Appendix 2
Safe micro-organisms

In May 1997, a safety conference was convened by the Association for Science Education (ASE). Various organisations were represented, including the ASE, CLEAPSS, SSERC, HSE, MISAC (Microbiology in Schools Advisory Committee), Society for Applied Microbiology, Society for General Microbiology, NCBE (National Centre for Biotechnology Education), SAPS (Science & Plants in Schools), the Wellcome Trust and the educational suppliers Philip Harris and Blades Biological. The principal aims of the conference were to consider clarification of guidance on the use of micro-organisms and biotechnology in the DfEE publication *Safety in Science Education* and to evaluate the list of micro-organisms considered suitable for use in schools and colleges following changes to the hazard categorisation of certain micro-organisms by the Advisory Committee on Dangerous Pathogens[1]. One of the outcomes of the conference is a revision of this list.

The accompanying tables give selected micro-organisms which present minimum risk given good practice. These tables supersede the existing lists found in the *CLEAPSS Laboratory Handbook* (1992), the *CLEAPSS Shorter Laboratory Handbook* (2000), *Microbiology: An HMI Guide for Schools and Further Education* (1990, now out of print), *Topics in Safety* (1988) and *Safety in Science Education* (1996). As well as naming suitable organisms, the new lists give points of educational use and interest and comment on the ease with which organisms can be cultured and maintained. The lists of micro-organisms are not definitive; other organisms may be used if competent advice is obtained.

It should be noted that strains of micro-organisms can differ physiologically and therefore may not give expected results. Where possible, fungi that produce large numbers of air-borne spores should be handled before sporulation occurs, so that the spread of spores into the air and possible risks of allergy or the triggering of asthmatic attacks are minimised. This is particularly important for some species, such as *Aspergillus* and *Penicillium*, which produce very large numbers of easily dispersed spores. It should be noted that certain species of these two fungi, previously listed as unsuitable for use in schools, are now not thought to present such a serious risk to health, given good practice in culture and handling.

[Note: This list of micro-organisms is also be available from other sources including the ASE, CLEAPSS, MISAC and SSERC and is published in the new edition of Topics in Safety *(ASE, 2001, ISBN 0 86357 316 9) with notes on other aspects of safety relating to microbiology and biotechnology in schools.]*

[1]*Categorisation of Biological Agents According to Hazard and Categories of Containment*, 4th edition, 1995, Advisory Committee on Dangerous Pathogens, HSE Books, ISBN 0717610381.

Bacteria

Bacterium	Educational use/interest/suitability	Ease of use/maintenance
Acetobacter aceti	Of economic importance in causing spoilage in beers and wines. Oxidises ethanol to ethanoic (acetic) acid and ultimately to carbon dioxide and water.	Needs special medium and very frequent subculturing to maintain viability.
Agrobacterium tumefaciens	Causes crown galls in plants; used as a DNA vector in the genetic modification of organisms.	Grows on nutrient agar, but requires 2-3 days' incubation.
Alcaligenes eutrophus	In the absence of nitrogen, it produces intracellular granules of poly-β-hydroxybutyrate (PHB); was used in the production of biodegradable plastics.	Grows on nutrient agar.
Azotobacter vinelandii	A free-living nitrogen fixer, producing a fluorescent, water-soluble pigment when grown in iron (Fe)-limited conditions.	Grows on a nitrogen-free medium.
Bacillus megaterium	Has very large cells; produces lipase, protease and also PHB (see *Alcaligenes*); Gram-positive staining.	Grows on nutrient agar.
Bacillus stearothermophilus	Thermophilic species which grows at 65 °C; produces lipase and protease. Also used to test the efficiency of autoclaves.	Grows on nutrient agar.
*Bacillus subtilis**	General-purpose, Gram-positive bacterium. Produces amylase, lipase and protease.	Grows on nutrient agar.
Cellulomonas sp.	Produces extracellular cellulase.	Grows on nutrient agar but also used with agar containing carboxymethylcellulose.
Chromatium sp.	A photosynthetic, anaerobic bacterium.	Requires special medium and light for good growth.
Erwinia carotovora (=*E. atroseptica*)	Produces pectinase which causes rotting in fruit and vegetables. Useful for studies of Koch's postulates.	Grows on nutrient agar.
*Escherichia coli**	K12 strain: general-purpose, Gram-negative bacterium. B strain: susceptible to T4 bacteriophage.	Grows on nutrient agar.
Janthinobacterium (=*Chromobacterium*) *lividum*†	Produces violet colonies. Grows best at 20 °C.	Needs frequent subculture and is best grown on glucose nutrient agar and broth.
Lactobacillus sp.	Ferment glucose and lactose, producing lactic acid; *L. bulgaricus* is used in the production of yoghurt.	Require special medium containing glucose and yeast extract and frequent subculturing to maintain viability.
Leuconostoc mesenteroides	Converts sucrose to dextran: used as a blood plasma substitute.	Requires special medium as for *Lactobacillus*.
Methylophilus methylotrophus	Requires methanol as energy source; was used for the production of 'Pruteen' single-cell protein.	Requires special medium containing methanol.
Micrococcus luteus (=*Sarcina lutea*)	Produces yellow colonies; useful in the isolation of the bacterium from impure cultures. Also used to simulate the effects of disinfectants, mouthwashes and toothpastes on more harmful organisms. General-purpose, Gram-positive bacterium.	Grows on nutrient agar.
Photobacterium phosphoreum	Actively-growing, aerated cultures show bioluminescence; grows in saline conditions.	Requires a medium containing sodium chloride.
Pseudomonas fluorescens	Produces a fluorescent pigment in the medium.	Grows on nutrient agar.
Rhizobium leguminosarum	A symbiotic, nitrogen fixer; stimulates the formation of nodules on the roots of legumes. Only fixes nitrogen in plants.	Grows on yeast malt agar; some authorities recommend buffering with chalk to maintain viability.
Rhodopseudomonas palustris	A photosynthetic, anaerobic, red bacterium. Also grows aerobically in the dark.	Requires light and a special medium, growing atypically on nutrient agar.

Bacterium	Educational use/interest/suitability	Ease of use/maintenance
Spirillum serpens	Of morphological interest.	May grow on nutrient agar but requires very frequent subculturing to maintain viability.
Staphylococcus albus (*epidermidis*)‡	A general-purpose, Gram-positive bacterium, producing white colonies.	Grows on nutrient agar.
Streptococcus (=*Enterococcus*) *faecalis*	Of morphological interest, forming pairs or chains of cocci.	Nutrient agar with added glucose can be used but grows better on special medium, as for *Lactobacillus*.
Streptococcus (=*Lactococcus*) *lactis*	Of morphological interest, forming pairs or chains of cocci. Commonly involved in the souring of milk; also used as a starter culture for dairy products.	Can grow on nutrient agar with added glucose; some authorities recommend buffering with chalk to maintain viability.
Streptococcus thermophilus	Ferments glucose and lactose, producing lactic acid; used in the production of yoghurt. Grows at 50 °C.	Can grow on nutrient agar with added glucose; some authorities recommend frequent subculturing to maintain viability.
Streptomyces griseus	Responsible for the earthy odour of soil. Grows to form a fungus-like, branching mycelium with aerial hyphae bearing conidia. Produces streptomycin.	Grows on nutrient or glucose nutrient agar but better on special medium which enhances formation of conidia.
Thiobacillus ferrooxidans	Involved in the bacterial leaching of sulphur-containing coal. Oxidises iron(II) and sulphur. Demonstrates bacterial leaching of coal samples containing pyritic sulphur.	Requires special medium.
Vibrio natriegens§ (=*Beneckea natriegens*)	A halophile, giving very rapid growth. Prone, however, to thermal shock with a sudden drop in temperature.	Requires medium containing sodium chloride.

*Some strains have been associated with health hazards. Reputable suppliers should ensure that safe strains are provided.

†Can be chosen for investigations that once required the use of *Chromobacterium violaceum* or *Serratia marcescens*.

‡This organism has been known to infect debilitated individuals and those taking immunosuppressive drugs. Some authorities advise against its use.

§A well-known supplier currently lists an unspecified species of *Vibrio* because of its morphological interest. This has a typical shape, better shown than by *V. natriegens*. However, this species is a Hazard Group 2 organism which may cause human disease. This bacterium should only be used in establishments that have containment facilities suitable for work with Hazard Group 2 micro-organisms.

Fungi

Fungus	Educational use/interest/suitability	Ease of use/maintenance
Agaricus bisporus	Edible mushroom; useful for a variety of investigations on factors affecting growth.	Grows on compost containing well-rotten horse manure; available as growing 'kits'.
Armillaria mellea	The honey fungus; causes decay of timber and tree stumps. Produces rhizomorphs.	Grows very well on malt agar. Some authorities recommend carrot agar.
*Aspergillus nidulans**	For studies of nutritional mutants. Produces abundant, easily-dispersed spores – may become a major laboratory contaminant!	Grows on Czapek Dox yeast agar. Special media required for studying nutritional mutants.
*Aspergillus niger**	Useful for studies of the influence of magnesium on growth and the development of spore colour. Used commercially for the production of citric acid. Produces abundant, easily-dispersed spores - may become a major laboratory contaminant!	Requires special sporulation medium for investigations.
*Aspergillus oryzae**	Produces a potent amylase; useful for studies of starch digestion. Also produces protease. Used by the Japanese in the production of rice wine (saki).	Grows on malt agar; add starch (or protein) for investigations.

Fungus	Educational use/interest/suitability	Ease of use/maintenance
Botrytis cinerea	Causes rotting in fruits, particularly strawberries. Useful for studies of Koch's postulates with fruit, vegetables and *Pelargonium* spp. Important in the production of some dessert wines ('noble' rot). Used in ELISA protocols.	Can be grown on malt agar or agar with oatmeal.
Botrytis fabae	Causes disease in bean plants.	Requires agar with oatmeal.
Candida utilis	Simulates behaviour of pathogenic *Candida* spp. in investigations of fungicidal compounds.	Grows on malt agar or glucose nutrient agar.
Chaetomium globosum	Useful for studies of cellulase production; thrives on paper.	Can be grown on V8 medium but survives well just on double thickness wall paper, coated with a flour paste.
Coprinus lagopus	For studies of fungal genetics.	Grows on horse dung.
Eurotium (=*Aspergillus*) *repens*	Produces yellow cleistocarps (cleistothecia) embedded in the medium and green conidial heads in the same culture.	Requires special medium.
Fusarium graminearum	Causes red rust on wheat; used in the manufacture of 'Quorn' mycoprotein.	Can be grown on V8 medium.
Fusarium oxysporum	A pathogen of many plants. Produces sickle-cell-shaped spores, a red pigment and pectinase.	Grows well on several media including malt, potato dextrose and Czapek Dox yeast agar.
Fusarium solani	Digests cellulose; macroconidia have a sickle shape.	Grows on potato dextrose agar.
Helminthosporium avenae	A pathogen of oats.	May not grow easily in laboratory cultures.
Kluyveromyces lactis	A yeast, isolated from cheese and dairy products. Ferments lactose and used to convert dairy products to lactose-free forms. Genetically-modified strains are used to produce chymosin (rennet).	Grows on malt agar or glucose nutrient agar.
Leptosphaeria maculans	For studies of disease in *Brassica* plants.	Requires cornmeal agar or prune yeast lactose agar to promote sporulation in older cultures.
Monilinia (=*Sclerotinia*) *fructigena*	For studies of brown rot in apples. Useful for studies of Koch's postulates.	Grows on malt agar or potato dextrose agar.
Mucor genevensis	For studies of sexual reproduction in a homothallic strain of fungus.	Grows on malt agar.
Mucor hiemalis	For studies of sexual reproduction between heterothallic + and – strains and zygospore production.	Grows on malt agar.
Mucor mucedo	Common black 'pin mould' on bread. For sporangia (asexual), mating types and amylase production.	Grows on malt agar.
Myrothecium verucaria	For studies of cellulose decomposition, but *Chaetomium globosum* is preferred.	Grows on malt agar.
*Neurospora crassa**	Red bread mould. Produces different coloured ascospores. Can be used in studies of genetics. Beware – readily becomes a major laboratory contaminant!	Grows on malt agar.
*Penicillium chrysogenum**	Produces penicillin; useful for comparative growth inhibition studies in liquid media or when inoculated on to agar plates seeded with Gram-positive and -negative bacteria. Produces yellow pigment.	Grows on malt agar, though some authorities indicate that it thrives better on liquid media.
*Penicillium expansum**	Does not produce penicillin; causes disease in apples. Useful for studies of Koch's postulates.	Grows on malt agar.
*Penicillium notatum**	Produces penicillin; useful for comparative growth inhibition studies in liquid media or when inoculated onto agar plates seeded with Gram-positive and -negative bacteria.	Grows on malt agar.

Fungus	Educational use/interest/suitability	Ease of use/maintenance
*Penicillium roqueforti**	Does not produce penicillin; the familiar mould of blue-veined cheese.	Grows on malt agar.
*Penicillium wortmanii**	Produce wortmin rather than penicillin.	Grows on malt agar.
Phaffia rhodozyma	A fermenting red yeast. Used to colour the food supplied to fish-farmed salmon.	Grows on yeast malt agar.
Phycomyces blakesleanus	Produces very long sporangiophores which are strongly phototropic.	Grows on malt agar.
Physalospora obtusa	An ascomycete fungus that grows on apples. Thought to produce pectinase.	Grows on potato dextrose agar.
Phytophthora infestans†	Causes potato blight. Produces motile zoospores.	Can be grown on V8 medium.
Plasmodiophora brassicae	For studies of disease in *Brassica* plants, particularly club root. Useful for studies of Koch's postulates.	May not grow easily in culture.
Pleurotus ostreatus	Edible oyster cap mushroom.	Can be grown on rolls of toilet paper!
Pythium de baryanum†	Causes 'damping off' of seedlings; cress is best to use.	Grows on cornmeal agar.
Rhizopus oligosporus	Used in the fermentation of soya beans to make 'tempe', a meat-substitute food in Indonesia.	Grows on potato dextrose agar, Czapek Dox yeast agar and other fungal media.
Rhizopus sexualis	Produces rhizoids and zygospores. Useful for studies of the linear growth of fungi.	Grows on potato dextrose agar and other fungal media.
Rhizopus stolonifer	Produces rhizoids. Produces lipase.	Grows on potato dextrose agar, potato carrot agar, Czapek Dox yeast agar and other fungal media.
Rhytisma acerinum	An indicator of air pollution: less common in industrial areas. On sycamore leaves, it forms 'tar' spot lesions, the number or diameter of which can be compared at different sites.	Difficult to maintain but laboratory cultures are not likely to be needed.
Saccharomyces cerevisiae	Valuable for work in baking and brewing, showing budding, for spontaneous mutation and mutation-induction experiments, and for gene complementation using adenine- and histidine-requiring strains.	Grows on malt agar or glucose nutrient agar.
Saccharomyces diastaticus	Able to grow on starch by producing glucoamylase.	Grows on malt agar and nutrient agar + 1 % starch.
Saccharomyces ellipsoideus	Used in fermentations to produce wine; can tolerate relatively high concentrations of ethanol.	Grows on malt agar.
Saprolegnia litoralis†	Parasitic on animals. Produces zoospores. Good illustration of asexual and sexual stages.	Culture by baiting pond water with hemp seeds.
Schizosaccharomyces pombe	Large cells, dividing by binary fission. Good for studies of population growth, using a haemocytometer for cell counts. Prone to thermal shock.	Grows on malt agar. For studies of population growth, a malt extract broth can be used.
Sordaria brevicollis	For studies of fungal genetics, including inheritance of spore colour and crossing over in meiosis.	Requires special medium for crosses between strains.
Sordaria fimicola	For studies of fungal genetics, including inheritance of spore colour and crossing over in meiosis.	Grows on cornmeal, malt and other agars but may not transfer readily from one medium to another. White-spore strain may not always grow normally on standard cornmeal agar.
Sporobolomyces sp.	Found on leaf surfaces. Spores are ejected forcibly into the air from mother cells.	Grows on malt, yeast malt and glucose nutrient agar but laboratory cultures may not be needed.
Trichoderma reesei	Commercial production of cellulase.	Grows on malt agar.

*Possible risk of allergy/asthma if large numbers of spores are inhaled.

†Now classed as a protoctist, so may not be listed under fungi by some suppliers.

Viruses

These are rarely used in schools and colleges but a selected list of those which might be considered is given below.

Bacteriophage (T type) (host *E. coli*)	Cucumber Mosaic Virus
Potato Virus X	Potato Virus Y (*not the virulent strain*)
Tobacco Mosaic Virus	Turnip Mosaic Virus

Algae, protozoa (including slime moulds) and lichens

Though some protozoa are known to be pathogenic, the species quoted for experimental work in recent science projects and those obtained from schools' suppliers or derived from hay infusions, together with species of algae and lichens, are acceptable for use in schools.

Unsuitable micro-organisms

A number of micro-organisms have in the past been suggested for use in schools but are no longer considered suitable; these are listed below. Some fungi previously considered unsuitable have been reinstated in the list of selected organisms now that it is thought that they do not present a major risk, given good practice.

Bacteria	Fungi
Chromobacterium violaceum	*Rhizomucor* (*Mucor*) *pusillus*
Clostridium perfringens (*welchii*)	
Pseudomonas aeruginosa	
Pseudomonas solanacearum	
Pseudomonas tabaci	
Serratia marcescens	
Staphylococcus aureus	
Xanthomonas phaseoli	