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July 9 - 12, 2018

Program and Abstracts



**22nd Congress of the
International Organization for Mycoplasmaology - IOM**

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The International Organization for Mycoplasmaology
Dedicated to the study of the Mollicutes

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Background: Two moderately halophilic and psychrotolerant new *Mycoplasma* species were isolated from cephalopods. Three isolates could be cultured from two European flying squids (*Todarodes sagittatus*) and two isolates from two common octopuses (*Octopus vulgaris*). **Methods:** Approximately two months of incubation were needed for the primary isolations. The strains were selected on the basis of their ability to pass through 0.45 µm diameter pore filters and their need of NaCl and a lower incubation temperature for growth. The optimal growth was achieved at 25°C in SP4-II medium containing 3% (w/v) NaCl. Analyses of their 16S-23S rDNA intergenic spacer regions, 16S rRNA genes, RAPD profiles and genomes and also by MALDI-TOF mass spectrometry were done. **Results:** It was concluded that the isolates represent a new branch within the hominis lineage, yet comprising two types (squid and octopus species), and both different from all previously described mollicutes. Colonies showed typical fried-egg morphology. The cells were non-motile and presented coccoidal forms and lack of cell wall. The isolates fermented glucose and mannose but did not hydrolyse arginine or urea. **Conclusion:** Genomic information and phenotypic properties demonstrated that these organisms represent novel species of the genus *Mycoplasma*, for which the specific names *Mycoplasma marinum* sp. nov. and *Mycoplasma todarodis* sp. nov. are proposed; the type strain are PE^T (DSM 105487^T, NCTC 13951^T) and 5H^T (DSM 105488^T, NCTC 13942^T), respectively.

Keywords: polyphasic taxonomy, psychrotolerant, moderately halophilic, *Mycoplasma* sp. nov., cephalopods

40 'CANDIDATUS PHYTOPLASMA ASTERIS' ISOLATED FROM GRAPEVINE: PRELIMINARY METABOLIC FEATURES

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Background. Phytoplasmas are phloem-limited bacteria that affect plant species worldwide, transmitted by plant sap-feeding insects and with restricted ability to grow in artificial media. Their identification and classification over time has been based on increasingly complex and accurate molecular analysis techniques on the 16S ribosomal gene, confirmed using various other genes conserved at the level of the '*Candidatus*' genus. In recent years, however, techniques have been developed that allow, thanks to the support of complex artificial media, the isolation and growth of phytoplasmas from infected plant material, both maintained in micropropagation (*Catharanthus roseus*, periwinkle) and sampled in the fields. **Methods.** Several liquid and solid media have been tested for the survival and growth of '*Candidatus* Phytoplasma asteris'- related strains isolated from infected symptomatic grapevine plants. Different combinations of antibiotics, carbon sources and NaCl concentration were evaluated by visual observation of the degree of turbidity of the liquid media, due to the presence of contaminating micro-organisms and / or symbionts present in the isolation tissue, and by the ability to form phytoplasma-like colonies in agar. To confirm the results obtained from visual observation and optical microscopy, selected colonies with characteristic morphology were subjected to nucleic acid extraction using commercial kits (DNeasy Plant Mini Kit, Quiagen, USA) and to "nested" PCR molecular analysis followed by RFLP and / or sequencing, which confirmed the phytoplasma presence and identity. Biochemical tests were also carried out to check the ability of the cultured microorganisms to ferment glucose and to hydrolyze urea and arginine. **Results.** The comparative performance evaluation in the tested liquid and solid media allowed to identify some biological and nutritional properties of these '*Ca. P. asteris*' strains. They were not surviving with sucrose as source of carbon and were very well differentiable from the *Acholeplasma laidlawii* strain used as control for the arginine hydrolysis ability. **Conclusion.** The '*Ca. P. asteris*' isolates biological characterization is relevant for field disease management and containment measures of their grapevine epidemics in South Africa in the frame of the H2020 EU founded project TROPICSAFE. Furthermore, these results will allow to improve and support ('*Candidatus*') Phytoplasma' species description within the class Mollicutes.

Keywords: Phytoplasma, cultivation