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Biodegradation of cyanide wastes from mining and jewellery industries

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Cyanide, one of the known most toxic chemicals, is widely used in mining and jewellery industries for gold extraction and recovery from crushed ores or electroplating residues. Cyanide toxicity occurs because this compound strongly binds to metals, inactivating metalloenzymes such as cytochrome *c* oxidase. Despite the toxicity of cyanide, cyanotrophic microorganisms such as the alkaliphilic bacterium *Pseudomonas pseudoalcaligenes* CECT5344 may use cyanide and its derivatives as a nitrogen source for growth, making biodegradation of cyanurated industrial waste possible. Genomic, transcriptomic and proteomic techniques applied to cyanide biodegradation ('cyan-omics') provide a holistic view that increases the global insights into the genetic background of cyanotrophic microorganisms that could be used for biodegradation of industrial cyanurated wastes and other biotechnological applications.

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Introduction

The vast majority of gold produced annually around the world is extracted with cyanide leaching techniques. The United States, China, Australia, Russia and African countries are the largest producers that contribute to the worldwide gold supply, and mining activities in Europe are currently increasing due to the demand for gold jewellery and technology sectors [1,2]. The mining industry releases several billion pounds of toxic wastewaters that, in addition to cyanide, contain arsenic, lead, mercury, cadmium, chromium and sulphuric acid [3]. Spills with cyanurated wastewaters that cause environmental disasters have been linked to mining activities, but cyanide is also used by the jewellery industry to selectively recover precious metals, silver and gold, from the jewellery wastes that are generated during electroplating. The large volumes of cyanurated wastewaters produced by the jewellery industry usually contain high concentrations of metals, such as copper, iron and zinc. These toxic liquid residues must be treated to minimize the health and environmental risks. In this context, microorganisms that can degrade cyanide and tolerate high concentration of metals have been described [3–10]. In recent years, knowledge of the complete genome information from different microorganisms, integrated into genomic and proteomic techniques, offers the possibility of designing metabolic maps and other strategies for using the appropriate microorganisms in a specific bioremediation or biotechnological process. This review emphasizes the environmental and health impact of the cyanurated industrial wastes and discusses the application of global analysis techniques for improving the biodegradation of these toxic residues.

Cyanide management: impact on the environment and human health

In recent years, global cyanide production has increased due to the introduction of new markets. Cyanide is mainly used for gold processing in mining activities. Gold is present in ores at very low concentrations, and the use of cyanide reagents in the so-called cyanidation process is the only economically viable method for extracting gold from ore [1,2]. High levels of cyanide-containing liquid wastes are also generated in precious metal recovery in the jewellery industry [3]. In addition, cyanide is required for many industrial applications, including the production of nylon, plastics, adhesives, cosmetics, drugs, fire retardants, anti-caking additives and road salts [2–5]. Cyanide is manufactured and distributed in a variety of physical and chemical forms, such as solid sodium cyanide briquettes, flake calcium cyanide and liquid sodium cyanide, and the chosen cyanide reagent form usually depends on the availability, distance from the source and cost. For safe use, cyanide reagents must be dissolved in alkaline solutions to avoid the volatilization of hydrogen cyanide, a potent hazardous gas, at high pH values [3].

Cyanide toxicity mainly occurs because it binds to and inactivates several metalloproteins, such as cytochrome *c* oxidase, blocking the mitochondrial electron transport chain. This inhibition of aerobic respiration results in

histotoxic hypoxia and increases acidosis from the anaerobic reduction of pyruvate to lactic acid, resulting in depression of the central nervous system and myocardial activity [11•]. Cyanide intoxication is usually suspected in the presence of metabolic acidosis, coma, shock, seizures, bradycardia, and lack of response to oxygen treatment. Cyanide is not stable in blood, but some derivatives such as thiocyanate and 2-aminothiazoline-4-carboxylate [12] may be detected in cyanide-induced deaths; the latter of these compounds is a stable metabolite that acts as an important forensic cyanide biomarker [13]. Different therapies to treat cyanide intoxication have been developed. These include the use of methemoglobin/nitric oxide generators, such as sodium nitrite and dimethyl aminophenol because cyanide has a higher affinity for the oxidized form methemoglobin than for haemoglobin; sulphur donors like sodium thiosulphate and glutathione to form the less toxic thiocyanate in the presence of rhodanase; and direct binding agents such as hydroxocobalamin and dicobalt-EDTA, which act as antidotes [11•].

Biological cyanide removal from industrial wastes

Compounds containing the cyano (−C≡N) group are present in many different forms in nature, but the toxicity of these compounds depends on their capacity to release free cyanide. Cyanide is frequently found in metal–cyanide complexes because of its high affinity for transition metals [3–5]. Complexes of cyanide with nickel, copper or zinc are weakly acid-dissociable, whereas strong complexes with iron and cobalt are very stable, displaying dissociation constants within the range 10^{-17} to 10^{-52} M. Other important cyanide derivatives include cyanate (OCN^-), which is formed by cyanide oxidation; thiocyanate (SCN^-), which results from the interaction between free cyanide and reduced sulphur forms present in ores, such as pyrite and pyrrothite; and nitriles or cyanohydrins, the organic forms of cyanide.

Despite the toxicity of cyanide, many organisms, including bacteria, fungi, plants and certain animals, synthesize cyanide, which is usually a defence mechanism (cyanogenic organisms), and some microorganisms can assimilate cyanide, using it as nitrogen source for growth (cyanotrophic organisms). These microorganisms have different cyanide degradation pathways that are based on hydrolytic, reductive, oxidative or substitution/addition reactions [3,8–10]. Therefore, cyanide biodegradation has become a suitable alternative to the less efficient and economically more expensive chemical treatments for removing cyanide from industrial wastes.

The bacterial strain, *Pseudomonas pseudoalcaligenes* CECT5344, isolated from sludge taken from the Guadalquivir River (Córdoba, Spain), can grow under alkaline conditions with cyanide, cyanate, different metal–cyanide complexes, and wastewaters from jewellery industry as the sole nitrogen source [14–16]. This strain has an optimal pH for growth of 9.5, and it has tolerance to metals, making it a suitable candidate for bioremediation of cyanide-containing industrial wastes [17]. In this bacterium, cyanide induces a cyanide-insensitive respiratory chain that is associated with a malate:quinone oxidoreductase that converts L-malate into oxaloacetate. This ketoacid reacts with cyanide to produce a cyanohydrin (nitrile) that is further converted into its respective carboxylic acid and ammonium by the nitrilase NitC, which is essential for cyanide assimilation [18,19•]. An added value to the process of cyanide removal from jewellery industry wastewaters is the accumulation of polyhydroxyalkanoates (PHA) by *P. pseudoalcaligenes* when it grows, in a reactor, with this toxic residue [20••]. Cyanide biodegradation in reactors has also been described in other bacteria, such as *Bacillus* sp. CN-22, which was isolated from a cyanide-contaminated electroplating sludge [21•]. Recently, a consortium of *Bacillus* species has been used for cyanide bioremediation of electroplating wastes with agrowastes as a carbon source [22••].

Cyan-omics: new generation techniques for cyanide biodegradation

New generation 'omic' techniques have revolutionized our knowledge of biological processes by generating substantial data for the global analysis of these processes. Genomics, transcriptomics and proteomics have been applied in just a few studies on bacterial cyanide degradation, although recent studies have provided a holistic view of this topic.

Although many microorganisms can use cyanide as a nitrogen source, only the genomes of three cyanide-degrading bacteria have been sequenced, *Pseudomonas pseudoalcaligenes* CECT5344, *Pseudomonas fluorescens* NCIMB 11764 and *Azotobacter chroococcum* NCIMB 8003 [23••,24–26]. The genome of *P. pseudoalcaligenes* CECT5344 was the first completely sequenced genome for a cyanide-assimilating bacterium [23••,24]. Four nitrilase genes, including the *nitC* gene essential for cyanide assimilation, and six additional C–N hydrolase/nitrilase superfamily genes are present in this cyanotrophic strain. In addition to cyanide and metal resistance genes, the genome analysis also revealed the presence of genes that have great biotechnological potential, such as those required for PHA synthesis and biodegradation of pollutants, including furanic or aromatic compounds [23••]. The production of PHA-derived bioplastics confers an added value to the cyanide degradation process in this strain [20••]. The *P. fluorescens* NCIMB 11764 genome sequencing is a draft with more than 800 contigs, although four genes coding for putative nitrilase superfamily proteins that are potentially involved in cyanide assimilation have been identified [25]. *Azotobacter chroococcum* NCIMB 8003 is a N_2-fixing bacterium that may reduce cyanide to ammonia by nitrogenase [27]. The genome sequence of this bacterium has been determined, but no additional

genes involved in cyanide metabolism have been described [26].

Genome analysis of cyanotrophic microorganisms provides vast information for elaborate metabolic maps that may help predict the potential use of a microorganism for bioremediation of a specific cyanide-containing industrial residue. Cyanurated wastewaters are produced by diverse industries; hence, the chemical composition of these wastes is different. In addition to cyanide, mining or jewellery wastewaters often contain thiocyanate, arsenic, mercury and heavy metals; as a result, the presence of several determinants for resistance to these compounds in the genome of *P. pseudoalcaligenes* CECT5344 is of special relevance. Due to the chemical heterogeneity of the different cyanide-containing industrial wastewaters, identification of new cyanotrophic bacterial strains with different catabolic capacities is also of interest. Database mining for genes that are involved in cyanide metabolism is a predictive tool for identifying potential cyanide-utilizing microorganisms, and these comparative genome analyses allow for global insights into the genetic background of cyanotrophic microorganisms.

In contrast to cyanide-assimilating bacteria, many cyanogenic bacteria, including *Chromobacterium violaceum*, *Burkholderia cepacia* and different strains of *Pseudomonas*, have been sequenced [28–30]. These bacteria produce cyanide by a hydrogen cyanide synthase complex that is encoded by the *hcnABD* genes, and they share cyanide resistance mechanisms with cyanotrophic microorganisms. As mentioned previously, cyanide is the most important gold-extracting chemical; therefore, cyanogenic bacteria could be useful for biomining, an attractive, environmentally friendly technology that applies biological systems to facilitate the extraction and recovery of metals from ores, as an alternative to conventional methods [31]. In this sense, cyanogenic bacteria are currently used for gold biorecovery from electronic wastes [32,33].

Global transcriptomic analysis of bacterial cyanide assimilation has only been achieved in *P. pseudoalcaligenes* CECT5344; in these bacteria, the DNA microarrays from cells grown in sodium cyanide, jewellery wastewater, ammonium chloride and nitrogen starvation were compared [34••]. In addition, a whole-genome transcriptional analysis of *Nitrosomonas europaea* was performed to identify the cyanide stress response genes [35]. This nitrifying bacterium is used to remove nitrogen from wastewaters, but the nitrification process is sensitive to very low concentrations of cyanide. At the proteomic level, few studies describing the effect of cyanide on the proteome of cyanide-assimilating bacteria have been published. A two-dimensional electrophoresis approach and matrix-assisted laser desorption/ionization-time of flight-mass spectrometry allowed for the identification of a complex response to cyanide in *Klebsiella oxytoca* [36,37] and *P. pseudoalcaligenes* CECT5344 [38,39]. In the latter strain, this response includes cyanide resistance and assimilation proteins, oxidative stress protection and repairing systems, iron acquisition mechanisms and nitrogen assimilation pathway regulation [38,39].

Metagenomics, the genomic analysis of a population of microorganisms, has emerged in recent decades as a powerful tool for elucidating the physiology and genetics of uncultured organisms [40]. Recently, culture-independent and sequence-based metagenomic methodologies have been applied to natural coking and artificial cyanide and thiocyanate-containing mining wastewaters, allowing for the reconstruction of the microbial genome and providing knowledge about the structure and metabolic potential of the complex bacterial community in these wastewaters [41•,42•]. Function-driven metagenomic analysis for identifying new cyanide-utilizing enzymes has focused on nitrilases [43–45], enzymes with important economical impact because organic cyanides have wide applicability in medicine, industry and environmental monitoring. New nitrilase superfamily proteins, including cyanide-degrading nitrilases, have been obtained with genome mining [46•,47–50]. The major aim of these studies was to identify enzymes with new properties or high specificity for industrially relevant nitriles.

Conclusions

Microorganisms that are able to degrade cyanide allow for the biological treatment of highly toxic cyanide-containing industrial wastes. In recent decades notable efforts to characterize the cyanide degradation pathways in different cyanotrophic organisms, and there has been a special emphasis on nitrilases, enzymes with great biotechnology potential. Complete genome sequencing of the cyanide-assimilating strain *Pseudomonas pseudoalcaligenes* CECT5344 and other cyanotrophic bacteria, together with high-throughput metagenomic and comparative transcriptomic and proteomic analyses, will set the basis for elaborate metabolic maps that can predict the physiology and metabolic potential of microorganisms used for the bioremediation of different cyanurated industrial wastes. The identification of new cyanotrophic strains with novel biotechnological capacities will improve the cyanide biodegradation process.

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