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NOTE

Bacterial Color Response to Hexavalent Chromium, Cr^{6+}

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A blue pigment-producing bacterium, *Vogesella indigofera*, was isolated and quantified for the relationship between its synthesis of a blue pigment and exposure concentrations of Cr^{6+}. The concentration of Cr^{6+} and the percentage of blue colonies on agar plates was negatively correlated (r^2 = -0.8683). Critical concentrations inhibiting bacterial pigment production were found to be between 100-150 μg Cr^{6+}/ml on agar plates and 200-300 μg Cr^{6+}/ml in liquid culture. As the blue color is characteristic and easily observable, the bacterium *Vogesella indigofera* may have potential applications in the detection and monitoring of environmental pollution.

Key words: bacterial pollution indicator, biomonitoring, chromium, heavy metals, pollution, *Vogesella indigofera*

Aquatic toxicity tests and monitoring are increasingly important subjects to the public, legislatures and scientists. As the amount of pollutants being produced and dispersed into the environment have increased significantly (Ayres, 1992), many regulatory agencies have produced guidelines that are detailed and quantitative. Following that development, acute biotoxicity tests have been used in many pollution monitoring programs (Adams, 1995). Nevertheless, several major questions remain: 1) test organisms of the same species should be genetically identical worldwide regardless of location, 2) large numbers of the test organisms must be available for toxicity tests on a routine basis without disturbing the natural ecosystem, 3) test organisms should be relatively uniform in size and age, and easily maintained in any laboratory without sophisticated facilities, and 4) the test organisms must be inexpensive.

Besides conventional physico-chemical analyses, which usually involve delicate instruments and/or chemical reagents, various bioassays have been developed to detect the amount of pollutants present based on the presence-absence, physiological, behavioral or genotypic expressions of indicator organisms (Bitton and Dutka, 1986; Peitzsch *et al.*, 1998). A wide variety of living organisms have been used in these bioassays, which include plants, animals, protozoa, and microorganisms (Hellawell, 1986). Among these organisms, bacteria have the advantage of being small and culturable, with high reproductive rates and short generation times (Gu and Cheung, 2001). Thus, bacteria could potentially be adopted as a quick and cheap pollution indicator.

The abundance of coliform bacteria (e.g. *Escherichia coli*) present is probably the most commonly adopted fecal contamination bioindicator. A number of commercially available toxicity testing kits, like the Microtox® test, also make use of the altered physiological and biochemical responses of bacteria under stressful environments (Dutka and Kwan, 1982). In this study, a new direction for biomonitoring using phenotypic responses of a unique blue-pigment-producing bacterium, *Vogesella indigofera*, following exposure to hexavalent chromium (Cr^{6+}) was investigated. This paper presents preliminary findings on the feasibility of using this commonly found species of bacteria as a metal pollution bioindicator.

The blue pigment-producing bacterium *Vogesella indigofera* was first isolated from tap water, and its pure culture was obtained by the transfer of bacteria from an individual colony grown on nutrient agar plate (Difco Lab., Detroit, MI, USA) to sterilized liquid nutrient broth (Difco Lab.), which was incubated in a rotary shaker (200 rev/min) at 22±1°C for 48 h. The detail procedures about

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isolation, purification and identification were published elsewhere by Gu and Cheung (2001). Thereafter, 100 μl pure culture of *V. indigofera* was transferred into a series of pre-sterilized liquid media (5 ml) spiked with 0, 50, 100, 200, 300, 400 μg Cr^{6+}/ml in the form of potassium chromate (K_2CrO_4). In addition, 100 μl pure culture of *V. indigofera* was inoculated and spread evenly onto nutrient agar plates amended with 0, 50, 75, 100, 150 μg Cr^{6+}/ml. All inoculated liquid media and plates were incubated at 22±1°C in triplicate.

Owing to the intense production of exopolysaccharides by *V. indigofera*, which affect the validity of direct spectrophotometric readings by diffracting light, visual assessments of the color of liquid cultures were carried out against a white background every 48 h with a series of dilutions made from the commercially available blue dye indigo [2-(1,3-dihydro-3-oxo-2H-indole-2-ylidene)-1,2-dihydro-3H-indole-3-one] (Sigma, St. Louis, MO). For colonies grown on agar plates, the total number of colonies and number of blue colonies were enumerated every 48 h; the percentage of blue colonies can therefore be calculated.

V. indigofera, previously known as *Bacillus indigofera* or *Pseudomonas indigofera* (Elazari-Volcani, 1939), was recently re-classified, characterized and molecularly analyzed (with 5S rRNA and 16S rRNA sequencing) by Grimes *et al.* (1997). *V. indigofera* was chosen in this study because of its novel blue pigment-producing property, making it easy to be monitored for any morphological changes in appearance. To our knowledge, this characteristic has not been reported for this particular bacterium.

Pigment production by *V. indigofera* is a constitutive property in both liquid cultures (Fig. 1) and on agar plates (Fig. 2) amended with zero or relatively low concentrations of Cr^{6+}. Nearly all colonies were blue in color when the concentration of Cr^{6+} was lower than 75 μg/ml; only 62% of colonies were pigmented at 100 μg Cr^{6+}/ml, while pigment production completely ceased at 150 μg Cr^{6+}/ml (Fig. 3). This clearly indicated that there is a critical inhibitory concentration of Cr^{6+}, which lies between 100 and 150 μg/ml, for pigment production by *V. indigofera* on agar plates. Further investigation may be needed to determine whether there is a gradual or a sharp decrease in the percentage of blue colonies between 75 and 100 μg Cr^{6+}/ml. Liquid cultures also showed a similar phenomenon in that the pigment production decreased progressively at higher concentrations of Cr^{6+}, and it was completely inhibited at 300 μg/ml (Fig. 4). Thus, a critical concentration of Cr^{6+} inhibiting pigment production by *V. indigofera* was between 200 and 300 μg/ml in liquid cultures.

According to Kettrup and Marth (1998), bioindicators are defined as organisms which can provide information

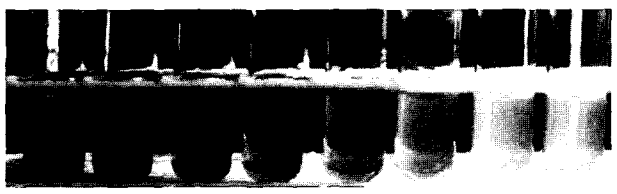

Fig. 1. Liquid cultures of *V. indigofera* illustrating the gradual change of color from dark blue (left) to pale yellow (right), with the concentrations of Cr^{6+} in medium increased progressively from 0, 50, 75, 100, 150, 200, 300 to 400 μg/ml (*left to right*).

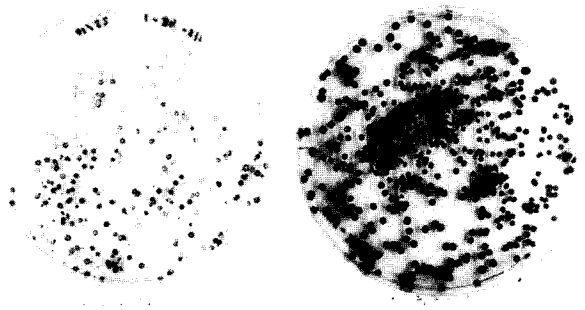

Fig. 2. Colonies of *V. indigofera* growing on nutrient agar plates. Pigment production of some colonies was inhibited at higher concentrations of Cr^{6+} (100 μg/ml) (*left*) and, all colonies produced blue pigment on agar plate without Cr^{6+} (*right*).

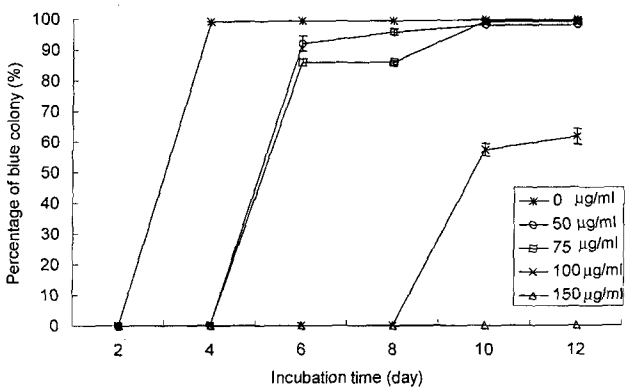

Fig. 3. Percentage of blue colonies of *V. indigofera* at various Cr^{6+} concentrations over incubation time, with error bars showing the standard deviation of three replications.

Time elapsed (day)	Concentration of Cr(VI) (μg/ml)					
	0	50	100	200	300	400
0	/	/	/	/	/	/
2					/	/
4						/
6						/
8						/
10						/
12						/
14						/

Fig. 4. Pigment production by liquid cultures of *V. indigofera* under different Cr^{6+} concentrations. The intensity of the dark color in this graph is directly related to the intensity of the blue color observed in the cultures; the white color in this graph represents no pigment production in the cultures, while'/'denotes no observable growth of bacteria.

on the quality of their environment (or a part of it). Pigment production of *V. indigofera* was found to be directly altered by ambient concentrations of Cr^{6+}, with a negative correlation (r^2 = -0.8683) between the percentage of blue colonies developed and Cr^{6+} concentration. Potentially, by assessing the intensity of pigment production of *V. indigofera* in either liquid cultures or on agar plates amended with the tested pollutant, the concentration of Cr^{6+} in the sample might be known. Unlike the already established biotoxicity tests, such as the Microtox® test which relies on the luminescent marine bacterium *Photobacterium phosphoreum* (renamed as *Vibrio fischeri*) and a delicate instrument (the photodetector in a completely cooled block holding the test tubes) measuring the minute change in light being emitted (Bastian and Alleman, 1998), using phenotypic responses of *V. indigofera* for biomonitoring has the advantage of being simple and needing only routine microbiological apparatus such as Petri dish/culture test tubes and nutrient broth/agar. In addition, this microorganism is an environmental isolate and is non-pathogenic posing no human health concerns. It can be easily maintained in large quantities in a deep-freezer and lower temperature refrigerator for storage. Bacteria also offer the advantage of quick multiplication and being genetically uniform. Combining this information, phenotypic responses of *V. indigofera* may warrant further consideration as a bioindicator for pollution monitoring.

Since only a preliminary investigation has been carried out so far, further experiments addressing the reproducibility and precision of using phenotypic responses of *V. indigofera* for biomonitoring will be carried out. Inhibition of pigment production by other heavy metals and toxicants (pollution priority organics, organometals, etc.) will also be examined in order to obtain detailed information on the specificity between toxicant level and pigment production by *V. indigofera*. Thus, the realization of using this bacterium as a standard organism to derive the effective concentration (EC_{50}) of a sample will be further supported by our investigation on the bacterial response to specific pollutants at the molecular level. From our results, phenotypic responses of *V. indigofera* provide potential applications in biomonitoring of pollution, and this new direction will make biotoxicity tests teasier to perform with high uniformity for comparison.

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