

## Chitosan: The Preliminary Research and the Host/Parasite System that Led to the Discovery of its Antifungal and Gene Inducing Properties

Lee A Hadwiger\*

Department of Plant Pathology, Washington State University, Pullman, WA 99164, USA

\*Corresponding author: Hadwiger LA, Department of Plant Pathology, Washington State University, Pullman, WA 99164, USA, Tel: 1-509-335-3751; E-mail: [chitosan@wsu.edu](mailto:chitosan@wsu.edu)

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### Commentary

Being in the terminal phase of my scientific career I am grabbing the literary license to tell a story of the discovery of two biological properties of chitosan, namely, its gene activating potential [1] and its anti-microbial properties [2]. The geneticist, Gregor Mendel, selected peas as a genetic tool and I garnered a pea pod system from an Australian pathologist, I.A.M. Cruickshank, as a biological tool [3]. When the immature pea pod is split, the epidermal cell layer surface, the endocarp is temporarily exposed without a cuticle layer. Thus these naked, undamaged cells rapidly respond to foreign cell, macromolecules, UV radiation, fungal spores of plant pathogens, to initiate (or not initiate) the synthesis of an antifungal isoflavonoid called pisatin. The accumulation of this easily quantitated product is associated with many of the plant immune responses such as the activation of genes coding for enzymes in a secondary pathway and pea defense genes termed DRR genes and later were called pathogenesis-related or PR genes.

These latter gene products are crucial to the development of the plant's immune response [4]. Thus this system is a simple, excellent assay system for evaluating the plant's response to an unlimited array of microbes, chemicals, compounds, and physical effects such as ultra violet light. This encouraged Professor Cruickshank's laboratory to pursue research investigating the action of heavy metals, salts etc, that could at some level increase or block pisatin production. My laboratory joined into this endeavor but quickly became interested in how these and other plant defense genes were activated [5]. First was this response actually a result of increase in mRNA? The RNA synthesis inhibitor of choice at that time was, actinomycin D (AD). AD is composed of planer rings that intercalate into DNA in between the base pairs of the DNA ladder and its attached peptide components settled into the DNA grooves preventing polymerase complexes from moving through the open reading frame of a gene. In microbial systems this blockage efficiently suppressed the synthesis of RNA. Surprisingly, when AD was employed to block pisatin production in pea, it actually super-enhanced pisatin accumulation, as well as the activity of enzymes in the pisatin pathway [6]. The long-story-short explanation was obtained by the use of labeled AD, which in microbial systems blocked transcription but required an intercalation rate of 1 molecule per 1000 base pairs of DNA. The amount intercalating into pea nuclear DNA was one AD molecule per 10,000 base pairs [7]. This brought into play the moderate intercalating action of AD as a distorter of the DNA helix [8]. This resulted in a loosening of the entire chromatin structure enabling the read-through by polymerase II complexes of the defense genes that were typically silent. The subsequent pea pod assays encompassed hundreds of both regulatory chemicals and many biologically active components. Many of the components that had positive enhancements also had some potential

to interact with DNA or other components of chromatin. Pure isolated DNA has an abundance of negative charges. Therefore polymers with an abundance of positive charges have an affinity to DNA. The positive charged polymers found to be pisatin inducers included polylysine, poly-arginine, poly-ornithine, protamine, histone and spermine [9]. Alternately, polymers of many other amino acids were not inducers. At that time, 1978, a new book appeared entitled, "Chitin" authored by Muzzarelli [10] described a deacetylated chitin molecule called chitosan. Even though chitosan was already a commercial product for industrial uses and reported as a component of some fungal walls, I was only now seeing it as a polycationic polymer with strong similarities with basic amino acid polymers. It was no surprise to find chitosan as a strong inducer of pisatin and the other defense responses. Since our laboratory was also assaying for antifungal compounds it was rewarding to know that chitosan also had anti-microbial properties [2]. As a refinement of the size optimums for chitosan oligomers as inducers of biological activity it was determined that a polymer of 7 glucosamine sugars was optimal for both the antifungal and immunity inducing properties [11,12] and that such oligomers were clearly major players in plant host/ fungal parasite interactions. This basic research made possible some innovations in agriculture, the most important was a wheat seed treatment "YEA" that both increased wheat yield and reduced symptoms of a root rotting disease called "eye spot". Chitosan was also found to induce a defense response in potato against the Potato Late Blight disease. However this response resulted in less crop protection than some commercial fungicides. Another property of chitosan already mentioned is its ability to complex with negatively charged components. This property enabled its use as a "sticker" for copper sulfate pentahydrate on potatoes to reduce late blight symptoms [13]. This chitosan "sticking" component enabled the antifungal properties of the copper sulfate compound to reside on the potato leaf surface and remain in a position to combat the incoming spores of *Phytophthora infestans*, the causal agent of Potato Late Blight. Other agricultural chitosan applications [14] and interaction signaling have been reported [15].

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