Microbiology Focus

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Pseudomonas a Communicative Bacteria

Pseudomonas, **the water bacteria, is psychrotrophic and can pose a significant food spoilage problem in refrigerated shell fish.**

Pseudomonas a Communicative Bacteria

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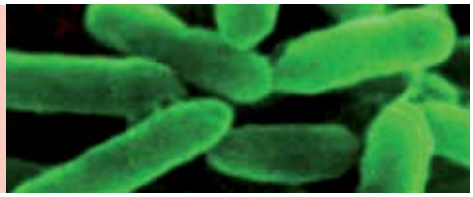
Nature, Identification and Differentiation of *Pseudomonas*

Pseudomonas are motile (one or more polar flagella), rod shaped and aerobic, Gram-negative, non-fermentative bacteria. The typical bacterial size is 0.5 – 1.0 x 1.5 – 5.0 µm. The catalase test gives a positive result, but in some rare cases species show a negative reaction in the oxidase test, e.g. *P. syringae*. To differentiate and identify by biochemical characteristics, Sigma-Aldrich provides the Gram staining kit (Fluka 77730) and other required tests and media (see **Tables 1 and 2**). Another known feature associated with *Pseudomonas* species (e.g. *P. aeruginosa, P. fluorescens, P. putida)* is the secretion of pyoverdin (fluorescein, a siderophore), a fluorescent yellow-green pigment under iron-limiting conditions [1]. *Pseudomonas* needs siderophores to build a complex with iron (III) and to be able to take up iron. Certain *Pseudomonas* species may also produce additional pigments, such as pyocyanin (blue pigment, a siderophore) by *P. aeruginosa* [2], quinolobactin (yellow, dark green in presence of iron, a siderophore) by *P. fluorescens* [3] or/and a reddish pigment called pyorubrin and pyomelanin (brown pigment) by *P. aeruginosa* [5]. On blood agar a hemolytic reaction can be observed.

Scientific classification of *Pseudomonas*

Kingdom:	Bacteria
Phylum:	Proteobacteria
Class:	Gamma Proteobacteria
Order:	Pseudomonadales
Family:	Pseudomonadaceae
Genus:	*Pseudomonas*



Sugars can be used as the energy source by using the Entner-Doudoroff pathway, the end product is pyruvate (Dissimilation). A different set of enzymes from those used in either glycolysis or the pentose phosphate pathway is used (sugars are degraded without acid production). Except for the alcaligenes group, most species can use glucose as substrate. No fermentation catabolism is used from *Pseudomonas* but some species like *P. stutzeri, P. denitrificans* and some species of *P. aeruginosa* are able to use nitrate as electron acceptor (instead of oxygen). The denitrification pathway is also used under anaerobic conditions.

Name and History

The name *Pseudomonas* comes from the greek and latin and means "false unit" (pseudo = false, greek; monas = single unit, latin). "Monas" was used in the early history of microbiology to describe single-celled organisms. In 1786 Otto Friedrich Müller, from Copenhagen, classified the bacteria and named the pseudomonads, they came into the group of vibriones (which was defined as group of shaking bacteria). Many years later it was detected that *Pseudomonas* are motile. They therefore were given the name "Pseudo", because they appeared to be shaking but in reality they were motile.

Because of their widespread occurrence in water, the pseudomonads were characterised and named as one of the first microorganism groups. The organism was defined in rather vague terms in 1894 as a genus of Gram-negative, rod-shaped and polar-flagella bacteria.

Natural Sources and Clinical Relevance

Pseudomonas can be found almost everywhere; in soil, water, plants and animals. In most cases it is not a pathogen and some strains (e.g. *P. putida*) are used for the biodegradation of diverse organic compounds in e.g. polluted air (bio scrubber), waste water and other polluted materials.

P. aeruginosa is the most famous opportunistic human pathogen most commonly affecting immunocompromised patients. Pathogenic *Pseudomonas* are found at almost every bodily site, most commonly they are isolated from specimens from the urinary tract, respiratory tract, blood and wounds.

Pseudomonas can use a broad range of nutritional sources; they even survive in very simple nutritional environments without any organic compounds. They can remain viable for long periods of time in many different habitats and under very adverse conditions. Species have been found in water, saline solutions, utensils and even in cosmetics, pharmaceuticals and disinfectants. The bacteria have been isolated from many natural and manufactured foods. Psychrotrophic *Pseudomonas* species, pose a significant food spoilage problem in refrigerated meat, fish, shell fish and dairy products. As well *Pseudomonas* can lead to problems in water systems, it can be the source of the contamination in food and beverage industry [4].

Did you know...

that *Pseudomonas aeruginosa* build a protective biofilm?

In a biofilm they avoid attack from the body's immune mechanisms and antibiotics. New studies have showed neutrophil-induced biofilm enhancement due to incorporation of F-actin and DNA polymers into the biofilm, while Poly(aspartic acid) and DNase were found to effectively prevent or disrupt biofilm formation.

(Source: Journal of Medical Microbiology, Q. Parks et al.)

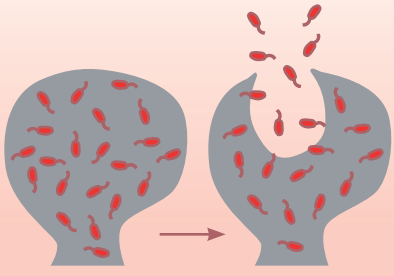
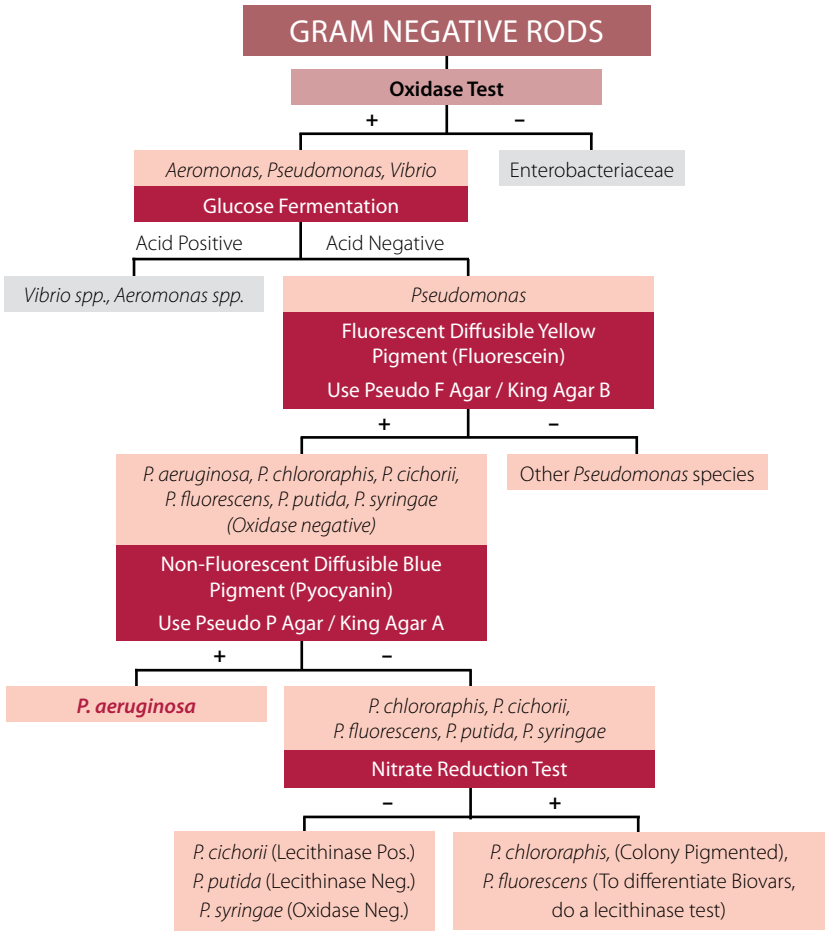


Figure 1: biofilm releasing some bacteria

Figure 2: Identification Flow Charts for *Pseudomonas*



(Bergey's Manual of Determinative Bacteriology 9th Edition)

Biofilm, Quorum Sensing and Antibiotic Resistance

It has been observed that *Pseudomonas* communicate from cell to cell via the production of small molecules called acyl homoserine lactones. This way they control the gene expression responsible for the growth, virulence factors and biofilm formation. This allows the bacteria to rapidly adapt to environmental changes. Biofilm formation is a major problem in cases of chronic opportunistic infections in immunocompromised patients and elderly people. *P. aeruginosa* is known to build biofilms and are able to proliferate within the biofilm. Within the biofilm the bacteria are protected from the immune system, from antibiotics and, outside the body, from other adverse environmental factors. As *Pseudomonas* have generally a low susceptibility to a diverse range of antibiotics, that begins to be a very serious problem. They are naturally resistant to penicillin and the majority of related β-lactam antibiotics. These resistance mechanisms are based on multidrug efflux pumps, the low permeability of the bacterial cell wall and other antibiotic resistance genes. It has also been observed that antibiotic resistance genes can be easily transferred into certain regions of the *Pseudomonas* chromosome. In recent studies compounds are being checked which inhibit the cell communication for possible future therapies for cases of chronic diseases [6]. More information about quorum sensing can be found on the web ***(http://www.sigma-aldrich.com/quorum-sensing)***.

Figure 3: Colorized scanning electron micrograph of a number of *Pseudomonas aeruginosa* bacteria.

(Soure: CDC / Janice Haney Carr)

Cultural Methods to Detect *Pseudomonas*

Generally *Pseudomonas* are not fastidious microorganisms and grow on very simple media like King Agar for example, which contains a protein hydrolysate, magnesium chloride, potassium sulphate and agar. For selective *Pseudomonas* media cetrimide, nalidixic acid, cephaloridine, fucidin, triclosan, penicillin G, pimaricin, bile salts, SDS, Tergitol®-7 sodium sulfite, fuchsin, malachite green and other inhibitory agents are used. The proteolytic activity, lipolytic activity, fluorescent pigment formation, nitrate utilization, glutamate utilization, hemolytic reaction and other biochemical reactions are used in the media for the identification and differentiation of *Pseudomonas* species. There is also a synthetic basal mineral medium for detection of ability to utilize acetamide (Acetamide Nutrient Broth; Fluka 00185). The acetamide is the substrate providing nitrogen and carbon and *Pseudomonas aeruginosa* possess the ability to use that substrate. The reaction can be made visible by yellow coloration using the Nessler's Reagent (Fluka 72190).

For the detection, identification, differentiation, enumeration and cultivation of *Pseudomonas* Sigma-Aldrich provides a broad range of specific agars and broths (see **Table 1**).

Table 1: Media and Supplements for *Pseudomonas*

Selective Media with Differential System	Cat. No.
GSP Agar	50875
HiFluoro™ Pseudomonas Agar Base	78996
Nonselective Media with Differential System	**Cat. No.**
Blood Agar (Base)	70133
Calcium caseinate Agar	21065
CLED Agar	55420
HiCrome™ UTI Agar, modified	*16636
King Agar A	60788
King Agar B	60786
Motility Nitrate Medium	14305
OF Test Nutrient Agar	75315
Pseudomonas Agar (for Fluorescein)	P1852
Tributyrin Agar	*91015
Selective by Defined Nutrients	**Cat. No.**
Acetamide Nutrient Broth	00185
Asparagine Proline Broth	17129
Selective Media	**Cat. No.**
Cetrimide Agar	70887
Cetrimide Agar	22470
Cetrimide Agar Plates (Diameter 55 mm)	14521
Cetrimide Broth	78886
Malachite Green Broth	63163
Milk Agar, modified according to Brown & Scott	17168
Pseudomonas Agar Base	P2102
Pseudomonas Isolation Agar	17208
Nonselective Media	**Cat. No.**
Brain Heart Infusion Broth	53286
Bushnell Haas Broth	B5051
Dey-Engley Neutralizing Broth	D3435
Lactose Broth, Vegitone	19057
Milk Agar	70147
Mueller Hinton Agar	70191
Mueller Hinton Broth 2 (Cation-Adjusted)	90922
Mueller-Hinton Agar 2	97580
Nitrate Broth	*72548
Nutrient Agar	70148
Nutrient Agar Plates (Diameter 55 mm)	44776
Nutrient Broth No 3	70149
Nutrient Broth No. 4	03856
Peptone Water	70179
Peptone Water, phosphate-buffered	77187
Peptone Water, phosphate-buffered (ready to use media in the bottle)	94217
Peptone Water, phosphate-buffered, Vegitone	40893
Plate Count Skim Milk Agar	80957
R-2A Agar	17209
Thioglycollate Broth (USP Alternative)	70157
Tryptic Soya Agar with Polysorbate 80 and Lecithin	51414
Tryptone Glucose Extract Agar	70159
Tryptone Glucose Yeast Extract Agar	T2188
Vegitone Infusion Broth	41960
Yeast Extract Agar	01497
Supplements for *Pseudomonas* Media	**Cat. No.**
Cetrinix Supplement	C8721
C.F.C. Supplement	53477

* Not sold in the U.S.

Table 2: Test for identification and differentiation of *Pseudomonas*

Test for *Pseudomonas* Diagnostics	Cat. No.
Catalase Test	88597
Nitrate Reagent A	38497
Nitrate Reagent B	39441
Nitrate Reagent Disks Kit	51138
Nitrate Reduction Test	73426
Nitrate Reagent Disks	51138
Oxidase Test	70439
Oxidase Strips	40560
Oxidase Reagent acc. Gaby-Hadley A	07345
Oxidase Reagent acc. Gaby-Hadley B	07817
Oxidase Reagent acc. Gordon-McLeod	18502

Figure 4: *Pseudomonas* on Cetrimide Agar

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6. H. Wu , et al., Synthetic furanones inhibit quorum-sensing and enhance bacterial clearance in Pseudomonas aeruginosa lung infection in mice, J. Antimicrob. Chemother. 53, 1054-1061 (2004)

Overview to *Bacillus cereus*

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Nature, Identification and Differentiation of *Bacillus*

Bacillus cereus is a typically motile soil bacteria (up to 1 million cells per gram) and are able to build resistant centrally located endospores. The cells are rod-shaped, have a size of 1 x 5-10 µm and usually occur in chains. The colonies on most media are dull and frosted. It is a Gram-positive and a facultative anaerobic organism, older cells may become Gram-negative as they age. *Bacillus cereus* builds together with *Bacillus anthracis, Bacillus thuringiensis, Bacillus cytotoxicus* and some other species the Bacillus-Cereus group, which share about 3100 genes.

Some strains are able to cause foodborne illness similar to those caused by *Clostridium perfringens* or *Staphylococcus aureus*.

The minimum infectious dose is about 10^{6-7} bacteria/g; at that range of cell concentration they start to build different enterotoxins. Four toxins are built, one is the heat and acid resistant toxin, called the emetic toxin, with short incubation time (0.5 - 6 hours) and mainly characterized by vomiting (similar symptoms caused by *Staphylococcus aureus*). The mechanism of this toxin in not completely understood, but the small molecule forms ion channels and holes in membranes (Ionophoric compound). The other three toxins are the heat-labile enterotoxins, the non haemolytic (Nhe), the haemolytic enterotoxin (Hbl) and the Cytotoxin K (CytK) need a longer incubation time (8 - 16 hours) and show diarrheic syndromes (similar to that caused by *C. perfringens*). Nhe, and Hbl form pores into the cell membrane and activate adenylate cyclase enzymes. The CytK is as well a pore-forming protein more related to other hemolysins, it has not been shown to be involved in food poisoning [2-4].

Did you know...

that *Bacillus cereus* can also be used as a probiotic?

Probiotic products consisting of bacterial spores of *B. cereus* strains are extremely sensitive to simulated gastric conditions and simulated intestinal fluids.

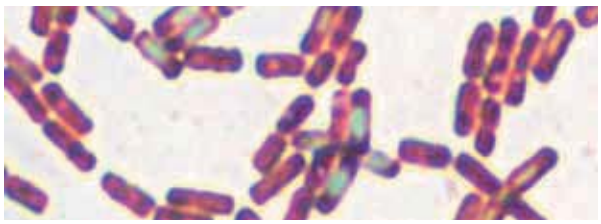


Figure 1: Bacillus cereus; Staining acc. Holbrook and Anderson (1980), Can. J. Microbiol. (spores with malachite green, sporangium with safranin and lipid bodies with sudan black B) for confirmation of suspicious colonies from Cereus Selective Agar.
(Source: H. Becker, Ludwig - Maximilian University, Tierärztliche Fakultät, Hygiene and Technology of Milk, Oberschleißheim, Germany)

Risk Food and Storage

Infection sources for the diarrhea type of *B. cereus* infection are sweets (puddings, vanilla sauce), meats (roast, goulash, sausages), vegetables, salads, soups and UHT milk products. Milk products are less dangerous as *B. cereus* and other bacilli degrade the casein to peptides which may be further degraded to amino acids which are responsible for alkaline reaction and bitter taste of milk.

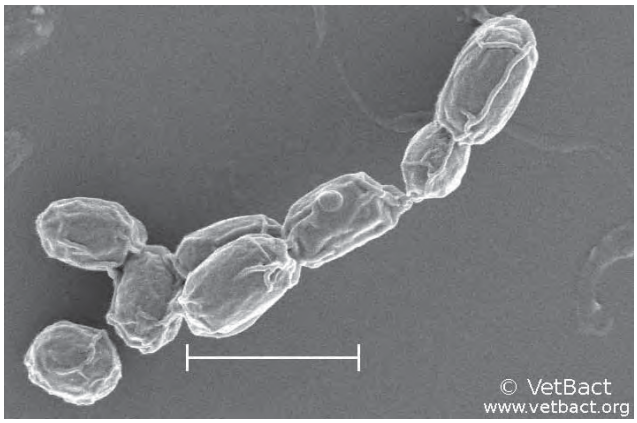
The vomiting type of *B. cereus* illness is caused mainly by contaminated cooked rice that has been reheated.

The risk for strong propagation and the resulting illness can be minimized by storing foods at <5 °C or >65 °C and by rapidly cooling down foods, thus lowering the pH value to <4.5 with a respective a_w-value of <0.95. Note: *B. cereus* spores are in most cases not eliminated by heating; in fact, heating activates spore germination. At the same time, however, the spoilage flora is eliminated, which results in no competition and no inhibition by microbial flora for *B. cereus*.

Table 1: Possible Identification and Differentiation Characteristics

Test/Properties	Reaction/Characteristics
Gram coloration	Positive
Respiration	facultative anaerob
Spores:	
Morphology	oval, do not appreciably swell cells
Location	central, or paracentral
motility	+
Hemolysis	β-haemolytic
Catalase	+
Oxidase	-
Lecithinase	+
Gelatine Hydrolysis	+
Indole	-
Carbohydrates:	
Glucose	+ (acid)
Xylose	-
Mannitol	-
Lactose	-
Sucrose	variable
Maltose	+ (acid)
Salicin	+ (acid)
sensitivity to acid	present
Antibiotic sensitivity [5]	Sensitive to: pefloxacin gentamycin chloramphenicol nalidixic acid clindamycin vancomycin erythromycin Resistant to: colistin polymyxin B
Fatty acid structure	80% Branched [1]

Figure 2: Spores of *Bacillus cereus*, strain ATCC 14579T.



This strain forms an exosporium (see Fig. 2), which is not as easily visible on the spores as on some other strains (c.f. Fig. 2). The length of the scale bar is equivalent to 2 µm. (source ***www.vetbact.org***; photographers Joakim Ågren, SVA, and Leif Ljung, BMC; images may only be used for non-commercial purposes)

Detection and Differentiation

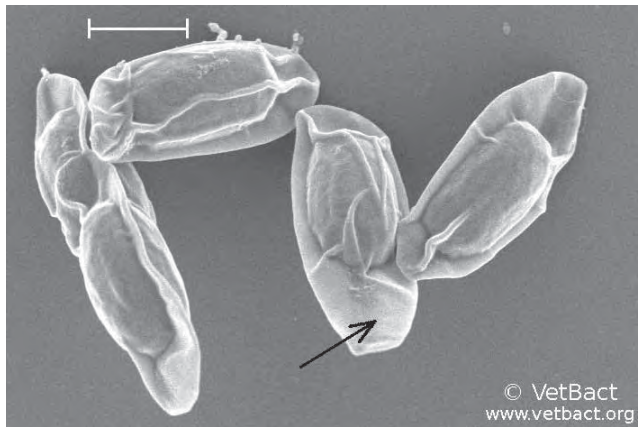
The heat-shock and ethanol spores selection procedure is an excellent way to do quantitative recovery of *Bacillus* species. Colonies of *B. cereus* show mostly (not always) on blood agar β-haemolytic reaction zones. The colonies are raised, irregular, greyish to greenish and with 3 to 8 mm in diameter. Probably the name cereus comes from the appearance of the colonies as in greek cereus means "wax-colored". The larger colonies with mycoid appearance are probably the non motile *Bacillus mycoides* (formerly *Bacillus cereus* var. mycoides). As written above, it is a Gram-positive organism and may older cells shows Gram-negative reaction but with the KOH string test there is a further possibility to check the uncertain Gram reaction. Lecithinase reaction, detected on Mannitol-Egg-yolk-polymyxine-Agar, is on of the most important differentiation characteristics. Together with colony morphology, haemolytic reaction and motility *B. cereus* can be confirmed [6].

Table 2: Media and Supplement for Differentiation of *B. cereus*

Media System	Cat. No.	Name
nonselective + differential system	70133	Blood Agar (Base)
	21065	Calcium caseinate Agar
	16447	Glucose Bromcresol Purple Agar
	70151	Nutrient Gelatin
selective + differential system	22310	Cereus Selective Agar (Mannitol-Egg-yolk-polymyxine-Agar)
	*92325	HiCrome™ Bacillus Agar
Selective Supplement	P9602	Polymyxin B Selective Supplement

* Not sold in the U.S.

Figure 3: Spores of *Bacillus cereus*, strain NVH 0597-99.



This strain produces an exosporium, which is clearly visible (see the arrow) and is smeared as a plastic bag around the spore (c.f. Fig. 2.). Spores of *B. anthracis* and *B. thuringiensis* are also surrounded by an exosporium, which thus, is another coat that overlies the normal spore coat. The length of the scale bar is equivalent to 1 µm. (source ***www.vetbact.org***; photographers Joakim Ågren, SVA, and Leif Ljung, BMC; images may only be used for non-commercial purposes)

Table 3: Test for Identification and Differentiation of *B. cereus*

Tests for *B. cereus* Differentiation	Cat. No.
Catalase Test	88597
Dextrose (Glucose) disks	63367
DMACA Indole Disks	05686
DMACA Reagent	49825
Kovac's Reagent for indoles	67309
Kovac's Reagent Strips	78719
Lactose disks	28816
Maltose disks	77653
Mannitol disks	94438
Oxidase Test	70439
Oxidase Strips	40560
Oxidase Reagent acc. Gaby-Hadley A	07345
Oxidase Reagent acc. Gaby-Hadley B	07817
Oxidase Reagent acc. Gordon-McLeod	18502
Salicin disks	92971
Sucrose disks	94309
Xylose disks	07411

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Win an iPod® in the 2nd Fluka Microbiology Photography Competition

This photography competition is sponsored by Sigma-Aldrich with the aim of encouraging microbiologists to promote something about their work and their science. The best photographic entries will win prizes like an iPod nano, swiss army knife, USB stick and laser pointer. The winning images will be published in Microbiology Focus and the best one will get pride of place on the cover.

Rules of the Competition and Conditions of Entry

1. The competition is open to all residents worldwide.
2. Entries should illustrate any microorganisms (living or dead) or a microbiologist in action at work.
3. Picture size should be at least 400 dpi and 90 x 120 mm (max 3 MB). The file format must be in jpg, tiff or pdf.
4. The entries will be judged on:
   - clarity of presentation
   - composition
   - illumination and contrast
   - congruency of subject matter and title of photograph
   - scientific interest and relevance
   - originality
5. Winning entries will be retained by Sigma-Aldrich, who will have sole rights of publication, reproduction and display.
6. Closing date will be 31st Jan. 2011.
7. Entries after the closing date will not be considered. Entries received incomplete, illegible, mutilated, altered or not complying exactly with the instructions and theme may be disqualified.
8. Decisions of the judges in all matters affecting the competition will be final and legally binding.

The competition will be judged by:

Dr. Antje Breitenstein,
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Prof. Mohammad Manafi
Medical University of Vienna,
Head of Department for Food Hygiene

Jvo Siegrist, Sigma-Aldrich,
Product Manager Microbiology

Method of Entry

There is no entry fee, but for each entry an entry form must be completed (two entries at the most).

The entry form is available from this website:
www.sigma-aldrich.com/mibi-competition

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