# Microbiology Focus

Volume 2.1, 2010

Fluka Analytical

# "Fusariosaurus" - The Discovery of a New Organism?

*Fusariosaurus in Wheat*

# Fusariosaurus (cover)

**By Jvo Siegrist, Product Manager Microbiology….** *ivo.siegrist@sial.com*

## *Who says that scientists don't have a sense of humor?*

**The interesting and intriguing microscopic image on the front cover of this issue of Microbiology Focus is the winning photo from the Summer 2009 Fluka Microbiology Photo Competition.** It captures a fungus of the species *Fusarium culmorum* with a notable example of a typical mycel-formed conidia with spores that have already germinated. Germ tubes, visible on image, develop later to abundant mycelium. Especially amusing was the description provided by **Dr. Ela Suchowilska** for that unique photo. She titled the image "Fusariosaurus", based on the Pterosaurs which dominated the skies during the mesozoic era (late Triassic to the end of the Cretaceous period; 220 to 65.5 million years ago).

Dr. Ela Suchowilska is an expert on Fusarium and wrote an interesting article in this issue of Microbiology Focus. To learn more about this noteworthy topic, see *"Dangerous Fungi of the Genus Fusarium"*.

**Figure 1:** Pterosaurs

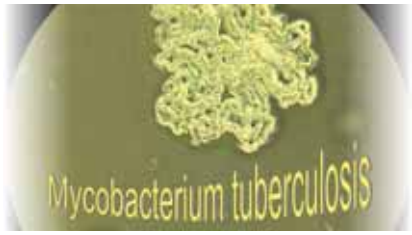

**Figure 2:** "Fusariosaurus"

## Winning Entries of Photo Competition

Last summer we held our Fluka Microbiology Photo Competition and received some fascinating images from around the world. The photographic entry winning Best of Show is featured in the previous article. The entries winning second, third, and fourth place prizes are showcased below. The aim of the competition was to encourage microbiologists to promote their work, with the condition that entries should illustrate any microorganisms (living or dead) or a microbiologist in action at work. Sigma-Aldrich would like to thank all who entered our competition and also our independent jury members Dr. Antje Breitenstein (Scanbec GmbH), Prof. Dr. Corinne Gantenbein (ZHSW), and Prof. Dr. Mohammad Manafi (Medical University of Vienna).

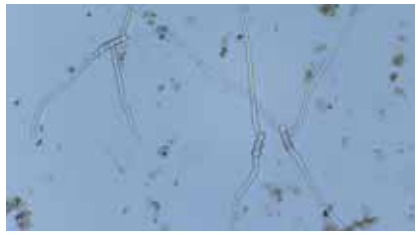
**Second Place Winner:**
This is the 1000X Microscopic view of two isolates from Indian Ocean nodules capable of metal oxidation from its sulphides and oxides.

**Abhilash Pillai**
National Metallurgical Laboratory, India




**Third Place Winner:**
Typical colony of Mycobacterium tuberculosis seen under a microscope with 10x magnification.

**Martin Anandi**
Institute of Tropical Medicine, Antwerp, Belgium



**Fourth Place:**
"Jogging with germinating Fusarium culmorum conidia"

**Ela Suchowilska**
University of Warmia and Mazury in Olsztyn, Poland

# Dangerous Fungi of the Genus *Fusarium*

**By Dr. Ela Suchowilska, Department of Plant Breeding & Seed Production, University of Warmia and Mazury in Olsztyn-Poland….**
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Fungi are heterotrophic organisms that were previously included in the Kingdom Plantae, and today are classified as a separate kingdom, MYCOTA. The Kingdom Fungi comprises unicellular organisms (e.g. yeasts), molds (e.g. fungi of the genera *Aspergillus, Penicillium, Fusarium*) and the Basidiomycetes, including the well-known edible and poisonous cap mushrooms. Molds are composed of filamentous hyphae that form an interconnected network known as mycelium, on which species-specific spores of the imperfect stage (most often asexual conidiospores) are produced, sometimes accompanied by sexual spores. Their typical size is from several to several dozen micrometers, so they are referred to as "microscopic fungi", since their structures can only be seen under the microscope.

Fungi of the genus *Fusarium* are of great economic significance due to their widespread occurrence and high pathogenicity to all crop species grown throughout the world. This is of particular importance in the case of cereals, as head and kernel infections may drastically reduce grain yield and quality. *Fusarium* head blight (FHB), caused by approximately 16 species of the genus *Fusarium* (Parry et al. 1995), can be devastating, with an overall decrease in yield reaching 70%. In addition, toxic secondary metabolites produced by *Fusarium* species can be found in FHB-affected grain. The most common among them are trichothecenes (mostly type B, deoxynivalenol and nivalenol) (Ueno 1977, Joffe 1986), zearalenone, fumonisins and other fusariotoxins (Nelson 1993) (**Table 1**). The above substances exhibit very strong phytotoxic and zootoxic effects. The development of FHB-resistant varieties is an important consideration and the main goal of numerous breeding programs across the world. This disease has been increasing in incidence and severity in recent years, due to the implementation of simplified crop production technologies including intensive monoculture systems and simplified crop rotation (in particular with respect to wheat and maize), the lack of effective fungicides for *Fusarium* control and the absence of resistant varieties (Mesterhazy 2002). Therefore, the current direction of research studies is to develop new, *Fusarium*-resistant varieties that could be relied on as a safe source of food for humans and animals.

| Mycotoxins | Fusarium species |
|---|---|
| Type A trichothecenes (e.g. T2-toxin, HT-2 toxin, diacetoxyscirpenol) and type B trichothecenes (e.g. deoxynivalenol and its acetyl derivatives, nivalenol) | *Fusarium sporotrichioides, F. poae, F. graminearum, F. culmorum, F. crookwellense, F. acuminatum* |
| Fumonisins | *F. moniliforme, F. proliferatum* |
| Zearalenone | *F. graminearum, F. culmorum, F. crookwellense* |
| Cyclodepsipeptides (enniatins, beauvericin) | *F. avenaceum. F. sporotrichioides* |

**Table 1:** Most common mycotoxins produced by Fusarium species, found in infected grain (Nelson 1993).

The programs aimed to breed *Fusarium*-resistant varieties involve determining pathogen-host interactions, the types and mechanisms of resistance, as well as genes responsible for resistance to fungi of the genus *Fusarium*. Due to the low host specificity of these pathogens, FHB resistance is conferred by multiple genes (Parry et al., 1995). Mesterhazy (2002) reported five different types of resistance to FHB, i.e. resistance to initial infection (type I), resistance to the spread of the pathogen (type II), ability to degrade mycotoxins (type III), resistance to kernel infection (type IV), and tolerance (type V). FHB resistance may be conditioned phytochemically by the presence of phenolic compounds inhibiting mycelial growth in host tissues. An analysis of their concentrations could facilitate the selection of resistant varieties (Suchowilska 2008).

Recent research has greatly expanded our knowledge about the role of fusariotoxins in pathogenesis and their impact on humans and animals. The presence of the above metabolites in the human food chain is a serious worldwide concern. Trichothecenes are known to act by blocking protein biosynthesis in the cell. The phytotoxic effects of trichothecenes, mostly DON and NIV, have been demonstrated in a number of experiments, but their role in pathogenesis seems to be most significant. A relationship has been found between the FHB resistance of crops and the accumulation of fusariotoxins, particularly DON. Although DON does not necessarily cause typical FHB symptoms, the fungi that produce this toxin are usually highly virulent. The presence of DON also contributes to the spread of the fungus within the spike. NIV-producing chemotypes are less aggressive than DON-producing chemotypes (Miedaner and Reinbrecht, 2001). Many programs have been initiated to prevent and reduce mycotoxin contamination of wheat and other cereals. The co-occurrence of multiple mycotoxins can produce a combined synergistic effect. Resistant cereal lines inoculated with pathogens of the genus *Fusarium* contained lower concentrations of DON in the grain than susceptible cultivars (Miller et al.,1985, Mirocha et al., 1994). Harris et al. (1999) reported that the trichothecenes (especially DON) may act as virulence factors to enhance the spread of *F. graminearum* on maize. Cultivars, breeding strains and lines show a great variability in susceptibility to infection, which is reflected in various levels of yield reduction in different traits (Wiśniewska et al., 2004). Contemporary common wheat cultivars are all susceptible to *Fusarium* infection to a greater or lesser extent (Mesterhazy et al.,1999). The ingestion of food containing trichothecenes may induce numerous diseases termed mycotoxicoses whose symptoms resemble

**Figure 1:** FHB symptoms- field experiments with *Fusarium culmorum* infection of spring wheat

**Figure 2:** FHB symptoms, zoomed

**Figure 3:** *Fusarium culmorum* mycelium on agar plate

those of radiation sickness, including diarrhea, vomiting, nausea, leucocytosis (Alimentary Toxic Aleukia– ATA), bleeding in the gastrointestinal tract, and – in extreme cases – circulatory shock leading to death. Exposure to low doses of trichothecenes (encountered most frequently) results in weight loss, loss of appetite leading to malnutrition, neuroendocrine and immunological changes.

## References


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# *Campylobacter*

**By Georgina Manning. Emerging Pathogen Group. School of Science and Technology, Nottingham Trent University, Nottingham, NG11 8NS, UK....** *georgina.manning@ntu.ac.uk*

## *Campylobacter is a major human pathogen worldwide.*

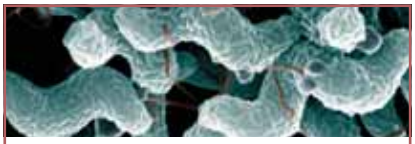
The two species *Campylobacter jejuni* and *C. coli* are responsible for the majority of human food-borne infections in the UK. In 2008 there were 49,880 reported cases of Campylobacteriosis in England and Wales (www.hpa.org.uk). Currently *C. jejuni* is known to account for approximately 90% of all reported cases. *C. coli,* accounting for 7-10% of the Campylobacteriosis cases, is still the fourth most common cause of gastrointestinal illness in England and Wales and therefore remains a significant health burden.

Human food poisoning is acquired through the consumption of contaminated food. Poultry is a major food source of infection and infection can occur through the consumption of undercooked poultry meat or through cross-contamination of other food products in the kitchen. In a recent report from the Food Standards Agency the prevalence of *Campylobacter* in chicken at retail was 65.2% (***www.food.gov.uk***). Other sources of infection include contaminated raw milk and water.

Symptoms of the disease can occur up to two weeks after consuming the contaminated food. They usually consist of a fever and general feeling of unwellness which is accompanied or followed by abdominal cramps and then watery, sometimes bloody, diarrhoea (Blaser and Engberg 2008). The disease is usually self-limiting after 2-7 days; however, in some cases, particularly in the very young, elderly or immuno-compromised, antibiotic treatment may be required. In more severe cases, hospitalization may result.

The mechanisms utilised by *Campylobacter* to cause disease are not yet fully defined. However, it is thought that the organism first colonises the small intestine before travelling to the large intestine, where it causes a local inflammatory response (Black et al. 1988). The organism is motile by way of polar flagella and it can propel itself through the mucous layer on top of the epithelial cells. Adhesion to the surface of the epithelial cells followed by invasion into the cells is also thought to occur before the onset of diarrhoea. *Campylobacter* does produce a cytolethal distending toxin, however the role of this toxin in disease is not fully characterized (Ketley 1997).

*C. jejuni* and *C. coli* are Gram negative, spiral, rod shaped cells (**Figure 1**). They are microaerophilic, requiring oxygen levels between 2 and 10% for growth. These two species are classed as thermophilic campylobacters as they have an optimum temperature for growth of 42 °C. *Campylobacter* can often be a difficult organism to isolate and grow in the laboratory. The current method used for detection of campylobacters from foodstuffs is the EN/ISO 10272-1 method. This method relies on homogenization of a known amount of material in Bolton Broth (BB) with selective supplement and 5% lysed horse blood either microaerobically or in a Schott bottle filled with Bolton enrichment broth (leaving a gap at the top of no more that 2 cm) aerobically at 41.5 °C for 48 hours. However in a recent publication (Moran et al. 2009) a change to the ISO method was recommended, as it was found that aerobic incubation during enrichment was not very successful in isolation of the organism. Therefore, microaerobic enrichment was suggested as the preferred method, with incubation for 24 hours being sufficient. Following incubation, a sample of the enrichment broth is then plated onto modified charcoal cefoperazone deoxycholate agar (mCCDA, see **Figure 2**) with the addition of a selective supplement. Identification is usually done by colony morphology and speciation by PCR-based methods.



### Did you know…

**what *Campylobacter* means?**

*Campylobacter* means "twisted bacteria" because these bacteria are spiral-shaped. (see **Figure 1**)

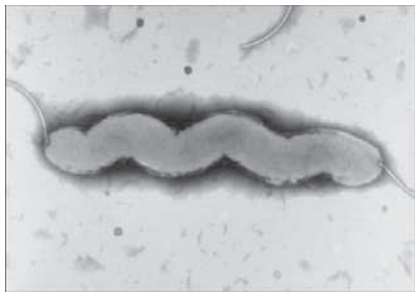


**Figure 1:** Electron micrograph of *Campylobacter jejuni* (Gaynor et al. 2004)

**Figure2:** *Campylobacter* colonies on mCCDA

### References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# More to *Campylobacter* Species…

**By Jvo Siegrist, Product Manager Microbiology….** *ivo.siegrist@sial.com*

## *… some helpful information for detection and differentiation of Campylobacter.*

### Media

*Campylobacter* are generally very fastidious microorganisms and grow only on complex media that has been amended with diverse essential amino acids and supplements, such as pyruvate, α-ketoglutarate, hemin, formate and other essential ions. For selective isolation of *Campylobacter*, the growth media can be supplemented with antibiotics such as cefoperazone, vancomycin, trimethoprim, amphotericin, cycloheximide, rifampicin, cefsulodin and polymyxin B sulfate. Typical agars and broths used for the detection, identification, differentiation, enumeration and cultivation of *Campylobacter* are listed in **Table 1**.

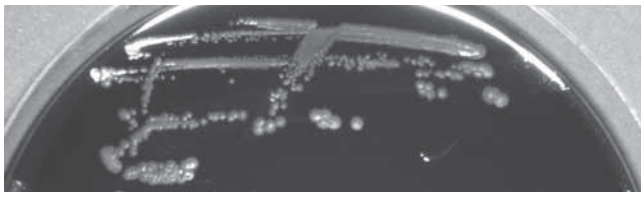

**Figure 1:** *Campylobacter* jejunii colonies on CCDA agar

### Biochemical Differentiation

The metabolism of *Campylobacter* is chemoorganotrophic, with amino acids and intermediates of the citric acid cycle serving as energy sources; typical carbohydrates cannot be used. *Campylobacter* reduce nitrate to nitrite, obtaining oxygen for their metabolism by this pathway. These distinctive metabolic reactions can be used for the differentiation and identification of *Campylobacter* species (see **Table 2**).

| Nonselective Media | Brand | Cat. No. |
|---|---|---|
| Columbia Agar | Fluka | 27688 |
| Tryptic Soy Agar | Fluka | 22091 |
| Tryptic Soy Agar (ready media in flasks) | Fluka | 79872 |
| Tryptic Soy Agar Plates (Diameter 55 mm) | Fluka | 57994 |
| Tryptic Soy Agar, Vegitone | Fluka | 14432 |
| Tryptic Soy Broth | Fluka | 22092 |
| Tryptic Soy Broth (ready media in flasks) | Fluka | 43592 |
| Tryptic Soy Broth No. 2 | Fluka | 51228 |
| Tryptic Soy Broth, Vegitone | Fluka | 41298 |
| **Nonselective Media for biochemical Differentiation** | **Brand** | **Cat. No.** |
| Blood Agar Base No. 2 | Fluka | B1676 |
| Hippurate Broth | Fluka | 53275 |
| OF Test Nutrient Agar | Fluka | 75315 |
| **Selective Media with Differential System** | **Brand** | **Cat. No.** |
| Mac Conkey Agar No 1 | Fluka | 70143 |
| ***Campylobacter* Selective Media** | **Brand** | **Cat. No.** |
| Blood Free Campylobacter Broth | Fluka | 59751 |
| Blood Free Campylobacter Selectivity Agar (mCCDA) | Fluka | B2426 |
| Bolton Broth Base | Fluka | 67454 |
| Brucella Broth Base | Fluka | B3051 |
| Campylobacter Selective Agar (Base) | Fluka | 21378 |
| Karmali Campylobacter Agar (Base) | Fluka | 17152 |

**Table 1:** Typical *Campylobacter* media

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| Species | | *C. jejuni* | | *C. coli* | *C. lari* | *C. fetus* | |
|---|---|---|---|---|---|---|---|
| | | subspecies *jejuni* | subspecies *doylei* | | | subspecies *fetus* | subspecies *veneralis* |
| Growth at | 25°C | no | No | no | no | yes | Yes |
| | 37°C | yes | Yes | yes | yes | yes | yes |
| | 42°C | yes | No | yes | yes | partial | no |
| Oxidase | | yes | Yes | yes | yes | yes | yes |
| Catalase | | yes | Yes | yes | yes | yes | yes |
| Nitrate Reduction | | yes | No | yes | yes | yes | yes |
| Hippurate Hydrolysis | | yes | Yes | no | no | no | no |
| Indoxyl Acetate Hydrolysis | | yes | Yes | yes | no | no | no |
| $H_2S$ Production | | no | no | yes | yes | no | |
| **Antibiotic Sensitivity** | | | | | | | |
| Nalidixic acid | | S | S | S | R | R | R |
| Cephalotin | | R | S | R | R | S | S |
| Penicillin | | R | R | R | R | R | S |
| TTC* | | R | R | R | S | S | S |

**Table 2:** Table of differentiating characteristics of *Campylobacter* species and subspecies

(* triphenyltetrazolium chloride)

| *Campylobacter* Test | Brand | Cat. No. |
|---|---|---|
| Catalase Test | Fluka | 88597 |
| Gram staining kit | Fluka | 77730 |
| Hippurate Disks | Fluka | 40405 |
| Hydrogen Sulfide Test Strips | Fluka | 06728 |
| Indoxyl Strips | Fluka | 04739 |
| Nitrate Reagent A | Fluka | 38497 |
| Nitrate Reagent B | Fluka | 39441 |
| Nitrate Reagent Disks | Fluka | 08086 |
| Oxidase Test | Fluka | 70439 |
| Oxidase Strips | Fluka | 40560 |
| Oxidase Reagent acc. Gaby-Hadley A | Fluka | 07345 |
| Oxidase Reagent acc. Gaby-Hadley B | Fluka | 07817 |
| Oxidase Reagent acc. Gordon-McLeod | Fluka | 18502 |

**Table 3:** Sigma-Aldrich tests for identification and differentiation of *Campylobacter*

## Detection of *Campylobacter* by rRNA

A further rapid and simple option to screen *Campylobacter* is the detection of specific rRNA. With oligonucleotide probes, specific sequences on the rRNA are detected and made visible with a sandwich hybridisation followed by a chromogenic reaction. It is a very easy method and does not need any PCR, is not sensitive to sample matrix and detects only living cells, as rRNA is destroyed within a few hours. No special expensive equipment is needed and the test is done within approximately 2 to 2.5 hours. The test format is based on a 96-well microplate with 12 strips of 8 wells so that small numbers of samples can be screened. A positive result is visible to the naked eye, however it is also possible by a standard microplate reader to quantify the number of cells at 450 nm. With a pre-enrichment step (44 - 48 hours in Bolton broth), a sensitivity of 1 CFU/25 g can be reached. The HybriScan®***D*** *Campylobacter* Kit specifically detects the most relevant *Campylobacter spp. (C. jejuni, C. coli, C. lari, C. upsaliensis)*. HybriScan***D*** *Campylobacter* was compared and validated (acc. to EN ISO 16140:2003) with the cultivation based method according to § 64 LFGB (official method in Germany). Five different food categories were tested. The results of the validation lead to a relative accuracy of 95.2%, a relative specificity of 97.5%, and relative sensitivity of 93%, respectively.

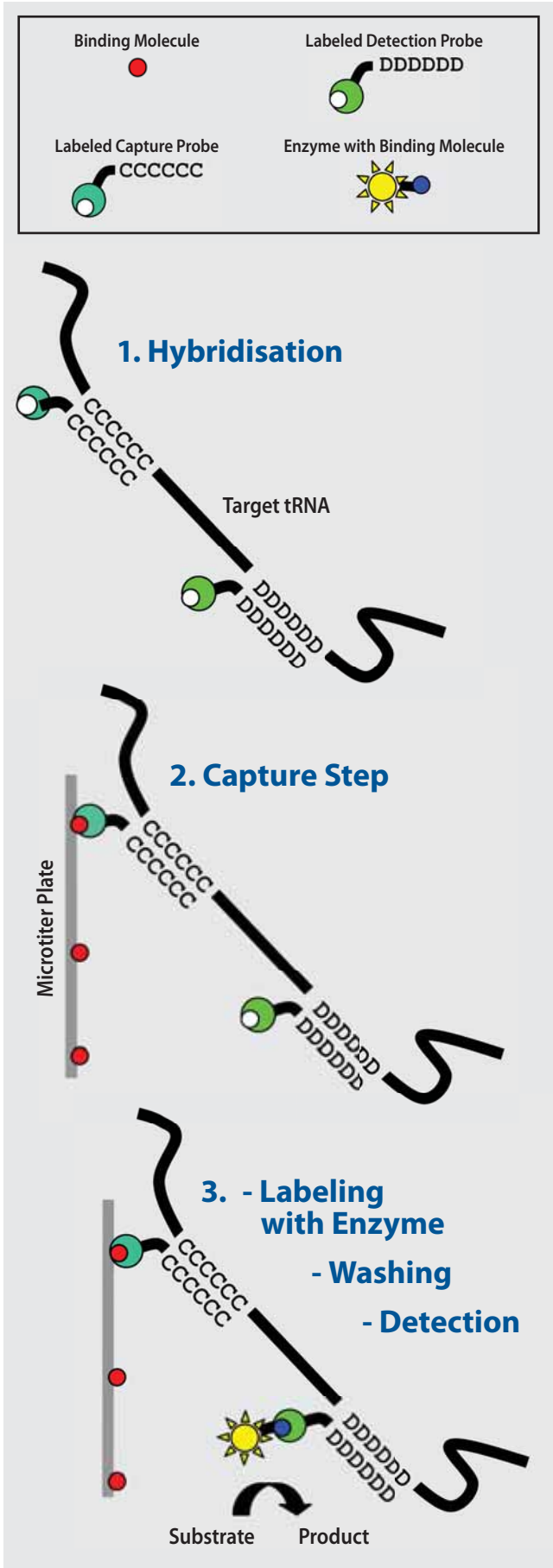


**Figure 2:** Scheme of the test procedure

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