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Use of Biological Database to Explore Microorganisms used in Bioremediation of Hexavalent Chromium

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Abstract

Bioinformatics is one of the newest emerging fields of life sciences. Number of biological database has been created to provide vital information regarding module of interest. The present study was aimed to utilize these biological databases to explore significant microorganisms that had got an ability to reduce hexavalent chromium. Using Text search tool available at Protein Information Resources database, total 906 entries were obtained, out of which 49 entries were filtered depicting different microorganisms having chromate reductase enzyme. Five microorganisms belonging to Pseudomonas, Bacillus, Geobacillus, Arthrobacter and Staphylococcus species show positive screening for reduction of hexavalent chromium. Thus, use of biological database provides an aid to explore the microorganisms having potential in bioremediation of chromium compounds. The bacterial isolates having ability to convert toxic form of chromium into their nontoxic form can be employed for bioremediation of hexavalent chromium.

Keywords: Hexavalent chromium, biological database, bioremediation, protein information resources.

Introduction

A large numbers of toxic chemicals (pollutants) are generated from various industries, releasing it into surrounding environment, where they contaminate soil and water[1-2]. Some of these toxic chemicals lead to cause severe problem to living organisms and required to be removed from environment[3]. Microorganisms due to their versatile nutritional requirement can be used to degrade such toxic compound[4-6]. Chromium compounds are one of such toxic chemicals generated from various industries like tannery, textile, steel, paint and galvanization units[7]. The chromium exists in nature primarily as highly toxic hexavalent chromium and least toxic trivalent chromium[8-9]. Chromium has its carcinogenic[10-11] and mutagenicity[12-13] effects. Hence, ranked 18th in priority list of hazardous substances issued by CERCLA, 2007[14]. USEPA has categories chromium as class a pollutant[15]. Various mechanisms were known for bioremediation of hexavalent compounds. One of such mechanism includes bioremediation by a group of enzyme chromate reductases[16]. Numbers of biological databases has been available consisting of number of information pertaining to molecule of interest. Hereby attempt has been made in this study to use information from biological database to incur potential microorganisms that can show effective bioconversion of hexavalent chromium into trivalent non toxic form.

Material and Methods

Exploring microorganisms with chromate reductase enzyme: Microorganisms having enzyme chromate reductase were explored using text search tools available at Protein information resources database[17]. The available list of microorganisms was further filtered to remove redundant entries having same genus and species. Selection of microorganisms was carried out on the basis of their availability from natural environment or culture collection centre and their pathogenic nature.

Pair wise sequence alignment: The pair wise sequence alignments were performed using pair wise alignment mode available at protein information resources[18] for selected entries to determine the similarity and dissimilarity between the chromate reductase which may contribute to variation in their activity to reduce hexavalent chromium into their non-toxic product.

Isolation of selected microorganisms: The microorganisms viz., *Pseudomonas* spp., *Bacillus* spp. and *Staphylococcus* spp. identified having chromate reductase enzyme were isolated from soil samples collected from vicinities of dyes and chemical industries near Palsana, Surat, Gujarat, India. The soil sample was mixed with sterile distilled water and aliquots of 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5} and 10^{-6} were prepared. Each dilution was plated onto Nutrient agar plate and incubated at 37°C for 24 Hr. *Geobacillus* spp was procured from HImedia whereas *Arthrobacter* was procured from culture collection centre. Pure culture of all the strain was prepared using nutrient agar medium.

Screening of microorganisms for chromium reduction: The isolate were precultured overnight on nutrient agar medium. culture flask was prepared with basal medium (Glucose 0.5 g,

calcium chloride 0.02 g, monopotassium phosphate 1.0 g, ferric chloride 0.05 g, dipotassium phosphate 1.0 g, magnesium sulphate 0.2 g, ammonium nitrate 1.0 g and distilled water 1000 ml) supplemented with $K_2Cr_2O_7$ having chromium (VI) concentration of 100 ppm. 3.5 ml of isolated culture was inoculated into 350 ml of basal medium with $K_2Cr_2O_7$. The inoculated flasks were incubated at room temperature on rotary shaker having speed of 150 RPM. Samples were drawn at an interval of 0 hr, 12 hr, 24 hr, 36 hr and 48 hr to determine growth of isolate measured in terms of optical density at 540 nm using a SHIMADZU UV-Spectrophotometer against uninoculated basal medium containing chromium as blank.

Determination of Hexavalent Chromium: The supernatant of each flask after centrifugation were analyzed for Chromium (VI) using 1, 5-Diphenyl Carbazide method as described by USEPA[19]. Chromium Standard with chromium concentration ranges from 100 ppm to 1000 ppm was prepared. 95 ml of the extract were centrifuge and 2.0 ml diphenylcarbazide solution was added. pH of 2 $\pm$ 0.5 was set with 10% H_2SO_4 solution and dilute to final volume of 100 ml. Colour developed was estimated spectrophotometrically at 540 nm using Basal medium as Blank.

Protein 3D Structure Prediction: Chromate reductase protein sequence of selected microorganisms was used for protein structure modeling using Swiss-Model[20-23] server available online at server of Swiss Institute of Bioinformatics. The structures were building using automated mode that identifies best templates based on Blast[24] and HHblits[25].

Multiple Structure Alignment: All the modeled protein structures were then superimposed using Mistral tool[26] for multiple structure alignment of protein to evaluate the similarity and dissimilarity with respect to protein structure. This help to identify the conserved and non-conserved region that may contribute for variation in chromate reductase activity. The Mistral tool performed multiple structure alignment of protein on the basis of energy minimization and translations of the given molecule.

Results and Discussion

Exploring microorganisms having Chromate Reductase: Using text search tools available at Protein Information Resources database, total 906 entries were obtained which was subjected to further screening for list of microorganisms possessing this enzyme. Further, filtration of obtained list give total 49 different microorganisms table-1 having chromate reductase. 5 different microorganisms table-2 were selected from the list of 49 microorganisms.

```
>>Seq2                                                    (228 aa)
 s-w opt: 500  Z-score: 612.5  bits: 120.7 E(): 2.6e-32
Smith-Waterman score: 500; 39.5% identity (66.5% similar) in 233 aa overlap (15-243:11-225)

               10         20         30         40         50
Seq1   SPQFFCHRRPSEPKCHR-MATEREDCCTASESPSECDMNASPCTIDASTRAINKTGNPPP
                     ::: ::::::::::::::. . : .    :.         . .::  :
Seq2       TRAIAIBACICHRKMATEREDCCTASEKSBACILL---CS--------SPBGNBBP
                   10         20         30                   40

      60         70         80         90        100        110
Seq1   ESVMSQVYSVAVVVGSLRKESYNRKVARALSELAPSSLALKIVEIGDLPLYNEDIEAEAP
       ::::    .:...:::.:::::: ..:. . .    .: :... . :::.::.::: :::
Seq2   ESVM----KVVAIVGSIRKESYNMQLAQFIEKRYTEKLDLEVLSLKDLPMYNQDIENEAP
              50         60         70         80         90        100

     120        130        140        150        160        170
Seq1   PETWKRFRDEIRRSDAVLFVTPEYNRSVPGCLKNAIDVGSRPYGQSAWSGKPTAVVSVSP
         .   :. ... .::::.:::::: .::: . ::::  ::   ...  :::. ....:
Seq2   QAVLD-FKAKVKAADAVLWVTPEYNGTVPGVMINAIDWLSRV--DKVMIGKPSIIMGASM
              110        120        130        140         150

     180        190         200        210        220        230
Seq1   GAIGGFGANHAVRQSLVF--LDMPCMQMPEAYLGGAASLFEDSGKLNDK-TRPFLQAFVD
       : .:   :.  .:. :    .. : ..  ..:.:.. . :.  :.:.:. :  ::.. .:
Seq2   GNLGTVKAQLHLRDILFSPGINSPLLSGNDVYIGAVHTKFDAEGNLTDEGTVKFLDVVID
      160        170        180        190        200        210

       240
Seq1   RFASWVKLNRAV
       : .:.:
Seq2   NFLNWAKKYI
      220
```

Figure-1
Pairwise Sequence Alignment [*Pseudomonas spp. Vs. Bacillus spp.*]

Table-1
List of microorganisms having chromate reductase

Protein AC/ID	Protein Name	Length	Organism Name	UniRef50
P0AGE6/CHRR_ECOLI	Chromate reductase	188	*Escherichia coli (strain K12)*	UniRef50_P0AGE7
P0AGE8/CHRR_SHIFL	Chromate reductase	188	*Shigella flexneri*	UniRef50_P0AGE7
Q88FF8/CHRR_PSEPK	Chromate reductase	186	*Pseudomonas putida (strain KT2440)*	UniRef50_Q93T20
A0A031QPU8/A0A031QPU8_SERMA	Chromate reductase, Class I, flavoprotein	188	*Serratia marcescens BIDMC 81*	UniRef50_V6A4U4
A0A060VC33/A0A060VC33_KLESP	Chromate reductase, Class I, flavoprotein	188	*Klebsiella sp*	UniRef50_P0AGE7
A1K9U9/A1K9U9_AZOSB	Probable chromate reductase	186	*Azoarcus sp. (strain BH72)*	UniRef50_V6A4U4
A3I992/A3I992_9BACI	Chromate reductase	180	*Bacillus sp. B14905*	UniRef50_D3ECI3
A6EA35/A6EA35_9SPHI	Putative chromate reductase	185	*Pedobacter sp. BAL39*	UniRef50_F5YA92
B0JDW3/B0JDW3_THESC	Chromate reductase	349	*Thermus scotoductus*	UniRef50_A8HNC2
B3VBK2/B3VBK2_ARTAU	Chromate reductase (Fragment)	75	*Arthrobacter aurescens*	UniRef50_P0AGE7
B3VBK3/B3VBK3_BACAT	Chromate reductase (Fragment)	75	*Bacillus atrophaeus*	UniRef50_P0AGE7
B4F0I4/B4F0I4_PROMH	Putative chromate reductase (NADPH-dependent FMN reductase)	182	*Proteus mirabilis (strain HI4320)*	UniRef50_A1VMH5
G8MY11/G8MY11_GEOTH	Chromate reductase	181	*Geobacillus thermoleovorans CCB_US3_UF5*	UniRef50_G8MY11
I5BT83/I5BT83_9RHIZ	Chromate reductase	186	*Nitratireductor aquibiodomus RA22*	UniRef50_I5BT83
I7MR59/I7MR59_STRCB	Putative chromate reductase	146	*Streptococcus canis FSL Z3-227*	UniRef50_I7MR59
J0MD04/J0MD04_9ENTR	Chromate reductase monomer	188	*Enterobacter sp. Ag1*	UniRef50_V6A4U4
J1GP88/J1GP88_9LACT	Chromate reductase	179	*Weissella koreensis KCTC 3621*	UniRef50_D3ECI3
J7LWV5/J7LWV5_9MICC	Putative chromate reductase	203	*Arthrobacter sp. Rue61a*	UniRef50_A0A059MSD7
J9YFA6/J9YFA6_LEUGJ	Chromate reductase	181	*Leuconostoc gelidum (strain JB7)*	UniRef50_M4KEA3
K2MJB1/K2MJB1_9RHIZ	Chromate reductase	186	*Nitratireductor pacificus pht-3B*	UniRef50_S5YVM0
K2QFK6/K2QFK6_9RHIZ	Chromate reductase	175	*Agrobacterium albertimagni AOL15*	UniRef50_K2QFK6
K5ZL66/K5ZL66_9PROT	Chromate reductase	180	*Acidocella sp. MX-AZ02*	UniRef50_K5ZL66
L7ZF37/L7ZF37_SERMA	Chromate reductase, Class I, flavoprotein	188	*Serratia marcescens WW4*	UniRef50_V6A4U4
M1GF39/M1GF39_MYCPM	Putative chromate reductase	166	*Mycoplasma pneumoniae M129-B7*	UniRef50_P47584

Protein AC/ID	**Protein Name**	**Length**	**Organism Name**	**UniRef50**
Q08VE8/Q08VE8_STIAD	Putative chromate reductase	291	*Stigmatella aurantiaca (strain DW4/3-1)*	UniRef50_F8CBU6
Q38W12/Q38W12_LACSS	Putative chromate reductase	185	*Lactobacillus sakei subsp. sakei (strain 23K)*	UniRef50_Q93T20
Q67S97/Q67S97_SYMTH	Putative chromate reductase	335	*Symbiobacterium thermophilum (strain T / IAM 14863)*	UniRef50_Q67S97
Q7VY03/Q7VY03_BORPE	Putative chromate reductase	184	*Bordetella pertussis (strain Tohama I / ATCC BAA-589 / NCTC 13251)*	UniRef50_A1VMH5
Q8CUR9/Q8CUR9_OCEIH	Chromate reductase	181	*Oceanobacillus iheyensis (strain DSM 14371 / JCM 11309 / KCTC 3954 / HTE831)*	UniRef50_D3ECI3
R4Y2X0/R4Y2X0_ALCXX	Putative chromate reductase	184	*Achromobacter xylosoxidans NH44784-1996*	UniRef50_A1VMH5
S5U1V3/S5U1V3_PROMI	Chromate reductase (NADPH-dependent FMN reductase)	182	*Proteus mirabilis BB2000*	UniRef50_A1VMH5
S6CM53/S6CM53_9PROT	Chromate reductase (Fragment)	103	*proteobacterium S3K*	UniRef50_P0AGE7
S7U7F5/S7U7F5_9BACI	Chromate reductase	272	*Geobacillus sp. WSUCF1*	UniRef50_P39605
T1Y622/T1Y622_STAAU	Chromate reductase	268	*Staphylococcus aureus subsp. aureus CN1*	UniRef50_Q8CMQ4
U2Y9E7/U2Y9E7_GEOKU	Chromate reductase	180	*Geobacillus kaustophilus GBlys*	UniRef50_G8MY11
V6MI66/V6MI66_PROHU	Chromate reductase	182	*Proteus hauseri ZMd44*	UniRef50_A1VMH5
V7EMG5/V7EMG5_9RHOB	Chromate reductase	178	*Rhodobacter sp. CACIA14H1*	UniRef50_V7EMG5
W0XIT2/W0XIT2_KLEPN	Chromate reductase, Class I, flavoprotein	188	*Klebsiella pneumoniae subsp. pneumoniae T69*	UniRef50_P0AGE7
W1SPF4/W1SPF4_9BACI	Chromate reductase	184	*Bacillus vireti LMG 21834*	UniRef50_G8MY11
W4N9W7/W4N9W7_9BIFI	Chromate reductase/NADPH-dependent FMN reductase/Oxygen-insensitive NA...	262	*Bifidobacterium moukalabense DSM 27321*	UniRef50_R5NFV9
W4Q5N1/W4Q5N1_9BACI	Putative chromate reductase	181	*Bacillus wakoensis JCM 9140*	UniRef50_R9X5A0
W5X189/W5X189_BDEBC	Chromate reductase, Class I, flavoprotein	183	*Bdellovibrio bacteriovorus W*	UniRef50_Q93T20
W5Y707/W5Y707_KOMXY	Chromate reductase	186	*Gluconacetobacter xylinus E25*	UniRef50_V6A4U4

Protein AC/ID	Protein Name	Length	Organism Name	UniRef50
W6EP16/W6EP16_BIFBR	Chromate reductase/NADPH-dependent FMN reductase/Oxygen-insensitive NA...	255	*Bifidobacterium breve 12L*	UniRef50_R5NFV9
W8FZ94/W8FZ94_9GAMM	Chromate reductase	176	*Thalassolituus oleivorans R6-15*	UniRef50_W8FZ94
W8X1A1/W8X1A1_BIFAN	Chromate reductase	376	*Bifidobacterium animalis subsp. lactis CECT 8145*	UniRef50_E4R026
X5IHB9/X5IHB9_BORBO	Chromate reductase	184	*Bordetella bronchiseptica (Alcaligenes bronchisepticus)*	UniRef50_A9HXL5
X7EE43/X7EE43_9RHOB	Chromate reductase	189	*Roseivivax halodurans JCM 10272*	UniRef50_X7EE43
UPI00029C4B42_587	chromate reductase	185	*Providencia rettgeri*	UniRef50_A1VMH5
UPI0003900F2B_562	chromate reductase, Class I, flavoprotein	176	*Escherichia coli*	UniRef50_P0AGE7

Figure-2
Pair wise Sequence Alignment [*Pseudomonas spp. Vs. Arthrobacter spp.*]

```
>>Seq2                                                    (328 aa)
 s-w opt: 134  Z-score: 158.0  bits: 37.1 E(): 5.4e-07
Smith-Waterman score: 134; 35.2% identity (56.2% similar) in 105 aa overlap (15-114:13-106)

              10        20        30        40        50
Seq1   SPQFFCHRRPSEPKCHR-MATEREDCCTASESPSECDMNASPCTIDASTRAINKTGNPPP
                    ::: ::::::::::::::. .: .     : .  :.      :: :
Seq2     TRSCFSCFBACICHRKMATEREDCCTASEKSGEKBA----CILLCSSPWSCCFGNIP-
                10        20        30            40        50

      60        70            80        90       100       110
Seq1   ESVMSQVYSVAV----VVGSLRKESYNRKVARALSELAPSSLALKIVEIGDLPLYNEDIE
       ::::.  . ..     .:: ....    .: ...  :   :    : .. : :: .:.:
Seq2   ESVMALSFLLSCSQKEAVGLFKEDIVMNQVIETI--LQHRS----IRRFEDQPLTDEQIR
           60        70        80          90          100

        120       130       140       150       160       170
Seq1   AEAPPETWKRFRDEIRRSDAVLFVTPEYNRSVPGCLKNAIDVGSRPYGQSAWSGKPTAVV

Seq2   TIVECAQAASTSSYVQAYSIIGVKDPEKKRKLAELAGNQSYVEHNGHFFVFCADFHRHEL
       110       120       130       140       150       160
```

Figure-3
Pair wise Sequence Alignment [*Pseudomonas spp. Vs. Geobacillus spp.*]

```
 >>Seq2                                                    (342 aa)
  s-w opt: 128  Z-score: 150.3  bits: 35.8 E(): 1.5e-06
 Smith-Waterman score: 128; 39.1% identity (51.7% similar) in 87 aa overlap (15-82:12-98)

              10        20        30              40
 Seq1   SPQFFCHRRPSEPKCHR-MATEREDCCTASESPS------ECDMNASPCTIDAST-----
                   ::: ::::::::::::::. :      .:    : :   .:
 Seq2      TRTYTYSTAACCHRKMATEREDCCTASEKSSTAPHYLKCKCCCSACRECSSCBSPAC
                 10        20        30        40        50

       50          60        70           80        90       100
 Seq1   RAINKTGNPP---PESVMSQVYSVAVV----VGSLRKESYNRKVARALSELAPSSLALKI
        :  . .::     ::::::.      .:    ::.. .. ::
 Seq2   RECSCNGNSAKKRPESVMQYEDFKLIVKYRGVGNVSEHVYNLVKKHHSVRKFKNKPLSED
         60        70        80        90       100       110

              110       120       130       140       150       160
 Seq1   VEIGDLPLYNEDIEAEAPPETWKRFRDEIRRSDAVLFVTPEYNRSVPGCLKNAIDVGSRP

 Seq2   VVKKLVEAGQSASTSSFLQAYSIIGIDDEKIKENLREVSGQPYVVENGYLFVFVIDYYRH
        120       130       140       150       160       170
```

Figure-4
Pair wise Sequence Alignment [*Pseudomonas spp. Vs. Staphylococcus spp.*]

Isolation and screening and characterization of selected microorganisms: 34 different isolates were obtained from collected sample and 5 screened for survival in Basal medium containing $K_2Cr_2O_7$. All the five strain were evaluated for tolerance in term of growth figure-5. The cell mass of *Pseudomonas* spp. show highest growth in the medium supplemented with chromium after 36 and 48 hours[27]. The B*acillus spp.* and *Arthrobacter* spp. show increase in growth after 36 hours of incubation[28]. *Staphylococcus spp.* show least growth pattern suggest less tolerance toward chromium[29]. The *Geobacillus* show increase in growth after 36 hr of incubation. Thus, these microorganisms possess ability to tolerate hexavalent chromium at the concentration of 100 ppm[7]. However difference in sequence may contribute for variation in their ability to reduce hexavalent chromium. The results obtained suggest all selected microorganisms had ability for reducing hexavalent chromium. The results were in accordance with different authors[7, 27-29].

The selected isolates were further evaluated for their activity for reduction of hexavalent chromium. Standard for chromium was prepared using concentration from 100 to 1000ppm.

Determination of Isolate ability to reduce hexavalent chromium: All the five isolate were evaluate for reduction in hexavalent chromium. *Pseudomonas species* show 42.3%, *Bacillus species* show 34.5%, *Arthrobacter species* show 29.8%, *Staphylococcus spp* show 28% and *Geobacillus* about 31.6% reduction of hexavalent chromium after 48 hrs of incubation with chromium concentration of 100 ppm[7] figure-6.

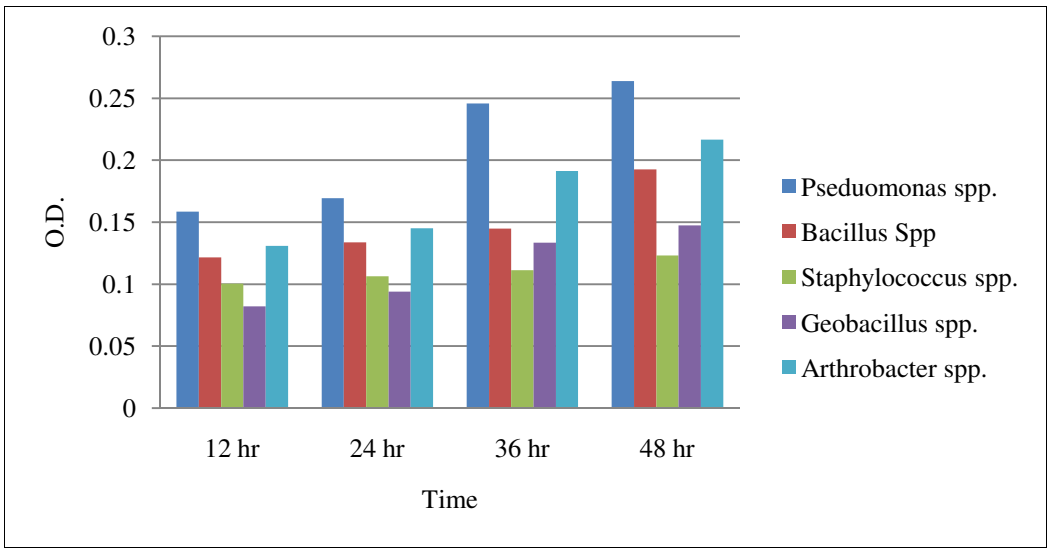

Figure-5
Biomass determination of selected isolates

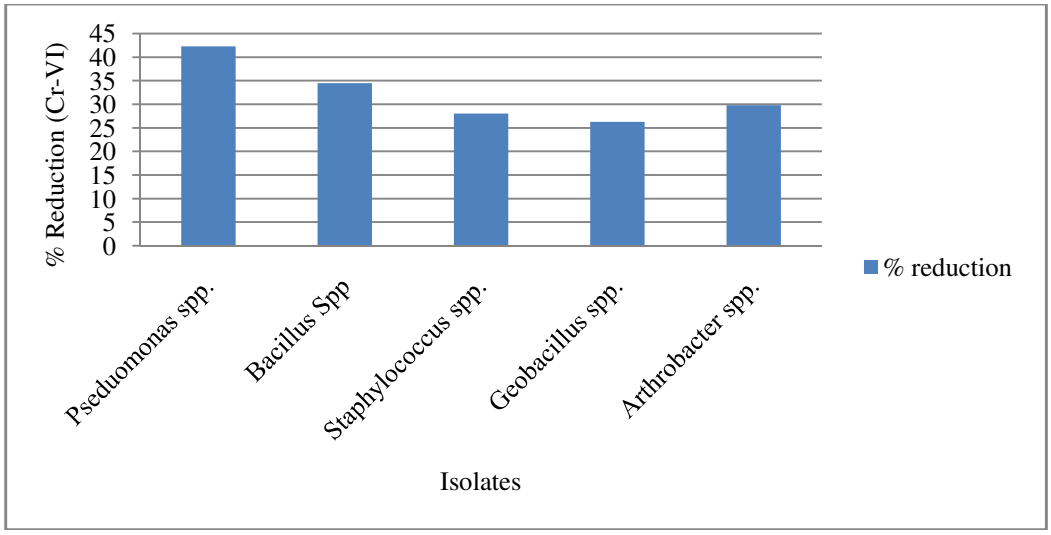

Figure-6
Activity of Isolate for Hexavalent chromium reduction

Protein 3D Structure Prediction: All the Structure prediction of chromate reductase were conducted using Swiss model template library search using Blast[24] and HHBlits[25] to determine evolutionary relatedness of the structures matching the target sequence of chromate reductase available in database. The templates found by Swiss model was overall 764 templates for *Pseudomonas spp,* 492 templates for *Bacillus spp.*,146 templates for *Staphylococcus spp.,* 667 for *Arthrobacter spp.* and 127 templates for *Geobacillus spp*.

Out of these entire template 03 models from each category was build on the basis of identity score. The best model from each category was selected on the basis of global and preresidual model quality expressed in terms of QMean4 value. *For Pseudomonas spp.,* QMean4 value of -4.67 with sequence identity of 49.72% and coverage of 95% were obtained figure-7. For *Bacillus spp.,* QMean4 value of -5.44 with sequence identity of 38.42% and coverage of 98% were obtained figure-8. For *Staphylococcus spp.,* QMean4 value of -1.50 with sequence identity of 41.13% and coverage of 93% were obtained figure-9. For *Athrobacter,* QMean4 value of -4.14 with sequence identity of 44.32% and coverage of 87% were obtained figure-10. For *Geobacillus spp,* QMean4 value of -1.50 with sequence identity of 59.51% and coverage of 91% were obtained figure-11.

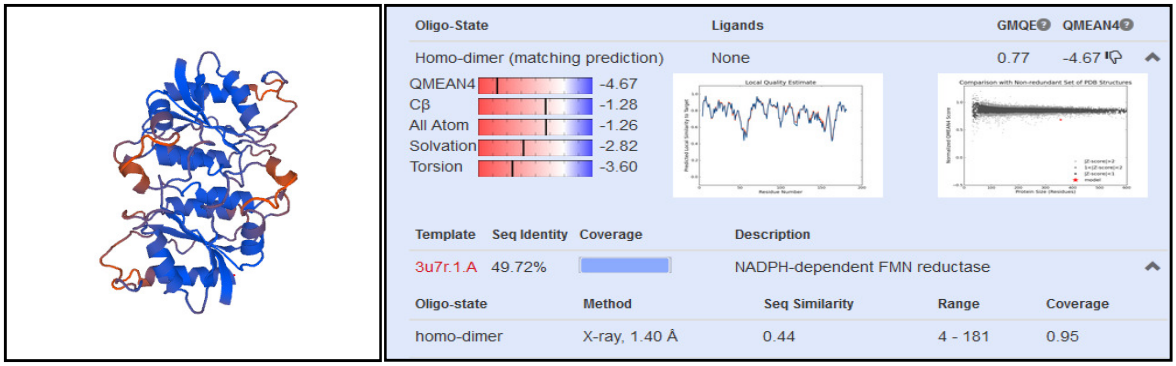

Figure-7
Chromate reductase 3D structure prediction of *Pseudomonas spp.*

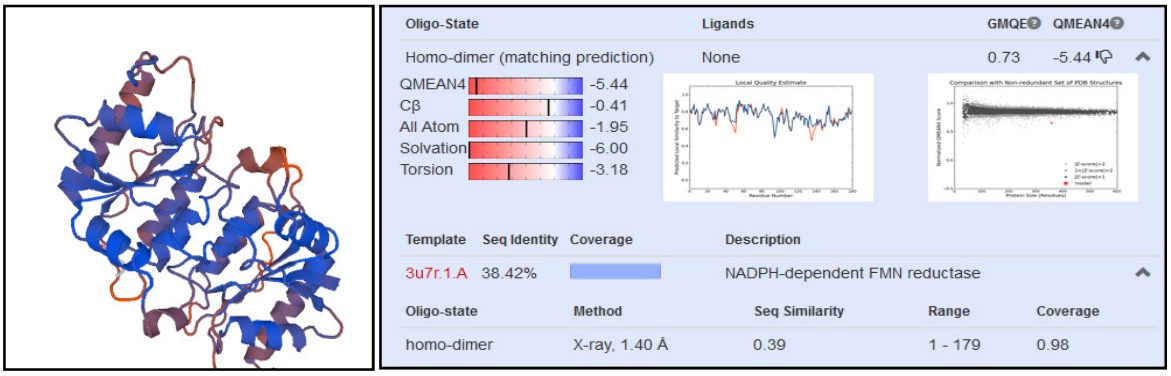

Figure-8
Chromate reductase 3D structure prediction of *Bacillus spp.*

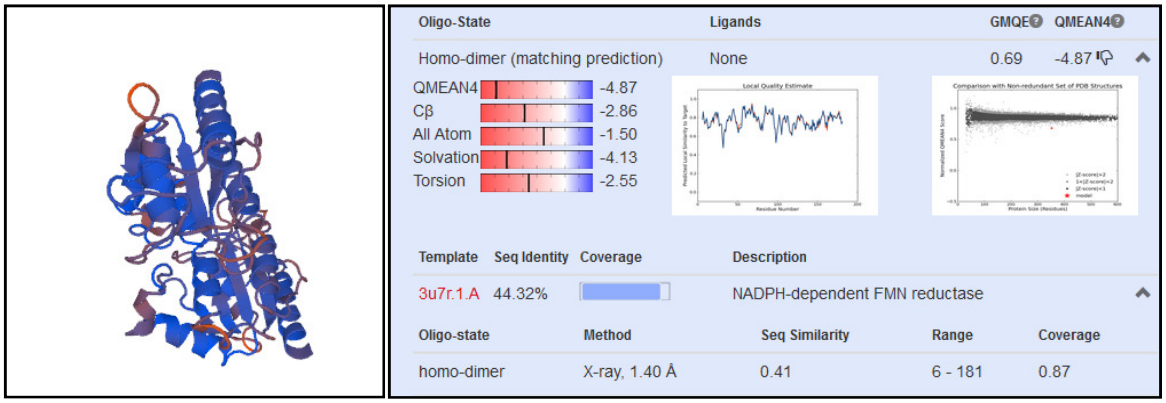

Figure-9
Chromate reductase 3D structure prediction of *Arthrobacter spp.*

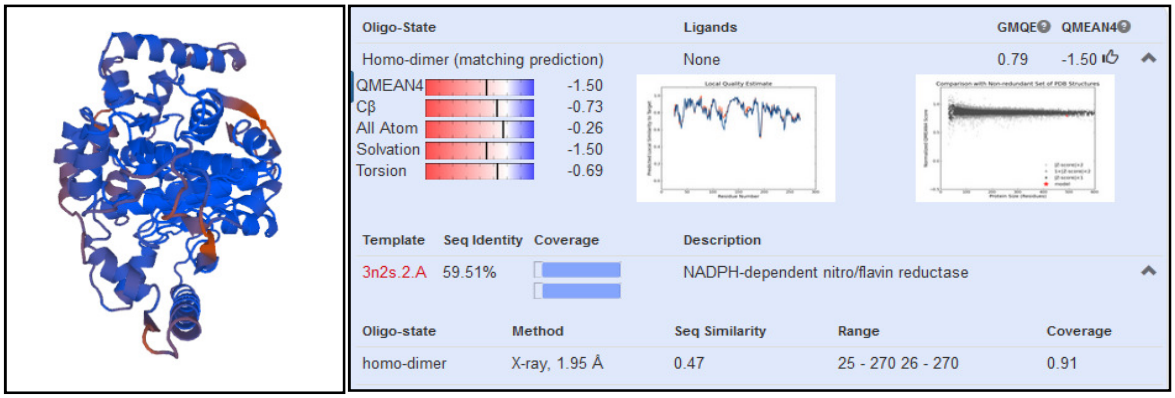

Figure-10
Chromate reductase 3D structure prediction of *Geobacillus spp.*

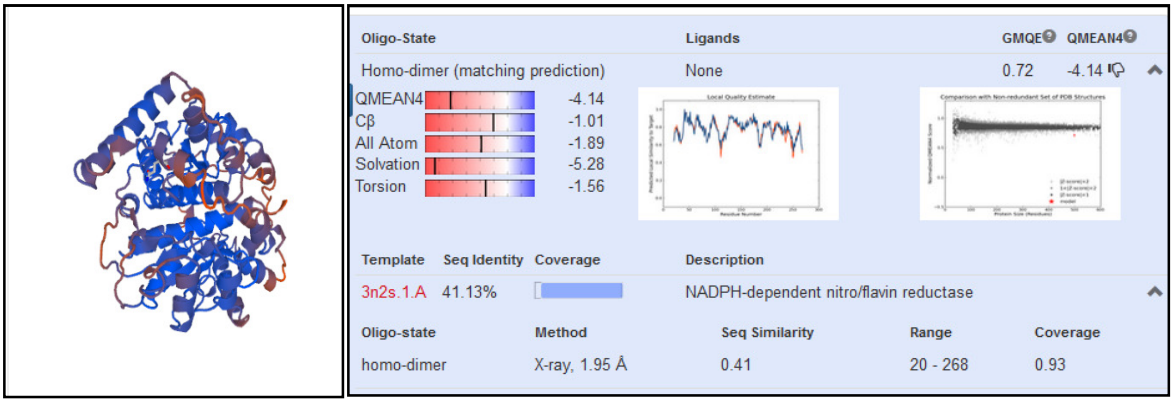

Figure-11
Chromate reductase 3D structure prediction of *Staphylococcus spp.*

Chromate reductase modeled of *Pseudomonas, Arthrobacter* and *Bacillus* was building using same template 3u7r.1.A as reference. Thus, all model predicted of *Pseudomonas, Arthrobacter* and *Bacillus* show similarity with NADPH-dependent FMN reductase whereas chromate reductase modeled of *Staphylococcus* and *Geobacillus* was build using 3n2s.1.A and 3n2s.1.B as reference. Thus, the model predicted show similarity with NADPH-dependent nitro/flavin reductase.

Multiple Structure Alignment: Mistral Software used for multiple structure alignment of all the modeled structure reveal that *Pseudomonas, Arthrobacter* and *Bacillus* species show majority of superimposition with minimum RMSD and least energy level. The best alignment was obtained showing good spatial arrangement and stability after superimposition[26].

Modeled chromate reductase of *Geobacillus spp*. and *Staphylococcus spp*. has higher RMSD and energy as compared to other above three chromate reductase table-3.

Conclusion

All the isolate show activity for chromium reduction contributed due to presence of chromate reductase enzyme. The efficiency to catalyze reaction varies due to variation in amino acid composition of chromate reductase. Using various tools and software of bioinformatics significant information can be obtained that aids in exploring microorganisms having ability to degrade such pollutants. Thus there is a need to develop specialized database showing potential candidates for waste cleanup.

Acknowledgement

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Table-2
Amino Acid Sequence of five selected microorganisms

>sp|Q88FF8|CHRR_PSEPK Chromate reductase OS=*Pseudomonas putida (strain KT2440)* GN=PP_4138 PE=1 SV=1 MSQVYSVAVVVGSLRKESYNRKVARALSELAPSSLALKIVEIGDLPLYNEDIEAEAPPETWKRFRDEIRRSDAVLFVTP EYNRSVPGCLKNAIDVGSRPYGQSAWSGKPTAVVSVSPGAIGGFGANHAVRQSLVFLDMPCMQMPEAYLGGAASLF EDSGKLNDKTRPFLQAFVDRFASWVKLNRAV
>tr|A3I992|A3I992_9BACI Chromate reductase OS=*Bacillus sp. B14905 GN=BB14905_07074 PE=4 SV=1* MKVVAIVGSIRKESYNMQLAQFIEKRYTEKLDLEVLSLKDLPMYNQDIENEAPQAVLDFKAKVKAADAVLWVTPEY NGTVPGVMINAIDWLSRVDKVMIGKPSIIMGASMGNLGTVKAQLHLRDILFSPGINSPLLSGNDVYIGAVHTKFDAEG NLTDEGTVKFLDVVIDNFLNWAKKYI
>tr|J7LWV5|J7LWV5_9MICC Putative chromate reductase OS=*Arthrobacter sp. Rue61a* GN=ARUE_c41610 PE=4 SV=1 MDTFKIGYFVGSLASNSINRVLSKALISVAPPELEFHEIAIKDLPLYSADYDADFPPAGRELKDAIAASDGILFVSPEYNR SIPGALKNAIDWGSRPWGTNSFARKPTGIIGASPGGIGTAVMQSSMRSVLSFLDAPQLNAPEAYIRFVADAYDDDGSV KDEGTAGLLRHYMEEYSAFVQRVLAANAPGHIGDPEPDSAKLTR
>tr|S7U7F5|S7U7F5_9BACI Chromate reductase OS=*Geobacillus sp. WSUCF1* GN=I656_00399 PE=4 SV=1 MALSFLLSCSQKEAVGLFKEDIVMNQVIETILQHRSIRRFEDQPLTDEQIRTIVECAQAASTSSYVQAYSIIGVKDPEKKR KLAELAGNQSYVEHNGHFFVFCADFHRHELIGELEGKDVLPSLESTEKFMVALIDTALAAQNAAIAAESMGLGICYIG GLRNNLPEVCALLNVPKRVIPLFGLAVGYPAQTPDQKPRLPFEHVYHEDEYDQDRARFIAQLQRYNETVSTYYEQRTN GRRRDTWTGQMADMLSRQVRMYMKEFVEGKGFNLR
>tr|T1Y622|T1Y622_STAAU Chromate reductase OS=*Staphylococcus aureus subsp. aureus* CN1 GN=SAKOR_00381 PE=4 SV=1 MQYEDFKLIVKYRGVGNVSEHVYNLVKKHHSVRKFKNKPLSEDVVKKLVEAGQSASTSSFLQAYSIIGIDDEKIKENL REVSGQPYVVENGYLFVFVIDYYRHHLVDQHAETDMENAYGSTEGLLVGAIDAALVAENIAVTAEDMGYGIVFLGSL RNDVERVREILDLPDYVFPVFGMAVGEPADDENGAAKPRLPFDHVFHHNKYHADKETQYAQMADYDQTISEYYDQR TNGNRKETWSQQIEMFLGNKARLDMLEQLQKSGLIQR

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Table-3
Multiple Structure Alignment of chromate reductase of selected microorganisms

Pair:	Lengths	Energy	N_matches	RMSD	sid	Z Score	P value
model_*Arthrobacter*.pdb model_*Bacillus*.pdb	352 358	1286.605	348	3.3	35	12.8	4.17e-08
model_*Arthrobacter*.pdb model_*Geobacillus.*pdb	352 492	-361.123	78	2.8	7	1.0	1.52e-01
model_*Arthrobacter*.pdb model_*Pseudomonas.*pdb	352 356	-296.951	348	**1.9**	122	13.0	3.22e-08
model_*Arthrobacter*.pdb model_*Staphyococcus*.pdb	352 497	-352.923	43	3.0	3	0.9	1.68e-01
model_*Bacillus.*pdb model_*Geobacillus*.pdb	358 492	-356.979	51	2.6	3	0.9	1.59e-01
model_*Bacillus.*pdb model_*Pseudomonas.*pdb	358 356	1290.714	345	**2.6**	72	12.8	3.91e-08
model_*Bacillus.*pdb model_*Staphyococcus.*pdb	358 497	-367.135	71	2.7	5	1.0	1.45e-01
model_*Geobacillus.*pdb model_*Pseudomonas.*pdb	492 356	-405.222	101	2.8	4	1.4	9.40e-02
model_*Geobacillus.*pdb model_*Staphyococcus*.pdb	492 497	-1769.578	489	**0.7**	208	13.6	1.45e-08
model_*Pseudomonas*.pdb model_*Staphyococcus*.pdb	356 497	-378.962	78	2.7	8	1.1	1.27e-0

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