

Conan the bacterium

Have you ever leafed through the pages of a book of Guinness World Records? You can find records of all kinds, from the most normal to the most odd. Among these one can also find Conan. "Who is Conan? Conan is a super-bacterium that is resistant to unthinkable doses of radiations, and for this reason it has been included in the Guinness World Records as the most radiation resistant life form in the world. But let us see, step by step, what it can do.

CONAN: the name is a guarantee

Deinococcus radiodurans, which has been nicknamed Conan the bacterium, was discovered in 1956 by Arthur W. Anderson at the Oregon Agricultural Experiment Station in Corvallis during the course of an experiment to prove the possibility of sterilizing canned food with high doses of gamma rays. During the experiment, a box of minced meat was radiated with a dose of γ -rays equal to 4000 Gy (in the International System of Units, the gray is the unit of measure of the absorbed radiation dose. Exposure of one gray corresponds to radiation that deposits one joule per kilogram of matter), a dose that is approximately 250 times greater than the dose generally used to kill *Escherichia coli*. At the end of the experiment, notwithstanding the high doses of radiation, they were able to isolate a bacterium that had survived. Initially they named it *Micrococcus radiodurans* because it was similar morphologically to members of the genus *Micrococcus*, but after subsequent studies, it was reclassified with the name *Deinococcus radiodurans*, i.e. terrible radiation surviving coccus.

Conan's Identity Card

Conan is a rather large spherical bacterium (diameter ranges from 1.5 to 3.5 μm). Usually it groups into a structure consisting of four cells, forming colonies that are smooth, convex and coloured from pink to red. It is a non-motile bacterium that does not form endospores, and it has a particular cell wall. In optimal conditions, *D. radiodurans* generation time is approximately 80 min. Optimal growth temperature is 30°C, however multiplication is still excellent up to a temperature of 37°C, but it stops if the temperature drops below 4°C or rises above 45°C. Up to date, scientists still have not been able to define the natural habitat of this "super bacterium" as members of this family can be found almost everywhere. They can be isolated in environments that are rich in organic nutrients such as soil, animal feces, processed meats and waste water, however they can also be found in dried foods, household dust, medical instruments and the textiles of clothes which are environments that are poor in organic nutrients. Therefore scientists have presumed that this bacterium has evolved to survive periods of prolonged environmental stress. It must be noted that *D. radiodurans* is exceptionally resistant to desiccation. In a study, it was shown that it was able to survive for six years in a dryer, maintaining a 10% vitality. It is because of this adaptability of *D. radiodurans* that it is defined a polyextremophilic bacterium (i.e. it is resistant to many conditions that are prohibitive for humans) as it is resistant to ionizing radiations, ultraviolet rays, dehydration, oxidation and exposure to electrophilic agents. The *Deinococcus radiodurans* genome is made up of two circular chromosomes, the first consists of 2.65 Mbp (million base pairs), the second consists of 412 kbp (thousand base pairs), a megaplasmid consisting of 177 kbp and a plasmid (portion of circular DNA, with double strand, present in the cytoplasm of bacteria, that can replicate independently from the chromosome) consisting of 46 kbp, which contain a total of approximately 3,195 genes. In the stationary phase, each bacterial cell contains four copies of its genome; while in the rapid multiplication phases, these become eight to ten copies. This combination of factors has made Conan a promising candidate for the studies on DNA damage and repair mechanisms, and it is also exploited for practical purposes such as cleaning and stabilizing sites where radioactive waste is disposed.

Radiation resistance: how and why

Ionizing radiation, that includes X-rays, γ -rays and part of the ultraviolet rays, is a type of radiation that has sufficient energy to ionize the atoms and molecules that it comes into contact with. In particular, this radiation can ionize DNA molecules, inducing adjacent thymine bases to form covalent bonds that can interfere with the mechanisms of replication, causing mutations that can cause severe damage to the cell. From studies carried out on victims of the atom bombs in Hiroshima and Nagasaki, and also from the results of experiments carried out on chimpanzees and other mammals, it is known that a person who is exposed to a dose of ionizing radiation equal to 10 Gy will die in a week or two. *Escherichia coli*, which is the bacterium that is most utilized in research experiments, does not survive with a dose of 60 Gy, however *Deinococcus radiodurans* is resistant to a dose of 5,000 Gy without losing vitality. If the dose increases to 17,500 Gy, despite all, it still maintains a vitality equal to 37%, even at 30,000 Gy some bacteria are able to survive. *D. radiodurans* is therefore resistant to doses of radiation that would kill any other organism. This however does not mean that the bacterium is not influenced by the radiations. Actually its genome is broken into hundreds of fragments, just as the genome of any other organism. How is it, then, that Conan is able to survive even if its DNA is fragmented? This bacterium is able to completely repair its DNA in 12-24 hours, as if nothing had ever happened. All living cells are able to repair their DNA to some extent. *Saccharomyces cerevisiae*, the yeast used to produce wine and beer, is able to do so, *Escherichia coli* is able to do so, also we are able to do so, but Conan does it better than any other. How is *D. radiodurans* able to repair its DNA so well? It is not easy to explain which are the mechanisms that allow *Deinococcus radiodurans* to survive. Surely the principal advantage that Conan has is that it has multiple copies of its genetic material. As already mentioned, this bacterium has four copies of its genome in the stationary phase, and eight to ten copies in the replication phase. The supplementary genetic material protects the cell in two ways: there are multiple copies of the genes that are fundamental for survival, so that even if one of these is broken by the radiation, there are other copies that guarantee survival. The multiple copies enable the bacterium to repair its DNA with mechanisms that will be explained soon. Many bacteria have multiple copies of their genome, and also *E. coli* is one of them, but this is not sufficient for survival. In 1996, scientists K. W. Minton and M. J. Daly demonstrated that *D. radiodurans* has a repairing system that is similar to that of other organisms, its advantage is that all its repairing mechanisms are more efficient. In case of **repair by homologous or classic or RecA-dependent recombination**, when a chromosome breaks, an enzyme called RecA binds to the broken fragment and searches complementary portions of DNA to use them as bases to replicate the missing part of DNA. Conan is able to carry out this search of homologous characteristics, in an excellent manner. In fact it is able to effortlessly reunite 500 fragments of DNA in a day. Comparatively, even though it is able to carry out this type of recombination, *E. coli* is unable to repair more than two or three breaks in the DNA. Minton and Daly also observed that classic recombination does not begin before four hours after exposure to radiation. In this interval of time the bacterium has already started to repair the DNA by a process called **single-strand annealing (SSA)**. If the repair is by **single-strand annealing**, two homologous fragments of DNA and a single strand are sufficient. This type of repair begins almost immediately after the break (it can be identified in one and a half hours). Minton and Daly state that they are not sure that *D. radiodurans* can carry out this kind of repair, but it is present in many bacteria, such as *E. coli*; in the case of Conan it would only be much more effective and efficient. Repair by SSA repairs approximately one third of all the breaks in the chromosomes caused by radiation, with a consequent decrease in the number of fragments that must be recomposed by means of classic recombination. These repair processes do not introduce a larger number of mutations than ordinary replication, therefore not only Conan can repair damages caused by radiation and they also enable survival without any further harmful mutations.

Where does Conan come from? Could it be from mars?

The multiple resistances of *D. radiodurans* have favoured a series of descriptions that are quite full of fantasies on the origins of this organism. Some suppose that this species was brought to Earth by comet, others who are even more whimsical suppose that it originated from mutations related to the tentatives made by humans to exploit nuclear energy. In favour of these theories there is the fact that the present levels of background environmental radiation are very low.

With the exception of the city of Ramsar in Iran (background radiation equal to 260 mGy per year) and Guarapari in Brazil (background radiation equal to 175 mGy per year), all the other regions of the Earth have levels that are greatly inferior (background radiation equal to about 0.4 mGy per year). With background radiation that is so low, it is normal to wonder what selective pressure gave an evolutive advantage to a bacterium with such a radiation resistance and investigate its possible extra-terrestrial origin. A team of Russian and American scientists proposed that the radiation resistance of Conan might have Martian origins. The evolution of the microorganism, according to these authors, could have taken place on the surface of Mars and then spread to the Earth following the impact of a meteor. In various experiments, they succeeded in making classic bacteria such as *E. Coli* radioresistant, by exposing them to multiple cycles of almost lethal doses of radioactivity, followed by periods of rest, during which the bacterial populations could grow and recover vitality. Natural cycles of this kind could only have taken place on Mars. Their hypothesis is based on the astronomic theory of the oscillations in the climate on Mars. The Martian biota could accumulate large doses of radiations during the periods of cold climate, while, during the periods of warm climate the bacterial populations could recover and multiply, thus favouring the possibility of adapting to radioactivity. However, besides its extreme resistance to radiations, *D. radiodurans* is genetically and bio-chemically very similar to other forms of terrestrial life, a circumstance that does not support the hypothesis of its Martian origin. Valerie Mattimore and John R. Battista of the University of Louisiana, instead, hypothesized that Conan's radioresistance is due to a side effect of prolonged cellular desiccation. From their experiments it was observed that mutant strains of the bacterium, that are sensitive to radiations, were equally sensitive to the damage caused by prolonged desiccation, while the wild-type strains (strains with a non-mutant wild-type phenotype) are resistant to both. Radioresistant strains, if desiccated for 6 weeks, survived in 63% of the cases, on the contrary, every radiosensitive strain that was tested was substantially more sensitive to desiccation. During dehydration, breaks in the double strand DNA accumulate in the bacterium, just as it occurs following radiations. From these data it is therefore evident that resistance to desiccation and radioactivity are functionally correlated phenomena, and that losing its capacity to repair cellular damage induced by ionizing radiations *D. radiodurans* appears to be sensitive to the lethal effects of desiccation.

Applications

1. Decontamination of radioactive sites

Hassan Brim and other collaborators of the Pathology department of the Bethesda University have successfully developed a radiation resistant bacterium that can be utilized for treating mixed waste containing ionic mercury, a highly toxic element. Most of the waste generated from the production of nuclear weapons from 1945 to 1986 was buried underground with the risk of contaminating the subsoil of thousands of sites. In the United States, approximately one third of the radioactive waste disposal sites has a radioactivity level equal to 370 MBq (Megabecquerel). The becquerel, is the SI unit of measure of the activity of a radionuclide – often incorrectly called radioactivity – and is defined as the activity of a radionuclide and its decay per second). It is estimated that this radioactive decay has contaminated an area of $7.5 \times 10^7 \text{ m}^3$ of the topsoil and subsoil and approximately $2 \times 10^{12} \text{ dm}^3$ of the ground water . This highly toxic waste contains inorganic and organic contaminants that include radionuclides (one nuclide, i.e. a single, unstable, nuclear species, that decays and emits energy in the form of radiation) such as ²³⁵Uranium, heavy metals such as mercury and solvents such as toluene. The cost of nuclear waste management of these waste disposal sites, with physical and chemical methods has been estimated around 265 billion dollars. This high cost has stimulated the development of bioreclamation strategies (removing contaminants from polluted environments by means of biological degradation processes) using specialized microorganisms that can be used for remediation of organic and metal contaminants. The development of microbiological methods for management of highly radioactive waste depends greatly on the capacity of the microorganisms to carry out the correct function to decontaminate the site and survive in a radioactive environment. Numerous microorganisms that have been studied are able to degrade metal pollutants, but in most cases the microorganism is unable to survive in a highly radioactive environment. For example, ionic mercury can be converted into elemental mercury vapour (that is much less toxic), by the mercury reductase enzyme. This enzyme is produced by the

merA gene that is present in various bacteria, among which *E. coli*; however as we already know these bacteria cannot be used to detoxify radioactive waste due to their sensitivity to radioactivity. On the other hand, *D. radiodurans* could be used but it does not have the merA gene, and consequently the mercury reductase enzyme. Brim and his colleagues have been able to clone (i.e. insert a gene or DNA fragment in another DNA molecule, with the help of particular enzymes that cut and then bind the nucleotide sequence) the merA gene from *E. coli* to *D. radiodurans* thus obtaining bacterial strains that could grow in the presence of radiations and of ionic mercury at concentrations that are much greater than those found in the radioactive waste disposal sites, and to effectively reduce the concentration of highly toxic mercury. In order to clone the merA gene in *D. radiodurans*, the scientists took the gene from *E. coli* and inserted it in a plasmid (small circular double-helix DNA strand, present in the cytoplasm of bacteria and distinguishable from the bacterial chromosome due to its small size. The genetic material that characterizes it enables the host organism to carry out various non-essential functions, which however give the cell a selective advantage). Subsequently the plasmid is inserted into Conan in order to enable it to express the merA gene, produce mercury reductase and therefore degrade ionic mercury. The gene expression (the amount of enzyme produced) can be regulated, by varying the number of copies of the gene per cell; therefore the greater is the number of copies of the gene in the bacterium, the greater it is ability to decontaminate high concentrations of mercury. Besides this, they also were able to demonstrate that various clusters of genes, i.e. groups of different genes, can be utilized to engineer Conan in order to utilize it for treatment of mixed radioactive waste containing mercury as well as toluene. Presently Brim and his colleagues are trying to engineer *D. radiodurans* to make it also decontaminate organic toxins present in radioactive waste, such as hydrocarbon.

2. Memory device

Conan could also have another important utilization: in fact it could be utilized as a memory device for data that are resistant to nuclear catastrophes: in other words a living hardware.

Doctor Hari Mohan and his collaborators used bacteria as memory devices, because with them there is no risk of losing data as may occur with magnetic data storage discs when they crash. Many scientists have also tried to use other biomaterials such as proteins or tissues, but these degrade too rapidly, instead, if the data are memorized in the bacterial DNA, they can be stored and transmitted to the progeny without any losses. The additional advantage of Conan, is its capacity to store data also in adverse environmental conditions. Pak Chung Wong and his collaborators have developed a protocol to utilize microorganisms as memory devices, subdivided into four principal stages: encoding the artificial DNA sequence, engineering the microorganism with the produced sequence, multiplication of the microorganisms, and extraction of information inserted by the microorganisms if required. One of the first steps is to choose the best bacterium to utilize – among the most utilized are *E. Coli* and particularly *D. radiodurans*. Subsequently the nucleotide sequence is produced artificially. Since the DNA is a digital sequence, it is possible to transform any text into a sequence of numbers, just like the binary system is used to encode ASCII characters. Then the number sequence is transformed using a quaternary numeral system so as to obtain a sequence of four different numbers, just like the number of nucleotides. Finally the quaternary sequence is transformed into a nucleotide sequence that can be produced artificially in a laboratory. Wong and his colleagues decided to use a song from Disneyland, "It's a small world" and to convert it into a nucleotide sequence. The DNA produced in this manner is cut into various fragments if it is too big, and each one is inserted in a plasmid and subsequently into the host bacteria, where it can remain forever without the risk of losing any information. If necessary, the sequence inserted in the bacterium can be extracted, united with the other fragments and reconverted in order to obtain the initial information once again, through a process that is practically the opposite of what we have just seen. One of the principal problems when utilizing microorganisms as memory devices, is the risk that casual mutations of the bacterial DNA may occur during its growth, that could alter the inserted information. The problem has partly been solved using Conan which, as we have seen before, has a formidable system for repairing errors in the DNA. This new protocol could therefore be used to permanently memorize any information of the bacterium. So we have seen how many things have been discovered in about 60 years of research on *Deinococcus radiodurans*, but many questions are still unanswered and we must continue our research work to discover all about this formidable bacterium, so that we will can use it in the most varied applications.