COST STSM Scientific Report- Nicoletta Contaldo
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STSM grant holder: Contaldo Nicoletta, DiSTA, Plant Pathology, University of Bologna, Italy

STSM host: Windsor David, Mycoplasma Exerience Laboratory, London, UK

STSM Topic: Microbiological techniques for axenic microbial growth

Background

Phytoplasmas are prokaryotes with phloematic habitat, associated with diseases in several hundred plant species throughout the world. They are cell-wall-less bacteria and represent a distinct clade within the class Mollicutes, a branch of the Gram-positive eubacteria that lack cell walls. Other mollicutes include mycoplasmas, acholeplasmas and spiroplasmas, and they are most closely related to bacteria such as *Bacillus*, *Clostridium* and *Streptococcus*. Phytoplasmas have small genomes ranging from 530 to 1,350 bp and low G+C content in their DNA and they were recently assigned to a novel taxon, '*Candidatus* Phytoplasma'. Thus far, none of them has been cultured in vitro.

Purpose of the visit

The main purpose of my visit was to attend a dedicated training course on Mycoplasma isolation and handling techniques. Conventional techniques for handling mycoplasmas are in fact relevant to the development and application

of techniques for possible handling of phytoplasmas which are, with haemoplasmas, are the two mollicute groups that cannot yet be cultured.

Description of the work carried out during the visit

The mycoplasma cultivation training course started with preparation of Mycoplasma Experience solid and liquid media from stored components.

All the different steps were discussed and studied: storage before use; thawing and preparation of complete media; suitable containers for different volumes; storage. Then, practical liquid and solid inoculation of different Mycoplasma species such as *Acholeplasma laidlawii*; *Mycoplasma arginini: Mycoplasma bovis*; *Mycoplasma felis*; *Ureaplasma urealyticum* were carried out, taking care of use of gas generating systems (kits or gas cylinder) - and the temperature range required.

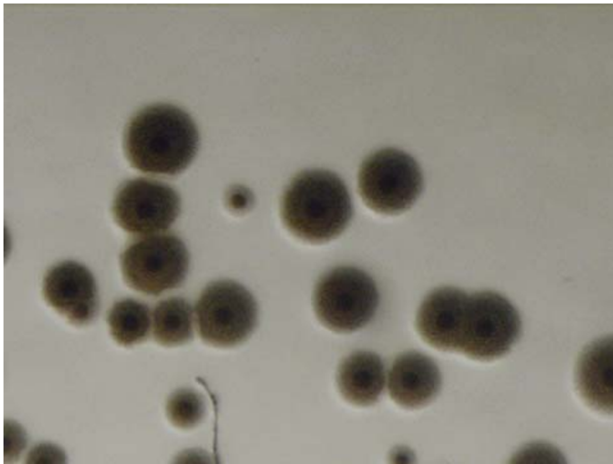
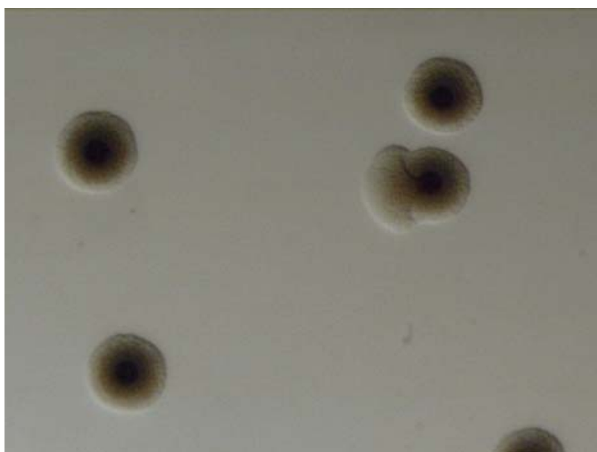
Liquid and solid subcultures from both solid and liquid media containing the same species and micro cloning of particular colonies from solid to solid were also handled. Different species have various rates of growth and colony forms, so practical inspection of 15 different species from different genera was done under microscope, with magnification x25 or x50. Using a digital camera in the microscope we were also able to photograph all the plates, in order to better understand the differences among the mycoplasma genera (see table and pictures below). Thus, colony appearance was quite different: classical 'fried egg' for *A. laidlawii*, dark for *M. felis* and *Mycoplasma hyorhinis*; atypical for A39 and *Mycoplasma hyopneumoniae*; motile for *Spiroplasma citri* and *Mycoplasma mobile*. Rates of growth was also very variable: 1-2 days (*U. urealyticum*); 2-4 days (*A. laidlawii*, *M. arginini*, *Entomoplasma entomophilum*, *Mesoplasma sominilux*, *Mycoplasma hyorhinis*, *M. bovis*); 6-10 days (*Mycoplasma orale*, *Mycoplasma pneumoniae*, *M. hyopneumoniae*, *M. mobile*); 12-14 days (*Mycoplasma amphoriforme*, *S. citri* and MELSA2). The mycoplasma genera growing with a longer incubation time were already prepared as subcultures and were ready for microscopical observation.

These examples were very useful for recognition of possible colonial growth in different plates and broth. Various media are designed for specific mycoplasma species so it is desirable to use different media as the optimal medium for uncultured organisms is unknown.

Different mycoplasmas are isolated from different animal species, so the temperature of growth and their general habitat and specific location must be taken into consideration before attempting *in vitro* cultivation. The course, held in one of the leading laboratories worldwide for routine cultivation of mycoplasmas, was then focused on acquisition of microbiological techniques already applied to cultivate human and animal mycoplasmas, to a greater understanding of possible future joint researches aimed to verify possibility of phytoplasma growth *in vitro* (Bertaccini *et al*., 2010).

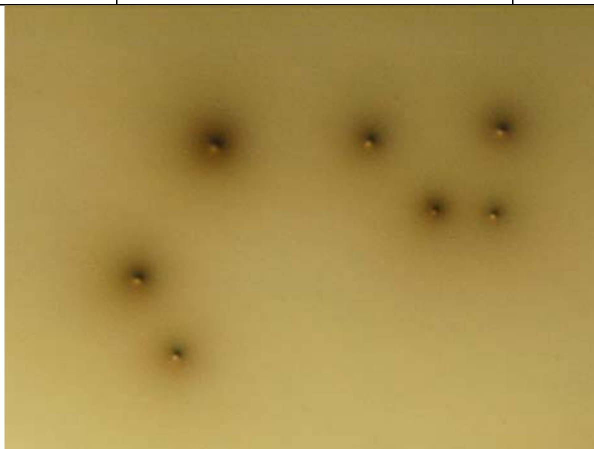
Table.

Genus	**Colony appearance**	**Rate of growth**
M. pneumoniae	Classical ‘fried egg’	6-10 days
M. felis	dark	2-4 days
M. hyorhinis	dark	2-4 days
M. hyopneumoniae	atypical	6-10 days
S. citri	motile	12-14 days
M. mobile	motile	6-10 days

M. pneumoniae	Classical ‘fried egg’	6-10 days
		
M. hyorhinis	dark	2-4 days
		
M. felis	dark	2-4 days

M. hyopneumoniae	atypical	6-10 days

S. citri	motile	12-14 days



M. mobile	motile	6-10 days