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JSM Environmental Science & Ecology

Editorial

Modeling Toxisome Protrusions in Filamentous Fungi

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Submitted: 11 January, 2014

Accepted: 13 January, 2014

Published: 15 January, 2014

ISSN: 2333-7141

Fungal export of secondary metabolites is an area of great importance to environmental sustainability, agriculture and human health. While the medicinal properties of many fungal secondary metabolites like penicillin and statins have benefitted human lives for decades, mycotoxins, such as aflatoxins and fumonisins, are detrimental to human and animal health. They contaminate our crops and result in billion dollar losses to agriculture every year [1]. Despite significant advances in research that have enhanced our understanding on the orchestration of fungal secondary metabolism, little is known about the underlying regulatory mechanisms of secondary metabolite export.

A model similar to exocytosis has recently been proposed for aflatoxin export in *Aspergillus parasiticus* [2], in which toxin release by the fungus occurs through a 3-step process (Figure 1a). The study suggests that the development of toxisomes (specialized vesicles that house secondary metabolite synthesis in fungi [3]) and metabolite export are co-dependent. Further, 90% of aflatoxin synthesized by the fungus is exported out from the cells [2,3], which implicates that secondary metabolite export rates are high. Hence constructing a strain, that has an altered export rate but with a background of uninterrupted secondary metabolite synthesis and toxisome development is extremely challenging. A feasible solution to this problem is to perturb the fungal cells temporarily and minimally by the use of either gene silencing techniques reported recently in Aspergilli [4,5], or by targeted low dose chemical perturbations of the export machinery. The difficult requirement for such an approach is achieving a data output that can efficiently determine and quantify the phenotypes for such perturbations. Genetically engineered Aspergillus strains that accumulate colored intermediates because of their impaired aflatoxin synthesis pathway provide an excellent opportunity to establish such outputs for studying fungal export. The *A. parasiticus* mutant B62 accumulates norsolorinic acid (NA) due to a mutation in the aflatoxin biosynthesis gene *nor-1* [6], and is an excellent example to illustrate this. This mutant has been used consistently in the past for understanding the effects of chemicals that inhibit secondary metabolism [7,8].

Our laboratory has recently developed a methodology using B62 to study aflatoxin export protrusions in real time (manuscript in preparation). The methodology involves live cell fluorescent light microscopy to monitor the occurrence of toxisome protrusions from the cell surface. As shown in Figure 1 b, fluorescent images overlaid on DIC images of B62 hyphae, collected from colonies grown in a secondary metabolite synthesis inducing media (yeast extract sucrose) have an increased number of toxisomes as NA synthesis reaches peak levels. The

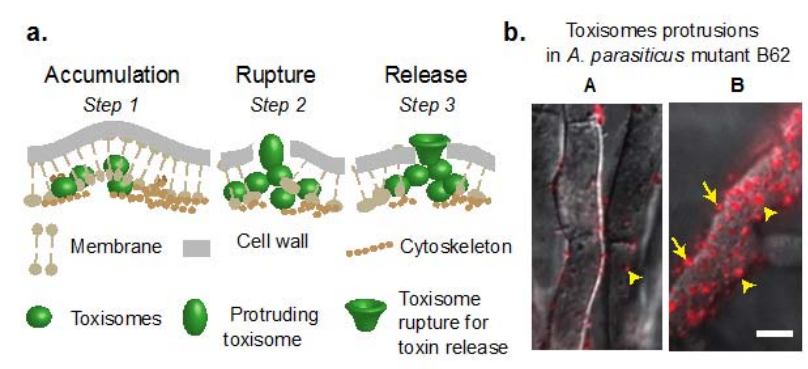

Figure 1 Toxisome mediated secondary metabolite export in Aspergillus parasiticus. Panel a) Theoretical (burst-and-blast) model for toxisome mediated aflatoxin export. Aflatoxins after synthesis in the toxisomes accumulate near the cell surface (step 1). During release, the toxisomes protrude out through the cell wall (step 2) and release the secondary metabolites into their environment (step 3) **Panel b)** Toxisome mediated export of Norsolorinic acid (yellow arrowheads) in A. parasiticus mutant, B62, during early (A) and late (B) stationary phases. Bar, 5mm.

presence of NA within the toxisomes allows for their observation as discrete spots on the images (Figure 1 b). However, in order to implement such a methodology for studying the effects of minimal perturbations on secondary metabolite export, or for conducting dose response experiments with novel molecules that modulate the export machinery, we envision that a predictive mathematical model for the rate of toxisome protrusions will be very useful.

The number of toxisome protrusions per unit hyphal length observed over time can be viewed as a stochastic process. Our proposed approach uses the Poisson random process [9], a popular modeling tool for counting processes, to model toxisome mediated export of secondary metabolites. The proposed model is as follows: suppose $\{X_t\}$ is a stochastic process that represents the number of toxisome protrusions per unit length in a hypha observed at time t, we assume that $\{X_t\}$ is a Poisson process with mean function $\mu(t)$. That is, the probability that there will be k protrusions per unit in a hypha at a particular time point t is given by:

$$P(X_t = k) = \frac{e^{-\mu(t)}[\mu(t)]^k}{k!}; \quad k = 0,1,2,3,\ldots$$

In a typical batch culture, starting from only a few protrusions upon the onset of secondary metabolism, the number of protrusions increases with time until the late stationary phase and then decreases again with progress of the death phase. A bell shaped Gaussian kernel for the intensity function of the mean measure can efficiently capture this trend. Hence, we propose the mean function $\mu(t)$, which is the expected number of protrusions per unit length in a hypha at time t on an average, can be modeled by a Gaussian function [10] as follows:

$$\mu(t) = K\int_0^t e^{-\frac{1}{2}\left(\frac{x-m}{\sigma}\right)^2} dx$$

The parameters of the mean measure K, m and σ should depend on the factors that control the mycotoxin export process. Generally speaking, m represents the time point when the number of protrusions is at peak, σ controls the spread of the time period during which the number of protrusions increases and then decreases and K is a scale factor. The parameters can be estimated from the observed protrusion data using regular maximum likelihood estimation methods [6]. The model can be used to predict the number of toxisomes protruding from the surface at a given time t. The experimental data for the number of protrusions reported at each time point within each field of view can be used as observed data to estimate the parameters of the mathematical model.

We believe that the Poisson model will provide an objective way of comparing toxisome protrusions, thereby allowing comparison of secondary metabolite export under different chemical and genetic manipulations. In such experiments, we can easily use the fitted model to test if the average numbers of protrusions under two conditions/treatments are statistically significantly different. Through these diverse cellular perturbations we can generate a large data pool for the mathematical modeling of the diversity and the temporal characteristics of the cellular parameters that regulate toxisome protrusions. Such studies have the potential to advance our mechanistic understanding of secondary metabolite export and help discover novel technologies to modulate the process.

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Cite this article

Kenne GJ, Chakraborty P, Chanda A (2014) Modeling Toxisome Protrusions in Filamentous Fungi. JSM Environ Sci Ecol 2(1): 1010.