human or food animal gastro-intestinal tracts via fecal isolates.

We note that use of a standardized culture in food fermentation does not necessarily mean that the microorganism is appropriate for use as a probiotic, and in fact, may not demonstrate any health benefit to the host at all. However, for microbial cultures known to have a probiotic effect, safety depends on the intrinsic biochemical and physiological nature of the organism and its use, including exposure levels and the target population group (e.g., healthy young adults, elderly, immunocompromised patients, etc.).

1.2. Commercial practice

Traditional food and feed fermentations relied on *autochthonous* microorganisms (i.e. microorganisms that occur naturally within the fermentation substrate itself) and were thusly referred to as "wild". Various additives, in particular salt, were included in traditional fermentation formulations, and we now know that these additives give certain bacteria a selective growth advantage. For example, the addition of salt favors the growth of *gram positive* over *gram negative* bacteria; hence salted meat becomes an acceptable fermented product due to the action of gram-positive bacteria, rather than being reduced to the unpalatable smelly slime that would result from the growth of gram-negative bacteria.

"Wild" fermentations relied on chance and were therefore neither predictable nor reproducible. Hence, maintaining consistent quality was all but impossible. In an effort to control the fermentation, small quantities of a "good" (i.e., organoleptically acceptable) fermentation were saved for use as inocula in future fermentations, a practice called "back-slopping". But this too had its problems, for example if the inoculum from an acceptable fermentation was to become contaminated with a pathogen, then the pathogen would carry over to the subsequent fermentations (Sandine, 1979).

1.2.1. Aseptic technique and pure cultures

Eventually the importance of utilizing aseptic technique in fermentation practice was recognized. This led to the isolation, characterization, and maintenance of the microorganisms