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Removal of chromium by *Staphylococcus saprophyticus* subsp. *bovis* strain 1

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The present study was taken up to carry out the removal of chromium by bacteria. Chromium is a heavy metal which is of concern as it causes chromium toxicity in both plants and animals. The tanneries are a major source of chromium release into the water bodies, and hence this study is aimed at identifying the potential chromium-tolerant bacterium and at studying the bacterium for its maximum tolerance and chromium removal capacity.

A total of 20 samples each of soil and industrial effluents were collected from the Peenya industrial area, Bangalore, India. The bacterial isolates were screened for chromium resistance by supplementing the nutrient media with 300 µg/ml of chromium. The growth of the bacteria was measured in terms of O.D. at 670 nm. The isolates were further screened by increasing the concentration of chromium from 300 to 3000 µg/ml. The isolate S10-5c which showed better resistance to chromium compared to other isolates was identified as *Staphylococcus saprophyticus* subsp. *bovis* strain 1 based on 16S rRNA sequencing. Optimization of temperature, pH, carbon sources, and nitrogen sources was carried out for the removal of chromium by *S. saprophyticus* subsp. *bovis* strain 1. Atomic absorption spectrophotometric analysis was carried out for the removal of chromium by *S. saprophyticus* subsp. *bovis* strain 1 in the mineral salts medium supplemented with 300 µg/ml of chromium.

Out of 40 soil and industrial effluent samples collected, a total of 51 isolates were obtained. Out of 51 isolates, 7 isolates gave the highest O.D. values in the presence of 300 µg/ml of chromium. The isolate S10-5c showed maximum tolerance up to 3000 µg/ml of chromium. *S. saprophyticus* subsp. *bovis* strain 1 was able to remove 2% of chromium in the medium at the end of 24 hours under optimized conditions.

It can be concluded that the organism *Staphylococcus saprophyticus* subsp. *bovis* strain 1 can be used for the removal of chromium from industrial effluents.

Key words: chromium removal, *Staphylococcus saprophyticus* subsp. *bovis* strain 1, atomic adsorption spectrophotometry analysis, heavy metal removal, bioremediation

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INTRODUCTION

The term "heavy metal" was first introduced in 1817 where Gmelin divided metals into non-metals, light metals, and heavy metals. The reason they are called heavy metals is because of them having a higher density and also due to the fact that they are a primary environmental concern due to their property of bioaccumulation and biomagnification. The metal concentration gets magnified as it reaches the higher trophic levels; this phenomenon is known as biomagnification (Packnikar et al., 2003). Not all heavy metals are of environmental concern. Heavy metals like lead, zinc, copper, arsenic, nickel, cadmium, chromium, mercury, etc. are of serious concern as these metals are usually toxic to the human body even at low concentrations. Chromium is one such metals which is used extensively and in large quantities in some of the industries. Chrome plating is often done to make a metal more anti-corrosive. Stainless steel and high-speed tool steel have 3 to 5% of chromium in them. Chrome yellow is a commonly used yellow pigment in paints. The industry which makes use of large quantities of chromium salt is a tannery. For the purpose of tanning leather, chromium salts are used which strengthen the cross linking between the collagen fibres. These above-mentioned heavy metals are present in the body in low concentrations through food and water we consume and also through the air we breathe. When concentration exceeds the tolerable amount, it poses a threat by being toxic and causes acute and fatal ailments (Babel, Kurniawan, 2004). Such toxicity is not confined to humans. Plants and animals are equally affected by these heavy metals.

Bacteria are considered to be an ideal choice for heavy metal removal due to their potential to adapt to stressful conditions. They have mechanisms which help them produce the necessary extracellular and intracellular enzymes which aid in the removal of such stress. In case of heavy metal stress, there is complexation of the heavy metal within the cell or outside the cell or the heavy metal is accumulated inside the cell after being reduced into a less toxic form (Nies, 1999). Some of the general mechanisms seen in bacteria are also of use in heavy metal removal. In some bacteria, the slime layer which is produced also brings about the surface adhesion of heavy metals.

Faisal and Hasnain (2004) investigated the conversion of Cr (VI) to Cr (III) in industrial effluents. They isolated the strains from the effluents based on their tolerance to chromium salt K_2CrO_4 which was around 40 mg/ml on nutrient agar and 25 mg/ml in nutrient broth. These isolates exhibited multiple metal (Ni, Zn, Mn, Cu, Co, Pb) and antibiotic (streptomycin, ampicillin, kanamycin, tetracycline, and chloramphenicol) resistance. It was also noted that their reduction potential was not affected by the different metallic salts present in the effluents. A bacterium isolated from tannery effluents was evaluated for its property to remove hexavalent chromium from the effluent. This strain showed tolerance to chromium to a range of 500 mg/L and could remove 35% of the metal in 72 hours (Karmakar, Ray, 2013). Sukumar et al. (2013) used Bacillus subtilis SS-1 strain isolated from the soil samples collected from an electroplating industry to remove chromium (VI). This strain of Bacillus showed a maximum resistance of 600 mg/L. 98.7% of chromium could be removed from the experimental setup. FTIR was performed which gave the results that carboxyl and amide groups were involved in chromium (VI) removal.

The objective of this study is to isolate, identify and screen the chromium-resistant bacteria and also to check for their efficiency in removal of chromium.

MATERIALS AND METHODS

Chemicals

Potassium dichromate was used as the source of chromium. The chemicals and media used were procured from Himedia Private Limited, Mumbai, India. The chemicals were of analytical grade.

Sample collection

Soil and effluent samples were collected from the Peenya industrial area, Bangalore. Twenty samples each of soil and effluents were collected and stored in plastic containers. These samples were refrigerated at 4 °C until use.

Isolation of bacteria

Each of the collected soil and effluent samples were serially diluted and plated on nutrient agar. Plates were incubated at 37 °C for 24 hours. Pure cultures of the isolates were maintained on nutrient agar for further use.

Screening of the isolates for chromium resistance

Each isolate was inoculated into 50 ml of nutrient broth supplemented with potassium dichromate at a concentration of 300 µg/ml and incubated at 37 °C for 24 hours. Growths of the isolates were measured in terms of O.D. at 670 nm. The isolates which showed better growth compared to other strains were further screened for their tolerance to increasing concentrations of potassium dichromate ranging from 300 to 3000 µg/ml (Karmakar, Ray, 2013). Tolerance to different concentrations of the heavy metal by the isolates was measured in terms of O.D. at 670 nm.

Identification of the bacteria

S10-5c which showed resistance to chromium was identified up to genus level based on biochemical tests (Krieg, Staley, 2010). The isolate was identified up to species level by 16S rRNA sequencing.

Optimization of temperature, ph, carbon and nitrogen sources for heavy metal removal by bacteria

The effect of various parameters like temperature, pH, carbon sources, and nitrogen sources on the removal of chromium by the isolate S10-5c was checked. For temperature optimization, four different temperatures of 25, 30, 35, and 40 °C were used. The pH was set to 5, 6, 7, 8, 9, and 10 using 0.1N NaOH and 0.1N HCl (Congeevaram et al., 2007). The carbon sources used were glucose, sucrose, starch, arabinose, maltose, and fructose at 0.2% concentration. The nitrogen sources used for the optimization were ammonium nitrate, beef extract, yeast extract, peptone, and sodium nitrate at 0.2% concentration. Mineral salts medium (MSM) was used to carry out optimization experiments. Flasks were incubated for 24 hours at 37 °C and then growth was measured in terms of O.D. at 670 nm.

The isolate S10-5c was grown in mineral salts medium (Sepahi et al., 2008) with chromium at a concentration of 300 µg/ml under optimized conditions. The flask was incubated for 24 h following which the samples were centrifuged at 10,000 rpm for 10 min. The supernatants were collected and diluted 50 times. The diluted supernatants were analyzed using an atomic absorption spectrophotometer (AAS).

The sequence was deposited in NCBI GenBank using BankIt as the tool for obtaining the unique accession number.

RESULTS

Out of a total of 20 soil and 20 industrial effluent samples which were collected, 51 isolates were obtained which were morphologically distinct. When all these 51 isolates were exposed to 300 µg/ml concentration of potassium dichromate, they showed difference in their growth in terms of O.D. at 670 nm. Seven isolates showed the potential to be chromium resistant (Table 1). Out of 51 isolates subjected to screening for growth in the presence of chromium, the isolates S1-6, E16-6b, E7-5c, E2-6c, and E5-6c showed an O.D. value of 0.01. The O.D. values for these 7 isolates ranged between 0.15 and 0.38. The isolate S10-5c showed a highest O.D. of 0.38.

These screened isolates showed a difference in the range of tolerance to the increasing concentrations of the metals. Among 7 isolates subjected to screening at different concentrations of chromium the isolate S10-5c could grow in a concentration up to 3000 µg/ml (Table 2). The isolate E15-5c being least resistant to chromium could not grow in a concentration beyond 300 µg/ml.

Table 1. Growth of isolates in the presence of chromium in terms of O.D. at 670 nm

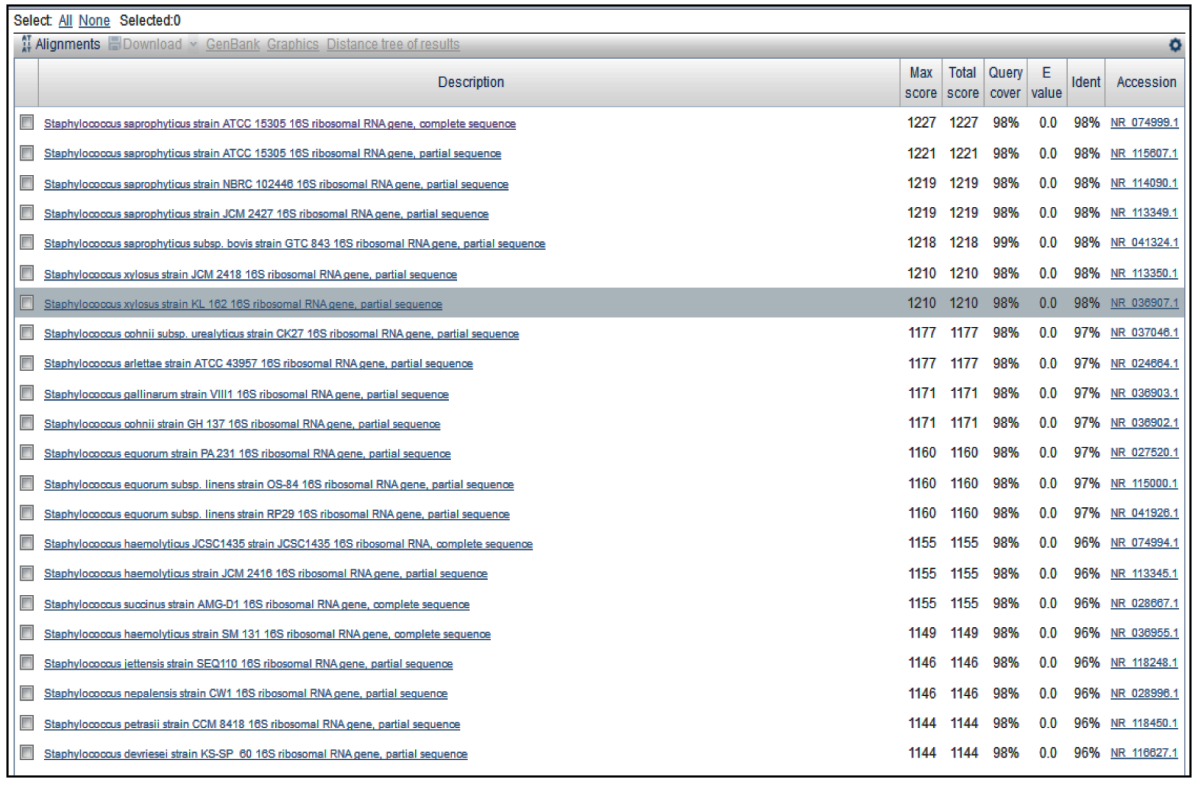
Sample	Chromium (Cr)	Sample	Chromium (Cr)	Sample	Chromium (Cr)
E2-6a	0.02	E9-6	0	S7-5	0.08
S8-5	0.06	S17-5	0.11	S2-5c	0
E2-6b	0.06	S1-5b	0.15	S13-5	0
S14-5	0	S7-6a	0.13	S16-6b	0.01
E12-5b	0.05	S4-5	0.19	E5-6c	0.01
E7-6b	0	S15-6	0	E12-5c	0
E8-6a	0.11	S16-6a	0.09	E7-5c	0.01
S1-5a	0.1	E5-6b	0.19	E5-6a	0.02
E8-5a	0.1	E7-5b	0.27	E15-5a	0
S16-6c	0.09	S8-6	0.01	E4-6b	0
E5-6c	0.02	E16-5b	0.06	S10-5a	0
S18-6	0.21	E7-5a	0.12	E17-5	0
S10-5c	0.22	E16-5a	0.01	S2-6	0.04
S9-6	0	E15-5c	0.15	S7-6b	0
S5-5	0.15	S2-5b	0.06	E2-6c	0.01
E12-5a	0.17	S1-6	0.01		
S10-5b	0.03	S6-5	0.04		
S18-5	0	S2-5a	0.15		

The isolate S10-5c was found to be a coagulase-negative organism. It gave a positive result for urease and catalase. Acid production was seen in fructose, glucose, glycerol, maltose, sucrose, trehalose, and turanose on performing a carbohydrate fermentation test. The organism gave a negative result for coagulase and DNAse and also showed a negative result for nitrate reduction. Acid production in arabinose, mannose, cellobiose, rhamnose, fucose, and galactose was not seen. Based on Gram's staining and biochemical tests, the isolate S10-5c was tentatively identified as *Staphylococcus* sps. On performing 16S rRNA sequencing the isolate S10-5c showed 98% similarity with *Staphylococcus saprophyticus* subsp. *bovis* and hence was identified as *Staphylococcus saprophyticus* subsp. *bovis* strain 1 with the accession number KT895962 (Photos 1, 2).

When optimization of different parameters was carried out for removal of chromium by *S. saprophyticus* subsp. *bovis* strain 1, the growth was higher at 35 °C (Table 3). Growth was also seen at a temperature of 30 and 40 °C for *S. saprophyticus* subsp. *bovis* strain 1. The optimum pH for the growth of the isolate *S. saprophyticus* subsp. *bovis* was found to be pH 8 (Table 4). The organism showed growth in both pH 6 and pH 7; however, the growth was comparatively slower. Among the different sugars checked, glucose was found to influence the growth of the isolate *S. saprophyticus* subsp. *bovis* strain 1 compared to other sugars (Table 5). Among

Table 2. Growth of chromium-resistant bacteria in terms of O.D. values at 670 nm in the presence of varying concentrations of chromium ranging from 300 to 3000 µg/ml

Sample	300 µg/ml	600 µg/ml	1200 µg/ml	1800 µg/ml	2400 µg/ml	3000 µg/ml
S18-6	0.22 ± 0.03	0.05	0.05	0	0	0
S10-5c	0.38 ± 0.03	0.2	0.15	0.18 ± 0.03	0.08 ± 0.03	0.07 ± 0.03
S5-5	0.05	0.05	0	0	0	0
S1-5b	0.23 ± 0.03	0.1	0.09 ± 0.02	0.04 ± 0.01	0.05	0.05 ± 0.005
E5-6b	0.1	0.05	0.05	0.05	0	0
E7-5b	0.35 ± 0.05	0.05	0.05	0.05	0.05	0.05
E15-5c	0.12 ± 0.03	0	0	0	0	0



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Alignments Download GenBank Graphics Distance tree of results

Description	Max score	Total score	Query cover	E value	Ident	Accession
Staphylococcus saprophyticus strain ATCC 15305 16S ribosomal RNA gene, complete sequence	1227	1227	98%	0.0	98%	NR_074999.1
Staphylococcus saprophyticus strain ATCC 15305 16S ribosomal RNA gene, partial sequence	1221	1221	98%	0.0	98%	NR_115607.1
Staphylococcus saprophyticus strain NBRC 102446 16S ribosomal RNA gene, partial sequence	1219	1219	98%	0.0	98%	NR_114090.1
Staphylococcus saprophyticus strain JCM 2427 16S ribosomal RNA gene, partial sequence	1219	1219	98%	0.0	98%	NR_113349.1
Staphylococcus saprophyticus subsp. bovis strain GTC 843 16S ribosomal RNA gene, partial sequence	1218	1218	99%	0.0	98%	NR_041324.1
Staphylococcus xylosus strain JCM 2418 16S ribosomal RNA gene, partial sequence	1210	1210	98%	0.0	98%	NR_113350.1
Staphylococcus xylosus strain KL 162 16S ribosomal RNA gene, partial sequence	1210	1210	98%	0.0	98%	NR_036907.1
Staphylococcus cohnii subsp. urealyticus strain CK27 16S ribosomal RNA gene, partial sequence	1177	1177	98%	0.0	97%	NR_037046.1
Staphylococcus arlettae strain ATCC 43957 16S ribosomal RNA gene, partial sequence	1177	1177	98%	0.0	97%	NR_024664.1
Staphylococcus gallinarum strain VIII1 16S ribosomal RNA gene, partial sequence	1171	1171	98%	0.0	97%	NR_036903.1
Staphylococcus cohnii strain GH 137 16S ribosomal RNA gene, partial sequence	1171	1171	98%	0.0	97%	NR_036902.1
Staphylococcus equorum strain PA 231 16S ribosomal RNA gene, partial sequence	1160	1160	98%	0.0	97%	NR_027520.1
Staphylococcus equorum subsp. linens strain OS-84 16S ribosomal RNA gene, partial sequence	1160	1160	98%	0.0	97%	NR_115000.1
Staphylococcus equorum subsp. linens strain RP29 16S ribosomal RNA gene, partial sequence	1160	1160	98%	0.0	97%	NR_041926.1
Staphylococcus haemolyticus JCSC1435 strain JCSC1435 16S ribosomal RNA, complete sequence	1155	1155	98%	0.0	96%	NR_074994.1
Staphylococcus haemolyticus strain JCM 2416 16S ribosomal RNA gene, partial sequence	1155	1155	98%	0.0	96%	NR_113345.1
Staphylococcus succinus strain AMG-D1 16S ribosomal RNA gene, complete sequence	1155	1155	98%	0.0	96%	NR_028667.1
Staphylococcus haemolyticus strain SM 131 16S ribosomal RNA gene, complete sequence	1149	1149	98%	0.0	96%	NR_036955.1
Staphylococcus jettensis strain SEQ110 16S ribosomal RNA gene, partial sequence	1146	1146	98%	0.0	96%	NR_118248.1
Staphylococcus nepalensis strain CW1 16S ribosomal RNA gene, partial sequence	1146	1146	98%	0.0	96%	NR_028996.1
Staphylococcus petrasii strain CCM 8418 16S ribosomal RNA gene, partial sequence	1144	1144	98%	0.0	96%	NR_118450.1
Staphylococcus devriesei strain KS-SP 60 16S ribosomal RNA gene, partial sequence	1144	1144	98%	0.0	96%	NR_116627.1

Fig. 1. Similarity index of the isolate S10-5c which was identified as *Staphylococcus saprophyticus* subsp. *bovis* strain 1

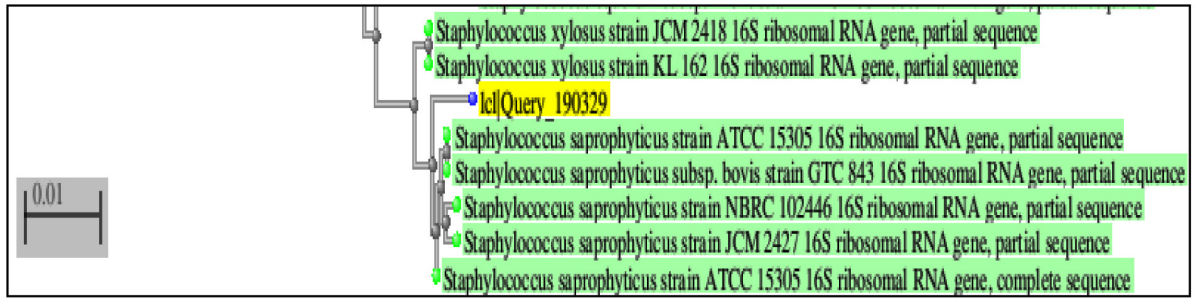

Fig. 2. Phylogenetic tree for *Staphylococcus saprophyticus* subsp. *bovis* strain 1

Table 3. Growth of *Staphylococcus saprophyticus* subsp. *bovis* strain 1 in terms of O.D. values at 670 nm at different temperature

Sample	25 °C	30 °C	35 °C	40 °C
S10-5c	0	0.03 ± 0.005	0.05 ± 0.01	0.02

Note: Values are the means of triplicates ± standard error

Table 4. Growth of *Staphylococcus saprophyticus* subsp. *bovis* strain 1 in terms of O. D. values at 670 nm at different pH

Sample	pH 5	pH 6	pH 7	pH 8	pH 9	pH 10
S10-5c	0	0.036 ± 0.005	0.05	0.056 ± 0.005	0.043 ± 0.005	0.07 ± 0.02

Note: Values are the means of triplicates ± standard error

Table 5. Growth of *Staphylococcus saprophyticus* subsp. *bovis* strain 1 in terms of O.D. values at 670 nm in the presence of different carbon sources

Sample	Sucrose	Glucose	Arabinose	Maltose	Starch	Fructose
S10-5c	0.033 ± 0.006	0.086 ± 0.01	0.023 ± 0.006	0.02 ± 0.01	0.03 ± 0.01	0.03 ± 0.01

Note: Values are the means of triplicates ± standard error

Table 6. Growth of *Staphylococcus saprophyticus* subsp. *bovis* strain 1 in terms of O.D. values at 670 nm in the presence of different nitrogen sources

Sample	$NaNO_3$	NH_4NO_3	Peptone	Beef extract	Yeast extract
S10-5c	0.01	0.03 ± 0.01	0.083 ± 0.005	0.10 ± 0.01	0.043 ± 0.01

Note: Values are the means of triplicates ± standard error

the different nitrogen sources checked for the growth of *S. saprophyticus* subsp. *bovis*, beef extract was found to be better compared to other nitrogen sources (Table 6).

After optimization of temperature, pH, carbon and nitrogen sources, the atomic absorption spectrophotometry revealed that the organism *S. saprophyticus* subsp. *bovis* strain 1 could remove 2% of total chromium content from the solution over a period of 24 hrs.

DISCUSSION

According to our study, *Staphylococcus saprophyticus* subsp. *bovis* strain 1 could grow in presence chromium compared to other isolates. Becker and Palsson (2005) reported glucose to be the best carbon source for the growth of *Staphylococcus* sps which is in agreement with the present study where *Staphylococcus saprophyticus* subsp. *bovis* strain 1 could grow the best when glucose was the sole carbon source. The optimum conditions for the growth of *Staphylococcus* sps is a pH of 6–7.5, 37 °C, and glucose as carbon source (Prakasham et al., 2006) which is close to what has been reported in this study where the optimum pH was seen to be 8 and optimum temperature was reported as 35 °C. *Staphylococcus* sps have been reported to be hexavalent chromium resistant by many researchers. Zahoor and Rehman (2009) reported that *S. capitis* is a hexavalent chromium reducing bacteria. It could tolerate up to 2800 µg/ml of Cr which is similar to that of *Staphylococcus saprophyticus* subsp. *bovis* strain 1 which could tolerate up to 3000 µg/ml. The optimum growth for *S. capitis* was reported at 37 °C which is similar to that of *S. saprophyticus* subsp. *bovis* strain 1 whose optimum temperature was reported to be 35 °C. *S. capitis* could remove 81% of Cr (VI) from the media after 96 h which is much higher than what has been reported for *Staphylococcus saprophyticus* subsp. *bovis* strain 1 which is 2% after 24 h. It was reported by Mistry et al. (2010) that *Staphylococcus* sp. could tolerate a concentration of 25 mM. The optimum pH was 7 and temperature was reported to be 32 °C. Shakoori et al. (2010) reported that *Staphylococcus* sps could tolerate chromium at a concentration up to 1.6 mg/ml. It was able to show optimum growth at a pH of 8 and a temperature of 37 °C. Using a diphenylcarbazide method it was determined that *Staphylococcus* sps could reduce 91% of chromium over 24 h which is higher than what has been reported for *Staphylococcus saprophyticus* subsp. *bovis* strain 1.

From the above discussion it can be seen that the optimum conditions of the present study are in agreement with the research performed by others. *S. saprophyticus* subsp. *bovis* strain 1 which is a chromium-resistant bacterium grew well under optimum conditions of 35 °C and pH 8. This organism used glucose as the carbon source and beef extract as the nitrogen source.

CONCLUSIONS

From the present study it can be concluded that the organism *Staphylococcus saprophyticus*

subsp. *bovis* strain 1 is a chromium-resistant bacterium with tolerance to a concentration of 3000 μg/ml of chromium, hence it can be used for the removal of chromium.

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STAPHYLOCOCCUS SAPROPHYTICUS BAKTERIJOS *BOVIS* PORŪŠIO 1 PADERMĖS PANAUDOJIMAS CHROMUI ŠALINTI

Chromas yra sunkusis metalas, toksiškas augalams ir gyvūnams, todėl keliantis rūpestį. Odų raugyklos yra pagrindinis chromo, išleidžiamo į vandens telkinius, šaltinis, todėl šio tyrimo tikslas buvo nustatyti chromą toleruojančias bakterijas, taip pat ištirti jų didžiausią toleruojamą chromo kiekį ir šalinimo gebą. Iš viso tyrimui surinkta po 20 mėginių dirvožemio ir pramoninių nuotekų Peenya industrinėje vietovėje Bangalore (Indija). Buvo tikrinamas bakterijų izoliatų atsparumas chromui papildant maitinamąją terpę 300 μg/ml chromo. Chromo koncentracija buvo didinama iki 3 000 μg/ml. Iš surinktų 40 mėginių gautas 51 izoliatas, iš kurių 7 turėjo didžiausias O. D. reikšmes esant 300 μg/ml chromo. Izoliatas S10-5c, pasižymėjęs didžiausia – 3 000 μg/ml chromo – tolerancija, 16S rRNR geno sekos kaitos metu identifikuotas kaip *Staphylococcus saprophyticus* bakterijos *bovis* porūšio 1 padermė. Remiantis gautais rezultatais padaryta išvada, kad *Staphylococcus saprophyticus* bakterijos *bovis* porūšio 1 padermė gali būti naudojama chromui šalinti iš pramoninių nuotekų.

Raktažodžiai: chromo šalinimas, *Staphylococcus saprophyticus* bakterijos *bovis* porūšio 1 padermė, atominės absorbcijos spektrofotometrinė analizė, sunkiųjų metalų šalinimas, bioremediacija