Tap water transmitted establishment of pathogenic microorganisms in household appliances

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1. Introduction

The understanding of the biological, medical and technical importance of microbial biofilms continues to increase dramatically. Despite the hostile conditions in municipal and household water systems and in some common household appliances, such as dishwashers and washing machines, rich communities of extremotolerant microorganisms can develop. Few of these selected microorganisms colonize these systems in a planktonic form, mostly they form persistent biofilms. Growth in these mixed biofilms enables both bacteria and fungi to persist on the interior surfaces of pipes of the water system as well as on different surfaces in household appliances. Some of the species in these mixed assemblages, especially among fungi, are known opportunistic human pathogens. The formation of biofilms on the surface of pipes in the water supply system and in household appliances is thus undesirable due to several reasons: on the one hand they increase the resilience of microorganisms against removal and possibly also against stress, while on the other hand biofilm-forming pathogens are associated with infections that respond poorly to conventional therapy. This makes biofilm-forming capability a particularly worrying trait from a medical point of view. While individual bacteria have been studied extensively, fungal and especially mixed fungal-bacterial biofilms have hardly received any attention. The same applies for prediction of relations between environmental conditions, microbial community structure and activity and biofilm properties. This information could have an important impact on human health as well as on certain industrial applications, in particular in treatments of municipal water system that is currently thought to be the source of contaminations of household appliances. The insights into the environmental biofilm formation are also highly relevant to medicine, as the ability of pathogens to form biofilms is associated with poor response to conventional therapy of various diseases.

2. Microorganisms are commonly present in water

Microorganisms are present in all natural habitats, as well as in human-made environments. Fresh water originates from glaciers, rivers, lakes and groundwater, and represents only 0.6% of global water supplies [1]. Among all of above listed types of fresh water usually groundwater and recycled surface water derived from rivers is used as a tap water. Water for drinking must be regularly checked for microorganisms and chemicals, as recommended by the World Health Organisation (WHO), the US Environmental Protection Agency (US EPA) and the European Union (EU) [2]. Limits set for chemicals and different microorganisms differ between countries. However, in Europe at least the total count of mesophilic bacteria, coliforms, *Escherichia coli* and spores of *Clostridium perfringens* must be determined [3]. Besides bacteria, WHO recommends also a regular check for Nematoda, Protozoa and viruses, but does not set limits for fungi in drinking water. As a consequence fungi in tap water are not monitored, and their potential influence on human health is thus poorly investigated [4]. Water is one of the main transmission sources also for algae. Due to their need for light, algae are mainly present in the upper layers of natural water resources. Some species from genera *Scenedesmus, Euglena, Anacystis, Coelastrum, Prototheca* and *Chlorococcum* are able to sustain the chlorination process in tap water systems, and can survive without light. Aditionally, the presence of algae from genera *Elakatothrix, Gomphosphaeria,* *Closterium, Cosmarium* and *Chlorella* in tap water systems can be problematic, since some species from the listed genera are known hosts or reservoirs for pathogenic bacteria [5].

Ecological role of protozoa in water systems is to control the number of bacteria and fungi. Like algae, some represent hosts for pathogenic bacteria of the genera *Legionella, Burkholderia* and *Mycobacterium*, while species from the genera *Cryptosporidium, Entamoeba, Microsporidia* and *Giardia* act as human pathogens causing diarrhoea, nausea, keratitis and encephalitis [4,5]. Additionally, species able to form oocysts represent particular problem, since they are less sensitive to chlorine water treatment [6].

Diversity of fungi in drinking water is high and is mainly represented by genera *Acremonium, Altenaria, Arthrinium, Aspergillus, Aureobasidium, Beauveria, Botrytis, Candida, Chaetomium, Cladosporium, Clavispora, Cryptococcus, Debaryomyces, Epicoccum, Exophiala, Fusarium, Galactomyces, Geotrichum, Gliocladium, Meyerozyma, Mucor, Paecilomyces, Penicillium, Phialophora, Phoma, Phomopsis, Pichia, Rhinocladiella, Rhizopus, Rhodotorula, Sporothrix, Stachybotrys, Trichoderma, Trichosporon, Verticillium* and *Yarrowia* [2,5,7]. Fungi in water are important components of mixed bacterial fungal biofilms, toxin producers and producers of bad taste and odour of water. Biofilms in tap water systems can contain up to 8.9 CFU/cm^2 of yeasts and 4.0 - 25.2 CFU/cm^2 of filamentous fungi [8].

β–Proteobacteria are typically found in groundwater in large amounts. Their number reaches its lowest limits in water tanks after a chlorination process, while in tap water the number of bacteria increases again due to formation of biofilms in plumbing systems [9]. For comparison, numbers of CFU in groundwater can be up to 10^6 CFU/mL, in well water up to 10^3 CFU/mL, in reservoir tanks after chlorination up to 10^2 CFU/mL and in household tap water between 10^2 and 10^3 CFU/mL [9]. In inappropriately treated water sources or due to faults in water supply systems, the number of pathogenic bacteria involved in infections of digestive and respiratory tract can exceed the limits set in standards. Most problematic bacteria in such cases are *Vibrio cholerae, Salmonella typhi, Shigella* spp., *Campylobacter jejuni, Escherichia coli, Yersinia enterolitica, Legionella* spp., *Aeromonas* spp., *Mycobacterium* spp., *Bacillus* spp., *Clostridium* spp., and *Pseudomonas aeruginosa* [5,10]. Also toxins producing Cyanobacteria of the genera *Microcystis, Planktothrix, Anabaena, Oscillatoria* and *Aphanizomenon* should be limited [11].

Transmission via water is known for many viruses, although only a few are known to be pathogenic for humans. Examples of such viruses are Adenovirus, Astrovirus, Calicivirus, Coronavirus, Enterovirus, Hepatovirus, Norovirus, Parvovirus, Reovirus, Rotavirus and Torovirus causing hepatitis, gastroenteritis, meningitis, fever, rash and conjunctivitis [4,10,12].

3. Water transmitted bacteria and fungi are forming stable biofilms

Biofilms are present everywhere in nature and have an important role in normal microbial life cycle. They are well organized microbial communities, formed on abiotic (e.g. plastic and metal) or biotic (skin and mucous membrane) surfaces. Approximately 15% of biofilms represent microorganisms, while the rest of a biofilm structure is build of extracellular polysaccharides (EPS), containing water, proteins, nucleic acids and lipids [8,13]. Biofilm constitutes protection for the cells in a stressful environment, allowing them to survive longer with low concentrations of nutrients and increases the chances for survival of chemical, physical and biological impacts from the environment [14,15]. Microbial cells within biofilms are physiologically different than in their planktonic form. Differences occur due to changes in gradients of oxygenated and anoxic areas and nutrient concentration in the biofilm. Due the microbial communication, known as “quorum sensing” microbes perceive a content of nutrients, ions, and cell density in biofilm. This affects the expression of virulence factors and the genes involved in the formation of EPS [14].

Roughly, bacterial biofilms are developed in three phases: an interface, basic and surface biofilm. The interface layer of the biofilm is the first layer made on the fresh material and contains different organic and inorganic molecules attached to the surface. Basic biofilm is formed on the interface layer and includes initial bacterial colonizers. Surface biofilm is represented by secondary microbial colonizers, attaching on the previous microbes in biofilm [14]. After the development and growth, parts of biofilm are released in water, mainly due to the shearing forces [16].

Not only bacteria, but also fungi can be primary colonizers of the surfaces. However, they are usually attached in the biofilm structure in later phases of biofilm formation. It has been investigated that the number of fungal cells inside biofilms is to up to 5000 times higher than in the running water [17]. Yeast cells in biofilms can range from 0 to 8.9 CFU/cm^2, while number of filamentous fungi is assesed to be between 4.0 and 25.2 CFU/cm^2. Fungal hyphae especially cross-link the biofilm structure, which makes the latter more difficult to remove [8]. Biofilms formed by yeasts are similar to those formed by bacteria. Formation of biofilms by yeasts was studied for species from genera *Candida, Saccharomyces, Cryptococcus* and *Aureobasidium*. Many biofilm-forming yeast species grow in dimorphic form, switching between the yeast and filamentous growth. This property allows them to quickly adapt in the environment and easily colonize a surface [18]. Biofilms formed by filamentous fungi were studied primarily in plumbing systems of hospitals. Filamentous fungi known to form biofilms belong to genera *Aspergillus, Penicillium, Coriolus* and *Trichoderma*, as well as to some dermatophytic fungi [5,19]. Filamentous fungi form biofilms in several stages. The initial phase involves the adsorption of propagules (spores and hyphae) on a surface. In the next phase, fungi adhere to the surface with the active attachment of sprouting spores. The last phase includes the apical growth and hyphae branching, forming a monolayer, followed by an invasive growth, which includes also the formation of EPS matrix. In maturation phase, hyphae are integrated into a compact mycelial network, from which fungal parts are later dispersed into the water [18].

4. Interactions between bacteria and fungi in biofilms

Interactions between microorganisms in a biofilm can be either antagonistic or synergistic, which leads to the formation of specific metabolic products, absent when microbes are in single cell colonies [20]. Known interactions between bacteria and fungi for instance include the inhibition of pyocyanin production in bacteria *Pseudomonas aeruginosa* in the presence of the yeast *Candida albicans* [21]. Also acidic metabolites formed by fungi from *Fusarium oxysporum* species complex have an effect on pyocyanin production in bacteria *Pseudomonas chlororaphis* [22]. Antagonistic effect against fungi from genera *Aspergillus* and *Candida* was reported by some bacteria from genus *Lactobacillus,* while the yeast *Saccharomyces cerevisiae* with its metabolism extends the survival of bacteria *Pseudomonas putida* in the environment.

Interactions between bacteria and fungi in biofilms are correlated to the presence of bacteria and fungi in drinking water [23]. Positive correlations between yeasts, total number of heterotrophic bacteria and the total number of coliform bacteria in drinking water were already determined [24,25], as well as the correlation between filamentous fungi and the total number of heterotrophic bacteria in water [7,24]. Negative correlation between fungi and bacteria was so far reported only for samples with a large bacterial biomass [26]. Such negative correlation is mainly the consequence of fast propagation of bacteria and direct competition for the available nutrients [23].

5. Biofilm communities in household dishwashers and washing machines

Although microbes are present inside a large number of different niches in our homes, inside each of them a different selection pressure leads to specific and limited diversity [27]. Increasing ecological awareness and low energy consumption among producers of household appliances lead to lowered temperatures of washing and reduced amount of water, as well as to the use of biodegradable detergents [27,28]. Substitution of aggressive detergents containing bleach with mild enzymatic mixtures leads to enhanced microbial growth in indoor biomes. Synthetic materials in household appliances, such as plastics and aromatic pollutants are prone to microbial growth in the form of biofilms. Different microbes are able to grow on glass, metals or silicon [29], while some are even able to use the complex phenolic hydrocarbons as the sole source of carbon [30].

Recent discoveries of fungal colonization of domestic dishwashers showed great consistence in fungal biota. On the global scale, dishwashers rubber seals were heavily colonized with polyextremotolerant black yeasts *Exophiala dermatitidis* and *E. phaeomuriformis*, white yeast *Candida parapsilosis*, red yeast *Rhodotorula mucilaginosa*, and filamentous *Magnusiomyces capitatus*, *Fusarium dimerum, F. oxysporum* and *F. solani* species complexes [27,31,32]. With the exception of *M. capitatus* all fungi colonizing dishwashers originated from water sources [2].

Mycobiota from different parts of washing machines differed from mycobiota of dishwashers; however they were consistent as well. It consisted primarily of members of the *F. oxysporum* species complexes, followed by *C. parapsilosis*, *R. mucilaginosa* and black yeast *E. phaeomuriformis* [28], as well as previously reported *Microsporum canis, Mucor* sp. and *Trichophyton mentagrophytes* [33]. Bacteria from genera *Micrococcus, Pseudomonas, Shewanella* and *Sphingomonas,* isolated from washing machines were recently connected to the unpleasant odor of different parts of machines and clothes [28,34]. Only very few fungi were isolated from machines with an odor in comparison to the non-smelly machines [28,35], which indicates possible antagonistic interactions between above listed bacterial and fungal species.

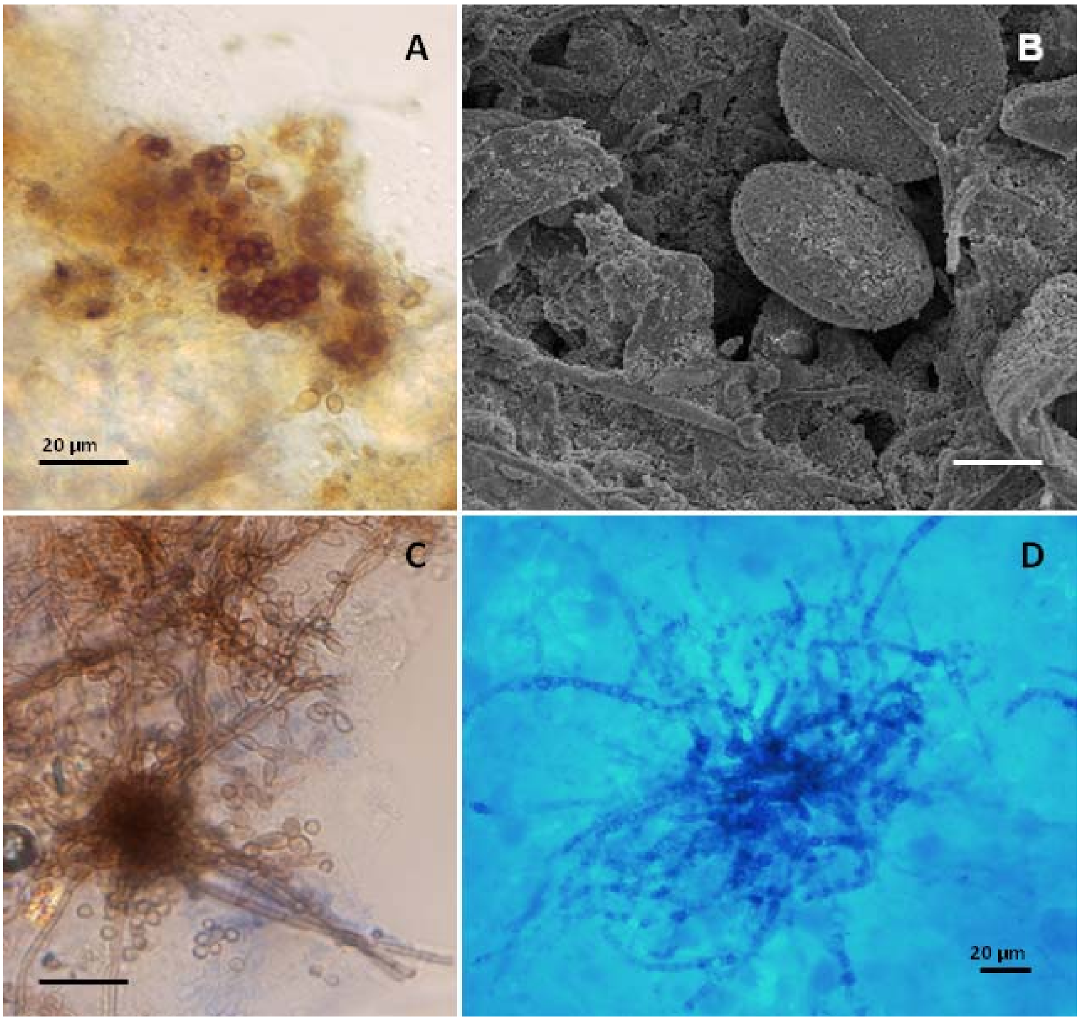

Figure 1: Fungal biofilms from dishwasher rubber and drawer for detergents in washing machine. A - *In vivo* morphology of brown muriform cells embedded in EPS; of a sample taken from a dishwasher; B – mixed biofilm community of a sample taken from a dishwasher, observed by scanning electron microscope; C - Fungal/bacterial biofilms from sample taken from a washing machine drawer; D - Autoflourescence of fungi and bacteria from washing machine drawer, observed by light microscope. Scale bar on a picture A valid also for pictures B and C.

6. Influence of material on biofilm formation

Besides temperature, pH, water flow and availability of nutrients, also the surface material has a significant effect on the biofilm formation [5]. Available reports show higher presence of fungi and bacteria on surfaces made of iron or steel in comparison to those made of PVC materials [36]. Some studies report the presence of fungi only on iron piping, but not in samples containing PVC [8,17]. It was also found that biofilm formation was more rapid in the polyethylene (PE) pipes than the copper pipes [37]. Biofilm formation occurs independently of the hydrophobicity or hydrophilicity of the material. Theoretically, biofilms should be generated faster on hydrophobic surfaces (plastics) than on hydrophilic (metal) surfaces [38]. Also roughness of materials, such as rubber seals plays an important role in colonization of materials with microbes. Surface area for microbial attaching becomes larger and water flow can not stay uniform throughout pipe systems, causing the reduction in shear forces [39].

Selection of the materials has also a significant effect on the quality of the disinfection. Materials made from different metals usually react faster with residual chlorine in water systems and thus lower its effect on microbes in biofilms [36]. Chlorination process was confirmed to be more effective in tubes made of polyethylene (PE) in comparison to the tubes built of copper [37].

7. Impact on human health

Microorganisms in water systems cause adherence and accumulation of biofilms and corrosion of materials. When they are present in large quantities, they can cause bad water taste and odour, and can affect human health via toxin production and ingestion of microbial pathogens [5,40]. Since water can not contain an excessive number of microorganisms or substances harmful to human healths, it is necessary to control the formation of biofilms and amount of suspended planktonic cells. Presence of certain bacteria, viruses and protozoa in drinking water affects human health, causing various diseases, including diarrhea, nausea, keratitis, encephalitis, hepatitis, gastroenteritis, meningitis, fever, rash and conjunctivitis [10].

Impact of fungi in water or household appliances on human health is so far poorly investigated. Fungal infections as a consequence might occur as an inhalation of released aerosols, trauma, direct contact with infected surfaces, or ingestion [27]. Presence of fungi in biofilms from different habitats are usually connected to allergies, pulmonary infections, superficial, or localised infections in healthy individuals, and more severe infections in immuno-compromised patients [5].

8. Conclusions

Standard microbiological tests developed for drinking water are with few exceptions performed on the most common bacteria that are known to represent health risk particularly in conditions of bad hygiene. Problems in the future will be more connected to the resistant emerging pathogens, both bacterial and fungal, occurring in disinfected water and in biofilms, formed inside tap water piping systems and on different surfaces in household appliances. Biofilms are usually formed between the liquid or aerial phase and the solid phase in natural and human-made environments, such as washing machines and dishwashers. Microorganisms more easily adapt to environmental changes when they are present in biofilm communities. Microbial growth in environment depends on conditions such as temperature, pH, changes in water flow, concentration of nutrients, building materials and disinfection process. Presence of microorganisms in biofilms affects human health, causing various diseases from allergic reaction to more serious systemic infections.

9. References

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