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How Genetic Engineering May Have Created *E. Coli* Outbreak

Greatly assisted horizontal gene transfer and recombination turned previously harmless bacteria into dangerous pathogens

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Rapid decoding in the new scientific commons

The *E. coli* O104:H4 genome was rapidly decoded within days of the initial outbreak in Germany by Beijing Genomics Institute (BGI)'s third generation technologies, and the raw data promptly uploaded to a public database (ftp://ftp.genomics.org.cn/pub/Ecoli_TY-2482) so geneticists all over the world could analyse and annotate the sequences and share their findings quickly in a new scientific commons on the internet.

It was clear that the outbreak *E. coli* O104:H4 is a new strain with a genome size of about 5.2 Mbp (million basepairs), which unusually, has both the properties of enteroaggregative *E. coli* (EAEC) that cause diarrhoea and enterohaemolytic *E. coli* (EHEC) that cause haemolytic uremic syndrome (HUS, or bloody urine), along with resistance to the widest range of antibiotics [1].

The outbreak strain is most similar to EAEC 55989, previously isolated in the Central African Republic from an HIV-positive adult, and since emerged as a major cause of diarrhoea in children and adults worldwide [2]. EAEC carry small extra units of genetic material called plasmids; the German outbreak strain has the typical plasmid genes of EAEC bacteria, as well as the Shiga toxin genes of EHEC carried on prophage (genome of bacteria virus) integrated in the bacterial chromosome.

Rampant horizontal gene transfer

Preliminary analyses using an algorithm that searches for protein similarity to define genes based on known proteins in *E. coli* and other bacteria, detected 6327 genes in all, 6156 coding for proteins and 171 coding for ribosomal and tRNA.

Of the proteins identified, 33 genes are toxins, 3 suspected haemolysins (proteins causing haemolysis), a putative hemolysin expression modulating protein, and a channel protein of hemolysin III family. In addition, 31 predicted genes are related to specific antibiotic resistance: beta-lactamic, aminoglycoside, macrolide, polymyxin, tetracycline, fosfomycin and deoxycholate, novobiocin, chloramphenicol, bicyclomycin, norfloxacin and enoxacin and 6-mercaptopurine [3]. The strain is also rich in adhesion, secretion systems, pathogenicity and virulence related proteins. It seems to have a restriction-modification system, many proteins involved in Fe transport and utilization (siderophores as aerobactin and enterobactin), lysozyme, one inhibitor of pancreatic serine proteases, proteins involved in anaerobic respiration, antimicrobial peptides, proteins involved in quorum sensing and biofilm formation that could confer competitive advantage to the strain. There are genes for tellurium resistance and resistance to other metals including mercury, nickel, copper, zinc and cobalt, and more than 170 phage proteins.

The proteins are from all major classes of *E. coli*, pathogenic and otherwise, and at least 21 bacteria of other genera. Most of the proteins (2810) are from *E. coli* O26:H11 (strain 11368/EHEC), while the second largest contribution (1166) are from *E.coli* O44:H18 (strain 042/EAEC). Only 51 proteins are recognizably from *E. coli* K12, the laboratory strain originating from the original 'wild-type' isolate, a harmless strain. Other bacteria with major contributions include *Salmonella typhi* (54 proteins), *Yersinia pestis* (29 proteins), *Shigella dysenteriae* (16 proteins) *S. flexneri* (20 proteins), *S. boydii* (9 proteins) and *Bacillus cereus*.

Judging from the fact that only 51 of 6156 proteins in the outbreak strain are identified with *E. coli* K12, the degree of divergence from the harmless 'wild-type' is more than 99 percent, and much of that could be due to horizontal gene transfer.

Another geneticist who has carried out extensive analysis of the sequences remarks [4]: "In the German outbreak bacteria, as in most *E. coli*, plenty of horizontal transfer has gone on to create the genome we are now looking at.."

She confirms that the chromosome of the outbreak strain is most similar to strain EC55989, sharing with it part of the EAEC plasmid carrying aggregative adhesion operons *aat*, the regulator *aggR* and some other bits, but has a different aggregative adhesion fimbrial complement (*AAF/I*). The outbreak strain has also acquired the *stx2*

phage carrying Shiga-toxin 2 genes *stx2A*, *stx2B*; a plasmid very similar to the IncI plasmid pEC Bactec, including blaCTX-M and blaTEM-1 beta-lactamase (antibiotic resistance) genes, and a lot of sequence similar to plasmid pCVM29188_101 from *Salmonella enterica* Kentucky. In addition, a 300-500 kbp do not match any known sequence.

Based on the single nucleotide polymorphism (SNP) analysis of the three outbreak isolates genomes carried out by Konrad Oaszkiewicz at University of Exeter in the UK, and ignoring about 10 percent of the genome that is obviously involved in horizontal gene transfer such as phage (bacteria virus), transposase and IS (insertion sequences), she drew a phylogenetic network (a network of evolutionary relationships) [4]. The network clearly shows that the outbreak genomes are very similar to EC55989, and very different from other sequenced *E. coli*. In particular, the group of EHEC O157:H7 are very distant from the outbreak strain. The current outbreak strain also has an EAEC plasmid carrying aggregative adhesion fimbrial cluster 1.All in all, the non-horizontally gene transferred regions of the outbreak strain differs only by 0.12 percent or less from EC55989.

"Natural GMO" or artificially enhanced by genetic engineering

Geneticist David Tribe has referred to the outbreak strain as a "natural GMO" in his blog [2], on account of the numerous horizontal gene transfer and recombination events that have gone into creating it; more or less taking for granted that rampant horizontal gene transfer is the natural order of things ever since bacteria began to populate the earth. However, there are some who claim that such profuse horizontal gene transfer is impossible within the timespan involved, and that the current outbreak strain must have been genetically engineered in the laboratory as a bioweapon. I take a somewhat different view from either of the two.

I do not believe anyone has intentionally created the outbreak strain. However, as in the case of *E. coli* O157:H7, in which nearly 20 percent of the genome is thought to have been derived from horizontal gene transfer [5, 6], one should be asking whether genetic engineerng has contributed, *unintentionally*, to creating it [7] (E. coli 0157-H7 and Genetic Engineering, *ISIS News* 9/10), in the same way that genetic engineering has contributed to accelerating the emergence of new pathogens and spread of antibiotic and drug resistance (see [8] Gene Technology and Gene Ecology of Infectious Diseases, ISIS scientific publication).

Horizontal gene transfer and recombination is a major route to creating new pathogens and spreading drug and antibiotic resistance. There is nothing natural about artificial genetic engineering, which has greatly expanded the scope and accelerated the rate of horizontal gene transfer and recombination. Furthermore, *E. coli* is the primary bacterium used in genetic engineering. Many new genes and combinations of genes were created and amplified and propagated in *E. coli*, because the originalbacterium was harmless. In the process, genetic engineers have turned an original harmless bacterium into deadly pathogens. The problem is surely that even when you have killed the bacteria, the recombinant (genetic engineered)DNA survives, and can be transferred into living bacteria in the sewage, soil, and water to create new strains.

Genetically engineered nucleic acids that slipped through the regulatory net

No, the current outbreak strain has not been intentionally created in the laboratory and released into the environment as a bioweapon. Much more isaccomplished by the bacteria themselves than can be dreamt of by human genetic engineers, when the genetically engineered DNA/RNA is released into the environment as waste, or worst, incorporated as 'fertilizer' for crops; as legally authorized by our regulators ever since genetic engineering began, on the mistaken assumption that the killed bacteria and genetically engineered nucleic acids are 'safe'.I and my colleagueshave warned regulators against such releases time and again since 1994, but in vain (see for example [8-11] Genetic Engineering Dream or Nightmare, Naked and Free Nucleic Acids - Unregulated Hazards, and Living with the Fluid Genome, ISIS/TWN publications).

It is time for such releases to stop. No more live GMOs should be released into the environment; and the GM DNA or RNA contained in laboratory and industrial wastes should be thoroughly broken down before they are discharged into the environment.

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Kevin Mayes Comment left 27th June 2011 21:09:31
Very circumspect of Mr Tribe to postulate that the bacteria was not genetically engineered as a "bioweapon". Of course, even if it was, it does no credit to anyone (at present) to rave on about it publicly as it allows "mainstream" to then dismiss those concerned with the issue as "conspiracy theory nutters". It still seems amazing how so many of the "right" characteristics for a dangerous and antibiotic resistant pathogen should all turn up in the same package, though- do you not think? Also, do you not find it strange how this just happens to turn up in *organic* produce. Is it just possible that this is intended to be used as a "trojan horse" for an attack on the organic sector because their produce is grown using animal manures, a potential source of E.coli? After all is it not a fact that a while ago a bill was proposed in the U.S., apparently the impetus for which emanated from Monsanto lobbyists, that would have effectively made the use of animal manures illegal on "public health grounds"? No doubt that particular attack on the organic sector being a part of their ongoing "dirty tricks" campaign. Could there not be a connection?

Paul Carline Comment left 27th June 2011 21:09:45
Dear Mae-Wan, why would you assume that the German strain had not been genetically engineered and released deliberately? Unless I've missed it in your article, early reports mentioned that fragments of plague DNA had been found in the EHEC samples. Doesn't that suggest a bio-weapon source? And how would an engineered strain "accidentally" contaminate a remote organic sprout farm? I haven't heard that there is a bioweapons lab in the vicinity. Ask the question cui bono? - and there are several suspects. Organic farming and gardening are taking increased market share from conventionally grown - including GMO - foods. Look at the effect the German 'outbreak' had on consumption of fresh and organic produce around Europe. And look at the Wall Street Journal article and video on "deadly organics" - and I smell a rat. Remember the Baxter case in 2009? Baxter Pharmaceuticals distributed swine flu vaccine contaminated with bird flu to several Labs in Europe. It was only by "chance" that a Czech lab worker tested the vaccine and found the contamination. The official response was that this was "an accident", but I'm sure you're aware of the incredibly stringent biosecurity provisions which would have made an accidental contamination impossible. The 'spiked' vaccine would have led to potentially thousands of deaths in Europe - needed to manufacture the "pandemic" which didn't exist. Recall also the anthrax mailed out after 9/11 - blamed on "the terrorists" but traced to the US Army's biowarfare labs at Fort Detrick. Rejecting conspiracy theories out of hand is irrational. There's abundant evidence of conspiracies organised by government agencies. Every time a conspiracy theory is rejected despite the evidence is a victory for those who are pulling the strings of world affairs to benefit a relative few. They need to be exposed, instead of being let off the hook - as happened with the

fake swine flu non-epidemic. Margaret Chan is still in place, instead of being behind bars as she deserved. Resisting conspiracy