

Black Biology-A Threat to Biosecurity and Biodefense

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The definition of Black Biology is the use of genetic engineering to enhance the virulence of a pathogen [1]. In the age of Do-It-Yourself (DIY) biology and synthetic biology [2-6], the reality of Black Biology has moved from science fiction to science reality. Furthermore, as the tools for genetic engineering become more common place and accessible, the reality of Black Biology becomes not merely reserved for nation states or rouge nations, but a real threat from terrorist groups and lone individuals (aka lone wolves).

One example of black biology was the work done by Sergei Popov, a department chief in the Soviet bioweapons program. Popov reported success in developing a strain of plague that was resistant to multiple antibiotics, and a strain of anthrax that was resistant to both the anthrax vaccine and multiple antibiotics [1,7,8].

The concerns regarding black biology focus on what this technology could do for pathogens, especially those that have been, or could be used for biological weapons (BW), or in acts of bioterrorism. The agenda to insert genes into pathogens could range from enhancing shelf-life of stored pathogens in ordinances [9]; to resistance of pathogens to antibiotics or vaccines; to capacity to evade innate immune mechanisms (e.g. resistance to fever or macrophage endocytosis); or to enhance the mortality rate of infected hosts (e.g. humans, farm animals, agricultural crops, etc.). For example, Daly [9] discusses how some extremophiles and their genes for specific traits could be used for genetically enhanced biological weapons. In one case, thermophiles (i.e. heat loving organisms) might provide traits to build a better heat resistant BW that would withstand explosive dispersal from a missile, or withstand the febrile state inside of human hosts. Furthermore, traits of barophiles (i.e. pressure loving organisms) would help design BW that could withstand the high pressures during the detonation of a BW warhead [9].

But, beyond these objectives, genetically engineered pathogens could be developed to create pathogens that evade detection by commercial or biodefense diagnostics (e.g. ELISA, qPCR); or creating pathogens with mixed symptoms to hinder diagnosis; or even create transgenic organisms with new exotoxins or endotoxins; or to infect new organ or tissue targets within the hosts.

Yet, the insertion of new genes, whether it is via transgenic plasmids or the cut and paste efforts into the pathogen's primary chromosome is not a guarantee for success. As Zilinskas [10] notes, many pleiotropic effects (some negative) may arise from the inserted genes, and/or gene products. Hence, the "engineered" pathogenic organism may exhibit reduced "fitness" in culturing, dispersal, or even in maintaining the epidemic after the initial outbreak. Yet, if used in a bioterrorism incident, the reduction in "fitness" might be a worthy tradeoff for a successful bioterrorist inspired outbreak, with lingering effects felt in society long after the organism dies out and becomes extinct.

Evidence for black biology of pathogenic agents are varied, but with the rapid expansion of genomic maps for many organisms (pathogenic and otherwise), as well as the explosion of bioinformatic tools to read, analyze and compare genetic maps; the capacity to detect actual black biology incidences will become easier. Some hints of genetic modification of pathogenic agents may include the presence of artificial

chromosomes from more complex organisms, but other simpler forms of "gene engineering" may demonstrate messy gene splicing into endogenous viral or prokaryotic genes (sans evidence of transposons). Other hints of black biology may involve unusual restriction splicing performed by rare restriction enzymes, not found within the genus or even kingdom of the pathogenic organisms, or incompatible gene combinations crossing phylogenetic boundaries (e.g. toll-like receptor mammalian genes found in an archean pathogen or plant toxins within a human virus genome).

Finally, as mentioned above, the explosion of genomic mapping of many organisms can also provide insight into a genetically engineered pathogen, by bioinformatically comparing the "new pathogen" strain's genomic database with genomic databases of known native pathogen strains.

Indeed, even without knowledge of the actors involved in the release of the genetically engineered organism, the evidence of black biology on a pathogen must be immediately reported to the Biological Toxins and Weapons Convention (BTWC), for further review and possible action. Also, international and national public health agencies and internet alert services (e.g. World Health Organization, Centers for Disease Control, Public Health Europe, ProMED [11], etc.) must be informed of the event and initiate discussion, as well as surveillance for similar events. The biosecurity of global public health depends on a well educated populace knowledgeable about the threats posed by the genetic engineering of biological warfare agents. It is hoped that this editorial helps to further the discussion on this topic.

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