Who are the culprits? – Potential contaminants of foods and beverages

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Who are the culprits? – Potential Contaminants of Foods and Beverages’

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Introduction

The ‘culprits’ of the title are actually extremely diverse, and include both living organisms and what might be referred to as ‘complex biochemical agents.’ This last, somewhat contrived, term is intended to cover viruses, viroids and prions. These latter may be thought of as occupying the evolutionary gap between molecules and living organisms.

All of these agents have the ability to contaminate foods and beverages, and the potential to cause food poisoning in different ways and with various degrees of severity ranging from mild enteric discomfort to death. Whilst methods of detection for known pathogens continue to improve, it is also the unknown that we need to be constantly vigilant over. The nature of the unknown is literally unknowable, but some guidance as to what new types of foodborne agents might emerge in the future may be gained by considerations of recent discoveries in microbiology that are helping to (re)define the boundaries of the possible. Consequently, whilst primarily focussing on well-characterised foodborne pathogens, some speculation about the ‘next generation’ has also been included where strictly relevant.

Filtration represents one method of removing potentially harmful pathogens from the human food chain. The physical dimensions of these agents is of particular importance in the context of filtration, and rather than attempting to formulate a new classification based on size, it will prove more efficient to make use of existing taxonomic divisions. We need not concern ourselves here with the foundations upon which the various sub-divisions have been arrived at, but merely attempt to draw general conclusions about the size ranges of the various taxons that are relevant here. Let us start with living organisms.

Living Organisms

The earliest systems for classifying living organisms comprised just two kingdoms – plants and animals. It was largely the discovery of micro-organisms that revealed this dichotomous system to be inadequate. Not surprisingly, many alternative schemes for classification have been put forward over the years, but the scheme proposed by Whitaker, which places organisms into one of five kingdoms, has gained general approval and will be adopted here (Whitaker, 1959).

Attention here is naturally restricted to micro-organisms, and although many organisms share the characteristic of being of microscopic dimensions, they can differ widely in morphology, structure and function. In the Five Kingdom Classification
there are 3 kingdoms that contain micro-organisms – these are the Bacteria, the Fungi and the Protists.

It is also relevant here to mention classification at the cellular level. Until about 25 years ago this was also viewed as being dichotomous, and cells were either prokaryotes or eukaryotes. This may be thought of as a level of classification above that of kingdom - a super-kingdom in fact, and under this scheme all bacteria were prokaryotes. However, new methods of comparing RNA sequences revealed that the prokaryotes contained within them a deep division yielding two new groups that were as distinct from one another as they were from the eukaryotes. These two new taxons came to be known as the Archaea and the Eubacteria. The Archaea, as their name suggests, are close descendants of the first living organisms on Earth. Many of the Archaea display extreme resistance to environmental parameters such as temperature and pH but fortunately they have never yet been found in association with foods and therefore they need not be considered further here.

**Bacteria**

Having eliminated the Archaea from consideration it is with the Eubacteria – or true bacteria - that we must concern ourselves with. Conventionally bacteria were described as having cell dimensions of the order of 1 μm, but, as I hope to illustrate below, this particular size qualification may no longer be useful, or indeed relevant, as a defining characteristic.

In 1993 the world of microbiology was rocked by reports that *Epulopiscium fishelsoni*, an organism previously classified as a protist and having a length of up to 600 μm, was in fact a bacterium (Angert et al., 1993). This was followed a few years later by the discovery of another even larger species with a diameter of 750 μm (Schulz and Jorgensen, 2001). Setting aside the vanishingly low probability of ever encountering this organism, its relatively large dimensions would make it eminently filterable! However, the crucial point to be stressed here is that the very existence of such bacterial giants calls into question previously held notions about the dimensions bacteria are ‘allowed’ to have.

This is important to keep in mind because at the other extreme of scale much controversy surrounds the question – ‘how small bacteria can be?’ There is as yet no definitive answer to that question. However, it is possible to examine the evidence at various graded steps downwards, as it were, from the nominal dimension of 1 μm.

Our first step down takes us to those bacteria universally recognised as being towards the lower end of the size range. Examples include *Ralstonia pickettii* (formerly *Pseudomonas pickettii*) and *Brevundimonas diminuta* (formerly *Pseudomonas diminuta*). The dimensions of *B. diminuta* were recently estimated as 0.39 μm wide and 1.54 μm in length (Xu and Chellam; 2005). Another frequently cited example in this context is presented by the mycoplasmas, an example of which is *Mycoplasma pneumonia*. This organism has spindle-shaped cells with dimensions 1 to 2 μm long and 0.1 to 0.2 μm wide (Waites and Talkington; 2004).

Mary et al. (2001) obtained what they termed ‘ultramicrocells’ of four *Aeromonas* spp. that were able to survive in autoclaved tap water for a few days. Unfortunately
other than specifying that their bacteria were able to pass through 0.2 μm filters, they
did not cite any actual dimensions. Notwithstanding, this bacterial genus is frequently
associated with water and it has even been isolated from bottled mineral water.

Next come the so-called ‘ultramicrobacteria’. These were defined by Hood and
McDonnell (1987) as having dimensions equal to, or below 0.2 μm. To date these
have only been found in environments where nutrients are in short supply – so-called
oligotrophic environments - and although the majority of them are unculturable using
conventional methods (Hahn et al., 2003), their existence has been confirmed by
molecular techniques that are also able to reveal the phylogenetic relationships
existing between them. It has been suggested that in oligotrophic environments there
is a distinct advantage to being small. This is because nutrients enter the cell by
diffusion through the cell envelope and surface area to volume increases as overall
size decreases. It also appears that bacteriovores preferentially prey on large rather
than small bacteria and that consequently there is a survival advantage in being small.
Rutz and Kieft (2004) isolated “dwarf” bacteria from soils and measured their cell
volumes: the smallest corresponded to cocci of diameters of 0.25 μm and for rods to
dimensions of 0.25 x 0.5 μm. Some of their isolates were shown to be closely related
to known bacterial genera.

The final category that must be considered here are the so-called ‘nanobacteria,’
which, it has been claimed, can be as small as 50 nm. The existence of bacteria of
such dimensions is a controversial area, not least because theoretical estimates made
on the basis of considerations of nutrient diffusion rates across the cell envelope as
well as the volume necessary to accommodate the organism’s genome suggest that the
lower size limit for a self-replicating cell lies within the range 100 –140 nm
(Maniloff, 1997). However, some researchers are not only ready to accept the
existence of nanobacteria, but also their role as causative agents of various urologic
conditions (Wood and Shoskes, 2006).

**Bacterial Spores**

Discussion of the eubacteria would be incomplete without the inclusion of bacterial
endospores: certain Gram +ve bacteria are able to produce endospores in response to
environmental stresses. Commonly referred to simply as ‘spores,’ some are highly
resilient to starvation, extremes of pH and temperatures etc. From a food perspective
the commonest spore-formers encountered belong to the genera *Bacillus* and
*Clostridium*. The spores of *B. subtilis* were measured by Leuschner and Lillford
(2000) as being 1.34 μm long and 0.57 μm in breadth, whilst those of *C. perfringens*
were almost spherical and ranged in diameter from 0.79 to 0.98 μm.

**Fungi**

These are eukaryotes and are characterised by a more complex cellular organisation
than prokaryotes and a significantly larger cell size. This is conventionally stated as
being of the order of 10 μm.

The fungi comprise both moulds and yeasts. The former grow in the form of branched
filaments referred to as ‘mycelium.’ The moulds produce fruiting bodies known as
exospores and it is these, rather than mycelium that presents challenges to filtration. The dimensions of exospores range in size from 0.5 to 30 µm (Nevalainen et al., 1992), however the dimensions of the spores of common spoilage moulds such as Penicillium melinii are approximately 3 µm in diameter (Cho et al., 2005) and those of Aspergillus flavus, 10 µm (Bock and Coty, 2006).

In contrast to moulds the yeasts do not produce spores but reproduce by budding. The smallest yeast is thought to be Schizosaccharomyces pombe that comprises cylindrically shaped cells with hemispherical ends. The mean diameter is 3.5 µm and the length can vary from 8 to 14 µm (Fantes, 1977).

The Protists

This is a highly diverse kingdom and includes such organisms as algae and protozoans. Some members of this kingdom are characterised by a relatively large cell size. After the revelations made above in relation to the eubacteria, it should come as no surprise to discover that protists have been isolated that defy conventions relating to size. The smallest eukaryote so far described is Ostreococcus tauri – a ‘picoeukaryote’ - with a diameter of 0.8 µm (Chretiennot-Dinet et al., 1995).

More relevant here is the fact that certain protists are able to produce oocysts (commonly abbreviated to ‘cysts’) which are equivalent to bacterial endospores described above. Medema et al. (1998) determined the dimensions of the cysts of two common water contaminants, Cryptosporidium parvum and Giardia lamblia. The former were spherical with a mean diameter of 4.9 µm, and the latter elliptical with a mean largest length of 12.2 µm and a mean smallest length of 9.3 µm. Both are resistant to a number of disinfectants and can remain viable for very long periods.

Complex Biochemical Agents

Viruses

Viruses range in size from 15 to 400 nm. Viruses need specific living cells in order to replicate and therefore cannot do so in food or water. However, they are typically quite stable outside their host cells and can survive for long periods. Viruses, similarly to living organisms, are classified as Families, and the family Caliciviridae contains within it noroviruses (formerly Norwalk-like viruses) that have been heavily implicated in the causation of enteric disease in humans. These viruses have diameters ranging from 30 to 35 nm (Goodridge et al., 2004).

Viroids

Viroids – or sub-viral agents as they are sometimes referred to - are un-enveloped stretches of covalently closed single strand RNA circles about 300-400 nucleotides in length that infect plants.

Prions

A prion is an infectious agent that is comprised only of protein. Prions are believed to infect and propagate by refolding abnormally into a structure which is able to convert
normal molecules of the protein into the abnormally structured form. Prions have shown themselves to be generally quite resistant to denaturation by protease, heat, radiation, and formalin treatments, although potency or infectivity can thereby be reduced. The prion responsible for Creutzfeldt-Jakob disease has a molecular weight of 21 kilodaltons (Telling et al., 1996).

Filtration of Cells & Other Biochemical Agents

Considerations of the smallest size that any given foodborne pathogenic agent can attain are crucial to the design of efficient filtration processes. It is important to recognise that these biological agents should not be viewed simply as inert rigid particles, but rather as complex entities capable of responding to their environments in ways that have not yet been fully elucidated. Haller et al. (1999) suggested that certain cells might have walls flexible enough to permit them to pass through pores smaller than their nominal dimensions. One would expect the mycoplasmas that lack cell walls completely to achieve this feat with comparative ease. Notwithstanding, Xu and Chellan (2005) have provided direct evidence of this phenomenon with B. diminuta. These cells have a width of 0.39 μm and a length of 1.54 μm and were shown to pass through a membrane with pores of 0.2 μm. More recently, Wainwright et al. (2002) showed that common bacteria, not normally viewed as particularly small, e.g. E. coli and B. subtilis, could also pass through membranes with 0.2 μm pores. The conditions under which this occurred was achieved by injecting nutrient agar to the underside of the membrane, and whilst this may be viewed as somewhat artificial, it suggests hitherto unsuspected mechanisms in these bacteria for drastically modifying their morphologies.

More conventionally, there is evidence that bacteria can undergo reductions in size when subject to environmental stresses such as starvation. Bossolan et al. (2005) achieved reductions in mean cell area of 15.8 % and mean cell volume of 13.4 % by maintaining cells of Klebsiella pneumoniae under starvation conditions for 91 days. Whilst earlier, Hood and McDonnell (1987) had shown that small, starved bacteria increased in size when incubated with organic substrates.

Certain bacteria are able to produce so-called exopolysaccharide substances (EPS) that serve to attach them to the surfaces of solid objects. These structured three-dimensional domains are referred to as ‘biofilms,’ and organisms that do not produce EPS may themselves become immobilised within biofilms. Biofilms offer organisms protection from disinfectants and antibiotics, and moreover, they can prove difficult to remove from surfaces – recourse has often to be made to harsh mechanical methods. Xu and Chellan (2005) showed that B. diminuta was a prolific producer of EPS when retained on the surface of polycarbonate membranes and this led to high backwashing resistances.

Fibrous filters are commonly used for air filtration and particularly for heating, ventilation and air conditioning (HVAC). The classical assumption made was that once an organism was retained on the surface of an individual fibre by such mechanisms as impaction or interception, it rapidly lost viability. However, this assumption has frequently been contradicted by experimental studies that have demonstrated that at certain relative humidities some spores can remain viable for many days (Maus et al., 2001). Additional studies have shown that there are
circumstances under which spores can germinate and the vegetative mycelium so produced can colonise the filter, obtaining nutrients both from materials leaching from the fibres themselves and other organic material retained within the filter matrix. (Price et al., 2005). Moreover, organisms retained by filters can, as a result of flow fluctuations, actually become re-entrained within the air stream (Jankowska et al., 2000).

**The Threat to Foods and Beverages**

Table 1 lists the principal foodborne diseases in the USA along with estimates of the number of ‘illness episodes’ that occurred in 1997. The totals represent only 19 % of all reported cases of foodborne infections and 36 % of the deaths. The great majority of the estimated cases remain unaccounted for and this suggests that there are many more foodborne pathogens yet to be identified.

**Table 1. Principal Foodborne Infections in the USA as estimated for 1997 (from Tauxe;2002)**

Some of the failures to identify the causative agents in the cases mentioned above might have arisen from difficulties in cultivating the respective agents in synthetic growth media. Many of the ultramicrobacteria referred to earlier do not lend themselves to cultivation by conventional methods and require sophisticated RNA-based techniques for their detection. Crucially, failure to cultivate them under such conditions should not necessarily be taken as an indication that they are non-viable or otherwise sublethally injured, it points rather at the inadequacy of existing synthetic media. Importantly, such organisms may still pose a threat to public health.

This is also the difficulty that besets virus detection: noroviruses, commonly implicated in foodborne diseases, have not yet been artificially cultivated in animal cells, and the methods currently available for their detection are relatively expensive and quite involved. However, this will undoubtedly be an important area for future research - not least because only a few virus particles ingested with foods can be sufficient to trigger enteric diseases (Koopmans and Duizer, 2004).

Moreover, some viruses are able to survive for long periods in the environment, for example, the enteric virus Adenovirus 41 has been shown to survive in tap water for over 300 days whilst sustaining only 2 log reductions in viability (Koopmans and Duizer, 2004). Of particular interest here is work done by Keswick (1983) who showed that poliovirus and bacteriophage f2 were able to survive on electropositive filters for as long as five weeks.

So far neither ultramicrobacteria nor nanobacteria have been detected in foods. To some extent this might be because no one has seriously attempted to look for them. If the current theories about the effect of environment on cell size are correct, then perhaps it would be unlikely that they would ever be found in foods. The same could not be said of conditions that might induce more common bacteria to undergo reductions in cell volume.

**Conclusions**
Foods were being imported from country to country long before the term ‘globalisation’ was ever coined, and it can surely come as no surprise to discover that the food industry has fully now fully embraced globalisation. It partly creates, and partly attempts to satisfy, consumer demands. A fine example is provided by the much-cited case of sugar snap beans cultivated in Kenya, and without which life in developed counties would scarcely be worth living. But what implications do such changes in production and distribution hold for previously unencountered foodborne diseases? Table 1 also lists agents of foodborne diseases that have emerged during the last 30 years. The emergence of these new threats to public health have arisen through changes to the patterns of agricultural practices (such as have been described above), food processing or changes to lifestyle and to how foods are prepared, eaten and even perceived.

Food processing is becoming increasingly centralised and whilst this may result in economies of scale, it poses completely new threats. For example, single incidents of food contamination can have consequences across wide geographical areas. A highly centralised food processing sector provides a particularly appealing target for bioterrorism. One wonders whether the ease with which Sudan red-contaminated Worcestershire sauce was able to be ‘cascade’ through into literally hundreds of food products was a sufficiently loud ‘wake up call’…..

Only one thing will be certain and that is that the nature of the culprits will change over time and that as methods for controlling the ‘old foes’ are developed new ones will take their place.

References


