Australasian Plant Dis. Notes (2013) 8:9–12
DOI 10.1007/s13314-012-0084-4

Neurospora contamination of cultures in Lao PDR — a sticky rice issue

P. Vongphachan · C. F. Villalta · P. Songvilay · K. Chittarhath · S. Sayapatha · J. L. Walsh · L. W. Burgess

Received: 4 April 2012 / Accepted: 12 November 2012 / Published online: 6 December 2012

Abstract A major problem with contamination of fungal and bacterial cultures by *Neurospora* in the diagnostic laboratory of the Plant Pathology Unit at the Plant Protection Centre in Vientiane, Laos, occurred in May 2009 and again in April-May 2010. The problem persisted despite sterilization of all contaminated cultures and stringent disinfection procedures. Initial attempts to identify the source were unsuccessful. However in May 2010 the senior author realised that the orange fungus common on old sticky rice in traditional baskets was *Neurospora*. A limited survey indicated that this was a key source of conidia in the precinct around the laboratory, and elsewhere in Vientiane. A sample was collected and forwarded to the second author at the Plant and Microbial Biology Department in the University of California, Berkeley, California, USA, for confirmation of the genus, and identification of the species. It was identified as *N. intermedia* (Tai Mycologia 27:289–294 1935), and the isolate used in the phylogenetic study was deposited in the Fungal Genetics Stock Centre as FGSC10868. This is the first report of a *Neurospora* species in Lao.

P. Vongphachan · P. Songvilay · K. Chittarhath · S. Sayapatha
Plant Protection Centre, Nahai Village, Hatxayphong District, Vientiane, Lao People's Democratic Republic

C. F. Villalta
Department of Microbiology and Immunology, University of California, San Francisco, 513 Parnassus S472, San Francisco, CA 94143, USA

J. L. Walsh · L. W. Burgess (✉)
Faculty of Agriculture, Food and Natural Resources, The University of Sydney, Sydney, NSW, Australia
e-mail: burgess.international@gmail.com

Keywords *Neurospora intermedia* · Sticky rice baskets · Lao PDR

Contamination of agar plates and other sterile media is a common problem in crop disease diagnostic laboratories in tropical countries. The problem is particularly acute in laboratories adjacent to cropping areas such as rice, the straw from which harbours a diversity of saprophytic fungi. Mould can also develop on laboratory walls affected by damp in the wet season. Indeed in such laboratories glass Petri plates are often used in preference to plastic Petri plates to minimise contamination from fungi and bacteria (Dau and Burgess, unpublished data). In May 2009 a major problem with widespread contamination of agar plates by *Neurospora* sp. was encountered in the relatively new diagnostic laboratory of the Plant Pathology Unit at the Plant Protection Centre (PPC) of the Lao Department of Agriculture, located in a semi-rural area on the outskirts of Vientiane. The high growth rate and bright orange colour of the conidia formed on the mature colony were indicative of this genus (Fig. 1). Moreover the fungus colonized many plates overnight and had grown out of the plate onto the surrounding surface. The fungus was not examined microscopically so as to minimise further contamination. The outbreak of contamination occurred during a disease survey and training program involving several hundred plastic Petri plates. Daily room temperatures were in the range 30–38 °C as it was the dry season and there was no air–conditioning. All contaminated plates were immediately wrapped in newspaper on discovery and autoclaved for 1 h. The laboratory was thoroughly cleaned before floors and tiled benches were disinfected with sodium hypochlorite. Other laboratory

Fig. 1 Section of agar Petri plate contaminated by *Neurospora* sp., with lid removed to show mycelium growing over edge of plate, and orange colour, the site of conidiation

benches were swabbed with 70 % ethyl alcohol (ETOH). Ceiling fans were also cleaned and swabbed with ETOH. Filters of the sterile work cabinets were thoroughly cleaned, and the UV sterilizing light left on for extended periods. All laboratory clothing was thoroughly washed and dried in the sun for several days. However, contamination by *Neurospora* sp. remained an intermittent problem over the next year, a period when fewer cultures were being grown on Petri plates.

A second training program was held over 5 weeks in April–May 2010 and involved the use of over 300 glass plates. Contamination by *Neurospora* sp. again occurred causing disruption to the training and survey activities. However, the problem was minimised using the procedures outlined above. We assumed that there was a significant external source of the *Neurospora* sp. and initiated another search for the source. The senior author then recalled commonly seeing an orange fungus growing on sticky rice residues in sticky rice baskets of various sizes that had been put aside with inadequate cleaning or forgotten. We readily found examples of contamination of sticky rice residue by *Neurospora* sp. in the PPC precinct and in the community (Fig. 2:a, b). A scrape of *Neurospora* sp. mycelium and conidia was made from a sticky rice basket at the PPC and forwarded to the second author at the Plant and Microbial Biology Department in the University of California, Berkeley, California, USA, for confirmation of the genus, and identification to species.

Neurospora contamination was not encountered in March 2012 during another major survey, involving the use of glass plates, and during which all appropriate precautions were put in place.

The *Neurospora* sp. samples from Laos were inoculated onto agar plates of Vogel's minimal medium (Vogel 1956). Agar slices from the outer portions of the suspected *Neurospora* colonies were collected, to prevent contamination from other fungi present in the field samples, and inoculated into slants of Vogel's minimal medium. They were grown until there was profuse conidiation. The cultures produced bright orange conidia expected from *Neurospora*. Instead of using tester strains and taking a biological species concept approach, we decided to use a phylogenetic species approach, which we expected to give a higher resolution of species identification (Dettman et al. 2003a; Dettman et al. 2003b; Villalta et al. 2009). Conidia were collected with a loop and serially diluted across water agar plates and left to germinate overnight. Single conidial isolates, assumed to be monokaryotic, were collected the following morning and inoculated into fresh slants of Vogel medium. Single conidial isolates were then used to inoculate tubes containing 5 mL of liquid Vogel's medium and grown at 30C for 2 days. Mycelium was collected from one culture, named P2, and polymerase chain reaction was performed on genomic DNA extracted using previously described methods and the

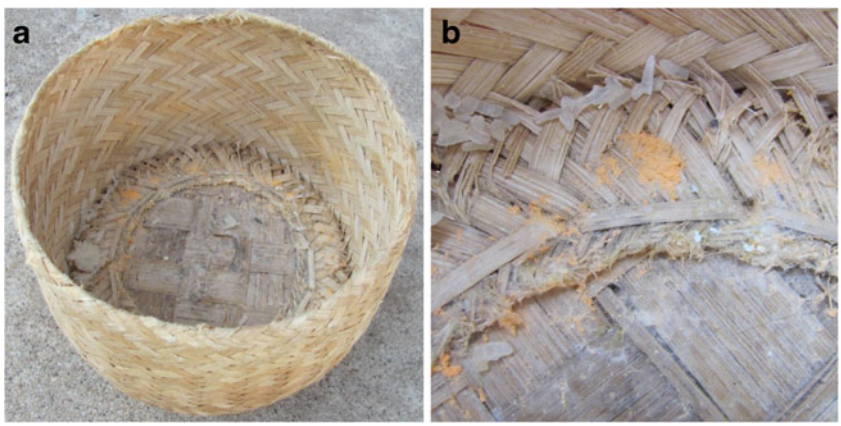

Fig. 2 Sticky rice basket contaminated by *Neurospora intermedia*, the source of culture P2 used in phylogenetic study. **a** Overview of basket. **b** Growth and conidiation

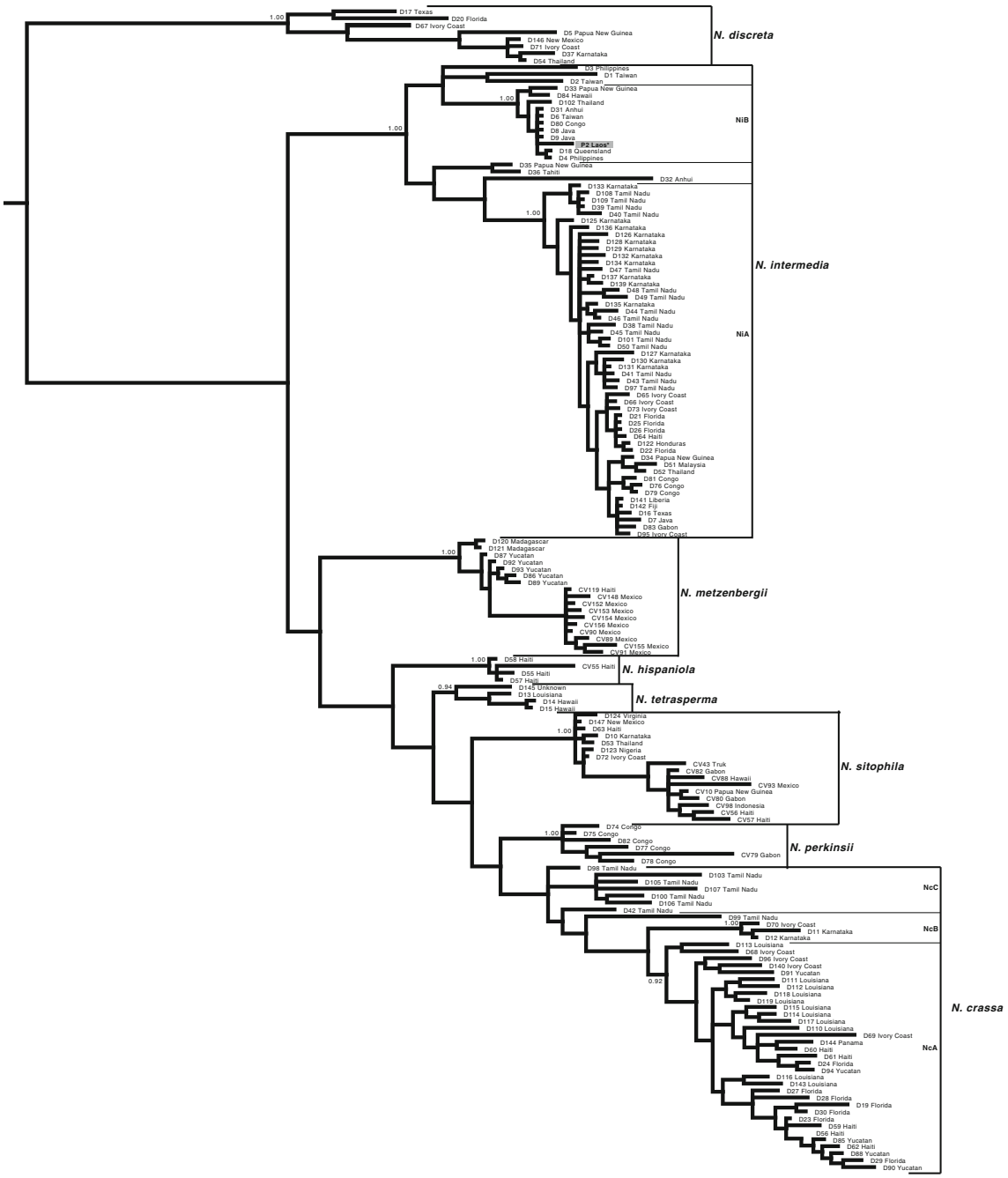

Fig. 3 The phylogenetic relationships of 157 outbreeding species of *Neurospora* characterized in Dettman et al. (2003a) and Villalta et al. (2009) with the inclusion of Lao P2 (*), an *N. intermedia* subclade B isolate collected from the Lao PDR. Bayesian posterior probabilities indicating confidence levels are displayed above major branches

four phylogenetically informative loci (DMG, QMA, TML, TMI) from Dettman et al. 2003a. We aligned each of our four informative loci with sequences from previous studies (Dettman et al. 2003b; Villalta et al. 2009) using the same methods into one consensus alignment of the four loci (The DMG sequence was truncated at 249 base pairs as a result of sequencing issues). We built a Bayesian inference tree using MrBayes version 3.2 and visually inspected the phylogenetic tree and found that species level clades were still well supported with Bayesian posterior probabilities and in agreement with Dettman et al. 2003b and Villalta

Fig. 4 Sticky rice baskets in Vientiane City, Lao PDR. **a** A variety of sticky rice baskets in a shop display. **b** Typical small basket with sticky rice as served in cafes

et al. 2009 (Fig. 3). Upon visual inspection it appeared that the P2 isolate belongs to the *N. intermedia* species and more specifically is a member of the *N. intermedia* B subclade made up of specimens from Southeast Asia and the Pacific islands. Isolate P2 was deposited in the Fungal Genetics Stock Centre as FGSC10868.

Sticky rice is a staple food in Lao. It is not only consumed in homes and cafes but is also carried to work and elsewhere as a regular snack (Fig. 4:a, b). Some containers are carefully woven baskets and re-cycled, while others are small roughly woven baskets that are often discarded after use. Consequently there is potential for a very large source of conidia of *Neurospora spp.* in the atmosphere around Vientiane, and other major urban areas of the Lao PDR. *Neurospora* regularly appears as the first colonizer of semi-burnt plant material after fires, (Perkins and Turner 1988; Turner et al. 2001). Consequently we were not surprised to later encounter abundant growth of *Neurospora* on burnt sugarcane residue in cane fields in Lao PDR which could be a potential source of inoculum to initiate the colonies growing on left over sticky rice, and another origin of the spores contaminating media in the diagnostic laboratories. The reader is referred to Perkins (1991) for further details on the history of this genus, and its original discovery on bread in 1843.

The potential for serious contamination by *Neurospora* spp. of culture media needs to be taken into consideration in designing future laboratories for crop disease diagnostic work, and other activities involving the use of culture media, in Lao PDR and in similar tropical regions. Furthermore the authors strongly recommend that glass Petri plates be used in such laboratories in the Lao PDR and other tropical regions to minimise contamination by *Neurospora*, and other airborne fungal contaminants (Burgess et al. 2008).

Acknowledgements The authors gratefully acknowledge the financial support from the Crawford Fund for the training programs in 'Crop Disease Diagnostics and Integrated Disease Management', and the provision of facilities and in-kind support by the Lao Plant Protection Centre, and in-kind support by the Royal Botanic Gardens and The University of Sydney, Sydney.

References

Burgess LW, Knight T, Tesoriero L, Phan HT (2008) 'Diagnostic manual for plant diseases in Vietnam.' ACIAR Monograph 129. Australian Centre for International Agricultural Research, Canberra

Dettman JR, Jacobson DJ, Taylor JW (2003a) A multilocus genealogical approach to phylogenetic species recognition in the model eukaryote *Neurospora*. Evolution 57:2703–2720

Dettman JR, Jacobson DJ, Taylor JW (2003b) Reproductive isolation and phylogenetic divergence in *Neurospora*: comparing methods of species recognition in a model eukaryote. Evolution 57:2721–2741

Perkins DD (1991) The first published scientific study of *Neurospora*, including a description of photoinduction of carotenoids. Fungal Genet Newsl 38:64–65

Perkins DD, Turner B (1988) *Neurospora* from natural populations: towards the population biology of a Haploid Eukaryote. Exp Mycol 12:91–131

Tai FL (1935) Two new species of *Neurospora*. Mycologia 27:289–294

Turner BC, Perkins DD, Fairfield A (2001) Neurospora from natural populations: a global study. Fungal Genet Biol 32:67–92

Villalta CF, Jacobson DJ, Taylor JW (2009) Three new phylogenetic and biological *Neurospora* species: *N. hispaniola, N. metzenbergii* and *N. perkinsii*. Mycologia 101:777–789

Vogel H (1956) A convenient growth medium for *Neurospora* (Medium N). Microb Genet Bull 13:42–43