Pseudomonas aeruginosa and nosocomial infections

Science, Biology

Bielecki et al. Research Paper Pseudomonas aeruginosa is a Gram-negative bacteria, particularly known for causing nosocomial infections. As a pathogen, it effectively causes disease by acquiring resistance to antibiotics that would otherwise inhibit growth. Reported rates of infection range from 0. 6 to 32% across various clinical environments because Pseudomonas aeruginosa has gained multi-drug resistance. Certain strains of Pseudomonas aeruginosa treated with gamma rays can break down the hydrocarbons in crude oil and are thus useful in cleaning up oil spills.

The genome of Pseudomonas aeruginosa is 6. 3 million base pairs long, which is the largest bacterial genome to be sequenced. It contains about 5, 570 open reading frames. Argyrin is a naturally synthesized antibiotic peptide extracted from myxobacteria. It has cytotoxic properties, suppresses the immune system, and is a highly active antibiotic used against Pseudomonas strains. Figure 1. Argyrin A structure. Bielecki et al chose to isolate these resistant clones in order to observe the mechanisms by which the P. eruoginosa acquires resistance to Argyrin A within the fusA1 gene. They isolated these clones by growing Pseudomonas aeruginosa strains on agar that contained Argyrin A. After incubation, the colonies that formed were able to grow in the presence of argyrin; these colonies were then streaked onto plates with Argyrin A again to ensure accuracy of obtaining resistant strains. A point mutation is an alteration of one base pair within a DNA sequence. The point mutations, which caused changes in the amino acid sequence within the fusA1 gene, were different among the six isolates.

They might have conferred resistance because the mutations caused the same impact on the resulting protein. The gene was identified by sequencing the whole genome of Pseudomonas aeruginosa strains with the bacterial target of Argyrin A, which showed mutations within fusA1 that encode for the elongation factor EF-G in resistant strains of Pseudomonas aeruginosa. The diagram below illustrates the process of elongation during the translational phase in EF-G along with EF-Tu. Figure 2. Elongation during ribosome-catalyzed translation. Bielecki et al confirmed the identity of the gene by using genetic maps. This required sequencing the resistant strain a second time to make a reference strain to compare the genes at a specific loci. Adding a mutation into the sensitive Pseudomonas aeruginosa strain demonstrated a resistance phenotype. Surface plasmon resonance is a lab technique that involves aiming a beam of light at a thin metal sheet, which catalyzes a reaction by causing movement in the molecules behind the metal sheet.

SPR was useful in this experiment because it confirmed that fusA1 is the target gene for Argyrin A, rather than fusidic acid, the antibiotic previously recognized. A heterologous protein, or a heterologue, is a protein that differs in structure and function relative to a given protein; not all proteins with different amino acid sequence necessarily differ in function. N-terminal His6-tags were fused to the fusA1 genes before undergoing the SPR experiments, causing the production of heterologous proteins in relation to the original fusA1.

According to Bielecki et al, the SPR procedures supported that Argyrin A binds to fusA1 by the resulting KD value. This shows that Argyrin A has a target on the heterologous protein. It is important to compare the variations made in the mutations because the other bacteria may have a different sequence that can still achieve resistance. It cannot be assumed that all bacterial strains will be identically resistant or sensitive because they all contain differences in their genomes. By mapping the mutated genes, the authors found the locations of the mutations in different domains.

They deduced that the mutations exhibiting resistance to Argyrin A in Pseudomonas aeruginosa are found on opposite sides of the domain, despite the fact that most mutations involving fusidic acid and Argyrin A are located on the same side of the domain. This shows that the binding sites for fusidic acid and Argyrin A must be independent of each other. Both fusA1 and the second gene, fusA2, encode for the elongation factor EF-G. The fusA2 gene was expressed 30 times less in the strains of Pseudomonas aeruginosa than in the fusA1 gene, as shown by RNA sequencing.

Homology modeling uses the model of a target protein to produce an estimated structure of a homologous template protein. After creating a homology model of Argyrin A’s protein structure, Bielecki et al concluded that it “ most likely binds to a site distinct from that of fusidic acid, indicating a new mode of protein biosynthesis inhibition by Argyrin A”. Multi-drug resistant pathogens pose a very big risk on the world because they can easily mutate their genomes to adopt resistance to a given antibiotic and persist in causing harmful diseases.

The authors used MDR clinic isolates in order to observe the mechanisms by which these pathogens mutate to build resistance to Argyrin. The fact that eleven of the twelve isolates showed sensitivity to Argyrin suggests that Argyrin is a useful antibiotic in preventing infections by Pseudomonas aeruginosa. There are other factors besides the uptake and export of Argyrin that affect Pseudomonas aeruginosa's sensitivity to Argyrin, such as efflux pumps; however, the uptake and export of Argyrin in other bacteria does play a role in its sensitivity.

A proteasome is a hollow protein complex with active sites that break down proteins by proteolysis. The degraded peptides that are produced can be used for other functions in the cell. Argyrin A is a factor used to inhibit proteasome function, yet there is no distinct evidence that Argyrin A binds to the site on the proteasome. This paper is important because it analyzes the resistance and sensitivity to Argyrin A in various strains of Pseudomonas aeruginosa.

This bacteria has been a leading cause in nosocomial infections, so it is important to determine which antibiotics best work to stop the spread of disease. About ten percent of patients in hospitals across the United States obtain a significant nosocomial infection. Although there are effective methods to prevent the spread of pathogens in clinical environments, it is important to study how bacteria acquire resistance, so that scientists can develop ways to inhibit the spread of nosocomial infections by multi-drug resistant pathogens.

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