ELSEVIER

International Journal of Food Microbiology 72 (2002) 73–76

INTERNATIONAL JOURNAL OF
Food Microbiology

www.elsevier.com/locate/ijfoodmicro

Fungal spoilage of bottled mineral water

Daniel Cabral [1], Virginia E. Fernández Pinto *

Departamento de Química Orgánica, Universidad de Buenos Aires, Ciudad Universitaria, Pabellón II (1428), Buenos Aires, Argentina

Received 12 December 2000; received in revised form 27 June 2001; accepted 14 July 2001

Abstract

The occurrence of filamentous fungi together with bacteriological parameters was assessed in 126 samples of still bottled mineral water of eight different commercial brands in Argentina. In spoiled samples with visible mycelium growth, the most frequently isolated fungal species were *Penicillium citrinum*, *P. glabrum*, other *Penicillium* species, *Cladosporium cladosporioides* and *Alternaria alternata*. In unspoiled samples, the genera found were *Penicillium*, *Cladosporium*, *Rhizopus*, *Aspergillus* and *Phoma*. Only three of the 126 samples failed to meet the required microbiological standards because they were found to contain faecal streptococci.

Keywords: Fungi; Bottled mineral water; Microbiological quality

1. Introduction

Bottled mineral water has long been consumed as a safer alternative in countries with reticulated water of uncertain quality. Sales of mineral water have been increasing all over the world. Despite the number of publications that have described the microbial flora of bottled waters and speculated on its public health significance, many questions remain to be answered. It is still not possible to know which components of the flora represent an increase in the flora of ground water and which represent contamination during or after extraction (Hunter, 1993). In Argentine legislation (CAA, 1998) and the European Community (EC, 1980), natural mineral water originates from an underground table or deposit, emerging from a spring tapped at one or more natural or bore hole exits. All natural mineral water must be recognized by the local authority and hydrological, physical, chemical and physicochemical surveys must be undertaken. At source and during marketing, a natural mineral water should be free from parasites and pathogenic organisms, *Escherichia coli*, coliforms, faecal streptococci and *Pseudomonas aeruginosa* in any 250-ml sample and sulphite reducing anaerobes in any 50-ml sample. Mineralised water is potable water with added salts and must meet the same microbiological criteria as natural water (CAA, 1998). In contrast, the US legislation does not distinguish between bottled water and bottled mineral water, classing them both as bottled water (EPA, 1989). U.S standards include requirements for microbiological quality that are based on coliform detection levels. If good manufacturing practice (GMP) is followed only a few micro-

* Corresponding author. Tel./fax: +54-11-4-576-3346.
E-mail address: virginia@qo.fcen.uba.ar (V.E. Fernández Pinto).
[1] Current address: Laboratorio de Micología. Departamento de Ciencias Biológicas. Facultad de Ciencias Exactas y Naturales. Universidad de Buenos Aires. Ciudad Universitaria (1428) Pab. II-4to. Piso. Buenos Aires, Argentina.

0168-1605/02/$ - see front matter
PII: S0168-1605(01)00628-6

organisms survive, resulting in very good microbiological quality (Cowman and Kelsey, 1992). Many samples of bottled water contain very high levels of bacteria and it is unknown whether this autochthonous flora has the potential to cause illness (Hunter, 1993).

The quality of drinking water is usually expressed in terms of numbers of bacteria present in a given volume of water. The estimation of fungi in drinking water has been infrequent (Nagy and Olson, 1982). Several problems have been detected in our laboratory in recent years related to fungal occurrence and growth in still mineral water in plastic containers.

The purpose of this work was to isolate and identify fungal species from spoiled bottled mineral water, to determine if viable propagules of those species were present in water without visible microbial growth and also to determine whether a relationship exists between the presence of fungal propagules and the microbiological quality of the product.

2. Materials and methods

2.1. Samples

In a first experiment, 36 samples of still mineral water belonging to three commercial brands were analysed. Of these, 12 appeared sound and 24 showed visible fungal growth. Following this work, 90 samples of eight commercial brands of still mineral water and mineralised water were analysed.

2.2. Fungi isolation and identification

Fungal species were isolated on Malt Extract Agar (MEA) (Pitt and Hocking, 1997). Fungal counts were carried out by membrane filtration of 100-ml samples and incubation of the membrane on MEA for 5 days at 25 °C.

Isolates were identified according to Pitt and Hocking (1997).

3. Bacteriological analysis

The presence of *Escherichia coli*, faecal streptococci and *Pseudomonas aeruginosa* was investigated by membrane filtration of samples (250 ml), followed by incubation on Lauril Sulfate Broth (BK 010; Biokar, Beauvais, France) for 4 h at 30 °C and 14 h at 44 °C for *E. coli* (EC, 1980) and on m-Enterococcus Agar for 24 h at 37 °C for faecal streptococci (APHA, 1992a). Detection of *P. aeruginosa* was carried out by membrane filtration, enrichment of the membrane in Malachite Broth, isolation on Cetrimide Agar and confirmation on P Agar, F agar and Acetamide Broth (DIN, 1991). Sulphite reducing clostridia were analysed by pouring samples (50 ml) over an equal quantity of double concentrate Differential Clostridium Medium (Merck 11699; Merck, Darmstdat, Germany). Medium and water were heated at 70 °C for 10 min. Incubation was carried out for 5 days at 37 °C in anaerobic conditions (DIN, 1991).

Total coliforms were determined by Most Probable Number on Lauril Sulphate Broth (APHA, 1992b).

Sterility controls of membranes and media were carried out by filtering sterile water (100 ml) and followed by incubation at corresponding time and temperature.

4. Results

The incidence of species from samples from batches with spoiled samples is shown in Table 1. Most samples contained only one species. However *Cladosporium* and *Penicillium* occurred together in 12.5% of samples and *Alternaria* and *Penicillium* in 4.2%. *Cladosporium cladosporioides* grew directly over the bottle surface, other species did not attach to the inner surface of the container. *Penicillium*

Table 1
Fungal isolates from batches of bottled water in which some samples showed fungal growth

Species	Samples in which the species was isolated (%)	
	Samples with visible mycelium	Samples without visible mycelium
Penicillium spp.	46	33
Cladosporium cladosporioides	50	17
Alternaria alternata	21	0

Table 2
Fungal species isolated from samples from batches of bottled water which showed no spoiled samples

Genera	Samples in which the species was isolated (%)
Penicillium	46
Cladosporium	32
Rhizopus	8
Aspergillus	3
Phoma	3

species included *P. citrinum* (4% of isolates), *P. glabrum* (4%) and other *Penicillium* species (38%).

None of the 36 samples was found to contain *E. coli*, coliforms, faecal streptococci, *P. aeruginosa* and sulphite reducing anaerobes.

The genera most frequently observed in samples from batches without spoiled samples are shown in Table 2.

Many of the samples (33%) from unspoiled bottles contained fungal contamination.

The fungal counts were in the range of 0–67 colony forming units (CFU) 100 ml^{-1}. The highest counts (20–70 CFU 100 ml^{-1}) were found in mineralised water and the lower ones (0–10 CFU 100 ml^{-1}) were in natural mineral water.

No samples were found to contain *E. coli*, *P. aeruginosa*, coliforms and sulphite reducing anaerobes; however, 11% of the samples were found to contain faecal streptococci.

5. Discussion

The results shown in Table 1 are in general agreement with those reported by Frankova and Horecka (1995), i.e. *Cladosporium* (44%), *Penicillium* (30%), and *Alternaria* (4.5%) in drinking water from the wells and reticulated water in Slovakia; by Nagy and Olson (1982) who found *Penicillium* (28%) and *Alternaria* (2.5%) and Hinzelin and Block (1985) who found *Penicillium* (23%) and *Cladosporium* (4.6%) in the reticulated water of Nancy and Metz, France. Lower counts in mineral water are expected as GMP must be followed during extraction and bottling. The water source must be protected against pollution and subjected to a hydrological survey. Disinfecting is not allowed, with purity depending only on the protected nature of the source (Fewtrell et al., 1997). Disinfection treatments such as chlorination and ozonation apparently do not affect the presence and distribution of fungal species because *Cladosporium*, *Penicillium* and *Alternaria* have often been isolated from chlorinated water (Nagy and Olson, 1982; Hinzelin and Block, 1985; Frankova and Horecka, 1995; Zacheus and Martikainen, 1995).

Although filamentous fungi in water are commonly thought to pose no potential public health problems, some of the fungi isolated from mineral water, i.e. *Alternaria alternata* and *P. citrinum*, have some toxigenic potential and could constitute some health risk. It is therefore advisable to count fungal propagules in routine microbiological studies of bottled mineral water and to establish baselines.

Further investigation is needed to find conditions under which fungi can grow and to establish if toxic metabolites could be released into the water under such conditions.

Acknowledgements

This research was supported by Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), PRHIDEB-CONICET (Publication No 147) and Universidad de Buenos Aires.

References

APHA (American Public Health Association), 1992a. Fecal *Streptococcus* and *Enterococcus* group. Standard Methods for the Examination of Water and Wastewater, 18th edn. American Public Health Association, Washington, DC, pp. 69–73.

APHA (American Public Health Association), 1992b. Multiple tube fermentation technique for members of the coliform group. Standard Methods for the Examination of Water and Wastewater, 18th edn. American Public Health Association, Washington, DC, pp. 45–51.

CAA (Código Alimentario Argentino Actualizado), 1998. Bebidas hídricas, agua y agua gasificada, Capitulo XII. Agua Mineral, Art. 985. (Eds.) De la Canal, S.R.L., Buenos Aires, Argentina, pp. 332–333.

Cowman, S., Kelsey, R., 1992. Bottled Water. In: Vanderzant, C., Splittstoesser, D.F. (Eds.), Compendium of Methods for the Microbiological Examination of Foods, 3rd edn. American Public Health Association, Washington, DC, pp. 1031–1036.

DIN (Deutsche Institut fur Normug), 1991. Deutsche Einheitsverfahren zur Wasser Abwasser und Schalammunterschung. Mik-

robiologische Verfahren (GruppeK). DIN 38411. Beuth Verlag, Berlin, Deustcheland.

EC (European Community), 1980. Exploitation and marketing of natural mineral waters. Council Directive No. 80/777. Off. J. Eur. Comm. L 229, 1–10.

EPA (Environment Protection Agency), 1989. National Primary Water regulation. Federal Register 54. Environment Protection Agency, Washington, DC, USA.

Fewtrell, L., Kay, D., Godfree, A., O'Neill, G., 1997. Microbiological quality of bottled water. Water Sci. Technol. 35, 47–53.

Frankova, E., Horecka, M., 1995. Filamentous soil fungi and unidentified bacteria in drinking water from wells and water mains near Bratislava. Microbiol. Res. 150, 311–313.

Hinzelin, F., Block, J.C., 1985. Yeast and filamentous fungi in drinking water. Environ. Technol. Lett., 101–106.

Hunter, P.R., 1993. The microbiology of bottled natural mineral waters. J. Appl. Bacteriol. 74, 345–352.

Nagy, L.A., Olson, B.H., 1982. The occurrence of filamentous fungi in drinking water distribution systems. Can. J. Microbiol. 28, 667–671.

Pitt, J.I., Hocking, A.D., 1997. Fungi and Food Spoilage, 2nd edn. Aspen Publishers, Gaithersburg, MD, USA.

Zacheus, O.M., Martikainen, P.J., 1995. Occurrence of heterotrophic bacteria and fungi in cold and hot water distribution systems using water of different quality. Can. J. Microbiol. 41, 1088–1091.