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New biosensor for detection of copper ions in water based on immobilized genetically modified yeast cells

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ABSTRACT

Contamination of water by heavy metals represents a potential risk for both aquatic and terrestrial organisms, including humans. Heavy metals in water resources can come from various industrial activities, and drinking water can be ex-post contaminated by heavy metals such as Cu^{2+} from house fittings (e.g., water reservoirs) and pipes. Here, we present a new copper biosensor capable of detecting copper ions at concentrations of 1–100 μM. This biosensor is based on cells of a specifically modified *Saccharomyces cerevisiae* strain immobilized in alginate beads. Depending on the concentration of copper, the biosensor beads change color from white, when copper is present in concentrations below the detection limit, to pink or red based on the increase in copper concentration. The biosensor was successfully tested in the determination of copper concentrations in real samples of water contaminated with copper ions. In contrast to analytical methods or other biosensors based on fluorescent proteins, the newly designed biosensor does not require specific equipment and allows the quick detection of copper in many parallel samples.

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1. Introduction

Due to both natural sources and certain industrial enterprises, the environment, including water resources, becomes contaminated by various pollutants including heavy metals. Such contamination poses serious problems for terrestrial and aquatic organisms and to human health. The heavy metals accumulated in the environment usually cannot be degraded naturally. Considerable amounts of heavy metals also penetrate the surface water system and accumulate in sediments. Among the heavy metals, copper is both an essential nutrient, as a constituent of some enzymes, and a drinking-water contaminant. The value recommended by the World Health Organization (WHO) guidelines is 2 mg of Cu^{2+} per liter of water. This is based on the presumption that adults consume 2 or 3 l of water per day and ingest additional copper from food. Copper consumption should not exceed an upper limit of 10 mg per day. However, copper concentrations in drinking water can range from ≤ 0.005 to > 30 mg/L, primarily as a result of the interior corrosion of copper plumbing (WHO, 2008).

Heavy metals can be detected using many analytical methods such as atomic absorption spectrometry (AAS), inductively coupled plasma optical emission spectrometry (ICP/OES) and inductively coupled plasma mass spectrometry (ICP/MS) (WHO, 2008), which are costly and time-consuming and require expensive specialized equipment and highly qualified staff. The limits of copper detection are 0.02 μg/L by ICP/MS (Zhu et al., 2009), 0.3 μg/L by ICP/OES (WHO, 2008) and 0.5 μg/L by flame atomic absorption spectrometry (FAAS) (Pourreza and Hoveizavi, 2005). These methods can reliably determine the total concentration of ions of heavy metals, including their insoluble forms, but they do not provide any information about the bioavailable concentrations of these metals, which is the amount that could be dangerous for living organisms. One way to address this problem is to use biosensors.

A biosensor is an analytical device that combines a biological component with a physicochemical detector, and it can be used for a detection of a specific analyte (Su et al., 2011). For example, one biosensor group is based on the ability of protein or DNA to bind heavy metals. In proteins, the binding of heavy metals predominantly to Cys residues can cause inhibition or activation of protein/enzyme activity.

Several important biosensors are based on intact cells (e.g., Belkin, 2003). Some of these biosensors can use the same enzymatic reactions as those based on isolated enzymes, but they are cheaper and can provide data on the bioavailability of pollutants and/or their effects on living systems. Intact cells provide ideal conditions for enzyme function. Cell-based biosensors include

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either unmodified or genetically modified microorganisms, along with detection based on either the measurement of changes in the intensity of bioluminescence or fluorescence or the measurement of electrical parameters.

Biosensors using unmodified microorganisms are often based on the inhibitory effect of heavy metals on microbial cells. Thus, the decrease in luminescence intensity of *Photobacterium phosphoreum* was used to detect chromium reaching I_{50} at 0.85 nM Cr^{6+} (Lee et al., 1992). A conductometric biosensor detects the decrease in alkaline phosphatase activity of immobilized blue algae *Arthrospira platensis* caused by the presence of heavy metals (Tekaya et al., 2013). The I_{50} values were 10^{-19} M for cadmium and 10^{-17} M for mercury. In addition, lyophilized biomass of yeast *Rhodotorula mucilaginosa* (Yuce et al., 2010) or dead biomass of algae *Tetraselmis chuii* (Alpat et al., 2007) have been used to construct a voltammetric biosensor used to detect Cu^{2+}. These biosensors were based on the ability of the biomass to adsorb heavy metals from aqueous solutions, and it reached detection limits of 10^{-7}–10^{-5} M and 4.6×10^{-10} M, respectively.

Biosensors that use genetically modified microorganisms are usually based on the production of specific reporter protein (s) controlled by a promoter induced by heavy metal(s). The genetically modified bacillus *Alcaligenes eutrophus* increases its bioluminescence based on the heavy metal concentration. That detection is based on the reporter operon *luxCDABE* from *Vibrio fischeri* in combination with bacterial σ-54 promoter regions that are inducible by heavy metals. Thus, in combination with the *chrA* promoter, *A. eutrophus* predominantly detects chromium Cr^{6+} (K_2CrO_4); the presence of the *copSRA* promoter region, the mer regulatory region (*merR* and *mer* promoter) or the *pbrR* promoter enable the detection of copper, mercury or lead ions, respectively, with detection limits of 1.0 μM for Cu^{2+} and Cr^{6+} and 0.5 μM for Pb^{2+}, respectively (Corbisier et al., 1999). A similar biosensor but combined with optical fibers was used later to detect copper ions (Leth et al., 2002). Copper ions can also be detected by biosensors using recombinant yeast *Saccharomyces cerevisiae*. This copper sensing is based on the presence of a plasmid containing the lacZ gene (from *Escherichia coli*) or a GFP gene controlled by the *CUP1* promoter, which is inducible by copper ions. The *CUP1* promoter is inducible specifically by Cu^{2+} or by silver ions (Dameron et al., 1991; Shetty et al., 2004). In the lacZ-based sensor, the enzyme β-galactosidase (encoded by the lacZ gene) was produced only in the presence of Cu^{2+} and enabled the yeast to utilize lactose. This sensor detected Cu^{2+} concentrations of 0.5–2 mM $CuSO_4$ (Lehmann et al., 2000). In the GFP-based biosensor, the production of GFP protein in a Cu^{2+}-dependent manner can be detected by monitoring the GFP fluorescence (Shetty et al., 2004). This system can detect Cu^{2+} with a lower limit of 0.5 μM.

Most of the biosensors described above require specific devices for the detection of a pollutant. Here, we present a newly designed biosensor for detecting Cu^{2+} concentration visually by the evaluation of the red coloring of immobilized yeast *S. cerevisiae*; the evaluation of this sensor requires a comparison of the unknown sample (liquid or solid) with a parallel sample containing a known Cu^{2+} concentration. The biosensor uses a *S. cerevisiae* strain with the *ADE2* gene deleted from the genome and with the natural promoter regulating the expression of the *ADE5,7* gene replaced with the *CUP1* promoter (submitted Czech patent, Vopalenska et al., 2014). The resulting strain produces red pigment only in the presence of Cu^{2+} and in a quantity proportional to the copper concentration when in the range of 1–100 μM. The intensity of the red coloring and therefore of the Cu^{2+} detection are optimal when the strain is immobilized.

2. Materials and methods

2.1. Strains and media

Strain BY4742 (*MATα; his3Δ1; leu2Δ0; lys2Δ0; ura3Δ0*) was obtained from the EUROSCARF collection. Strains BY-*ade2* (*MATα; ade2Δ*) and BY-*ade2*-P_{CUP}-*ADE5,7* (*MATα; ade2Δ; Pcup1-ADE5,7*) were prepared in this study. The BY-*ade2* knockout strain was constructed using the kanMX replacement cassette amplified from plasmid pUG6 (obtained from the EUROSCARF collection) by PCR (see Table S1 for the primers). The BY-*ade2*-P_{CUP}-*ADE5,7* strain was derived from BY-*ade2* using the *natNT2*-P_{CUP1} cassette amplified from the plasmid pYM-N2 (EUROSCARF) (Table S1). The transformation was performed as described previously (Gietz and Woods, 2002).

Yeast cells were cultivated in liquid complete YPD (0.5% yeast extract, 1% peptone, 2% glucose), liquid YPAD (YPD, 40 mg/L adenine sulfate) or using agar media YPDA (YPD, 2% agar) and YPADA (YPAD, 2% agar). For selection of transformants, the media were supplemented with geneticin (400 mg/L) or nourseothricin (100 mg/L).

Red coloring of the BY-*ade2* and BY-*ade2*-P_{CUP}-*ADE5,7* strains was monitored in minimal SD medium (0.19% yeast nitrogen base without amino acids and without copper, 0.5% $(NH_4)_2SO_4$, 2% glucose) with concentrations of Cu^{2+} (ranging from 0 to 100 μM) with shaking. Amino acids supplementing strain auxotrophies were added at concentrations of 50 mg/L (L-histidine, L-leucine, L-lysine), 30 mg/L (uracil) and 4 mg/L (adenine sulfate).

Red coloring of immobilized BY-*ade2*-P_{CUP}-*ADE5,7* cells was monitored in SD medium with amino acid and uracil supplements and without adenine sulfate. Cu^{2+} was added in various concentrations (0–100 μM).

2.2. Preparation of alginate beads with immobilized yeast cells

Yeast cells were cultivated for 6 days in YPAD with shaking at 28 °C to reach the stationary phase. Then, 0.2 mL of the culture was mixed with 5 mL of 3% sodium alginate in distilled water (final cell concentration from 4 to 8×10^6/ml). Alginate beads with yeast cells were prepared by dropping the yeast cell-sodium alginate suspension into 5% $CaCl_2$ and incubating for 30 min at 4 °C. Yeast cells within the beads were propagated by cultivation in YPAD at 28 °C with shaking for 36 h and then stored in distilled water at 4 °C.

2.3. Visual documentation of the red coloring

Red coloring of the cell suspensions in liquid media was documented by photographing 10 mL cultures in Petri dishes or beakers in transmitted light. Alginate beads with immobilized yeast cells were placed on microscope slides, and images were captured in transmitted light. Image capture used a ProgRes® CT3 CMOS camera with a Cosmicar TV zoom objective, the Kaiser illumination and NIS Elements software (Laboratory Imaging).

2.4. Incubation of copper pipes

Individual pieces of new plumbing copper pipe fittings (T Cu 5130, 22 × 22 × 22 mm). were immersed into 90 ml of different buffers or to distilled water. After 13 h of the incubation, the buffers/water were used for detection of Cu^{2+} concentration.

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