Induction of Recombinations in *Saccharomyces Cervisiae* via Horizontal Gene Transfer for Bioremediation of Heavy Metal Toxicity from Factory Effluents

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Abstract: Bioremediation is an interesting alternative for restauring the ecological equilibrium in polluted environments, based on microbial population. This investigation aimed to apply microbial genetic techniques to induce recombinants *Saccharomyces cervisiae* [*S. cerevisiae]* to be used for increase the efficiency of removal heavy metals from factory effluents. Five *S cerevisiae* strains were used in this study. *S cerevisiae* strains were marking using 10 antibiotics and 7 heavy metals to be use as a selectable markes in conjugation process. The available markers obtained were used in 5 mating. All matings between *S cerevisiae* strains were successes. Two from *S cerevisiae* strains were mated and the hybrids were isolated to be use in uptake experiments. Most of yeast hybrids appeared higher levels for all heavy metals uptake than the parental strains. The hybrid strain no 2 resulted from mating between the parental yeast strains (Y-566 X Y-154) was appeared more efficient in copper uptake than all strains when YPD medium supplemented with wastewaters. On the other hand, when used wastewaters supplemented with 0.01% glucose as a carbon source the hybrid No. 3 appeared a good uptake of heavy metal ions than other *S cerevisiae* strains and the hybrids obtained. Whereas, the potential role of yeast hybrid No. 6 that resulted from mating between (Y-572 x Y-154) in uptake of copper ions is very high than other *S cerevisiae* strains. Modern ecological biotechnology attempts to solve the problems of pollution by screening for and molecularly breeding microbial strains that are capable of degrading recalcitrant. This enhancement the biosorption which shall resulting in a decrease of environmental loading, i.e., in lesser contamination of groundwater and also receiving surface waters. The results appeared that the biosorption capacities for all heavy metals determined in this study was higher for some metals than others.

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1. Introduction

Effluents from textile, leather, tannery, electroplating, galvanizing, dyes and pigment, metallurgical and paint industries and other metal processing and refining operations at small and large-scale sector contains considerable amounts of toxic metal ions. These metal ions from metal mining pose problems to the water environment by discharging mine water from underground and open pit mines (Moncur *et al*., 2005). The contamination of the environment by heavy metals is an important environmental problem due to the toxic effects of metals, and their accumulation throughout the food chain leads to serious ecological and health problems. Common sources of metal polluted wastes include electroplating plants, metal finishing operations, as well as many mining, nuclear and electronics industries. All of these contribute to anomalously high concentrations of metals in the environment relative to the normal background levels (Neytzell-De Wildes, 1991). According to the World Health Organization (WHO, 1984), the metals of most immediate concern are cadmium, chromium, cobalt, copper, lead, nickel, mercury and zinc. The presence of such metals (>5 g cm3, Mahavi, 2005) in aquatic environments cause severe damage to aquatic life, killing microorganisms during biological water purification process. Moreover, these metals have exacting consequences on humans such as brain damage, reproductive failures, nervous system failures, tumour formation, etc (Mahavi, 2005).

Conventional processes for removal of metals from industrial wastewaters include chemical precipitation, oxidation- reduction, filtration, electrochemical techniques and other sophisticated separation procedures using membranes. These processes are expensive when metals are found in relatively moderate concentrations, such as 1 - 100 mg/L.

Biological methods such as biosorption or bioaccumulation strategies for the removal of metals ions may provide an attractive alternative to existing technologies (Preetha and Viuthagiri, 2005). So far, the biomass from filamentous fungi such as

Aspergillus niger and *Rhizopus oryzae*, yeast-like *Scerevisiae*, have demonstrable capability for the uptake or binding of several metal ions. The yeast *S cerevisiae* as a promising biosorbent has been used to remove Cr(VI), Fe (III) (Goyal *et al*., 2003), Cd (II) (Liu *et al*., 1997), Cu (II) (Machado *et al*., 2009) from aqueous solutions. Moreover, It can distinguish different metal species based on their toxicity, such as selenium, antimony and mercury. The study aimed to transfer induce recombinant in *Saccharomyces Cervisiae* strains via conjugation as a horizontal gene transfer for better bioremediation of heavy metal toxicity from factory effluents.

2. Material and Methods

Genetic Material:

Organism and culture conditions:-

Five *S. cerevisiae* strains (Table 1) were used in this study, they are kindly obtained from National Center for Agriculture Utilization Research, USA. All strains used in this investigation are wild type strains.

Table 1. *Saccharomyces cerevisiae* strains used in this study.

No.	Strains	Designation	Origin
1	*S cerevisiae*	*Y-566*	USA
2	*S cerevisiae*	*Y-564*	USA
3	*S cerevisiae*	*Y-154*	USA
4	*S cerevisiae*	*Y-572*	USA
5	*S cerevisiae*	*YB-399*	USA

Media:

1- Maintenance medium:

Stocks of strains were maintained on standard yeast extract/peptone/dextrose (YPD) rich medium comprising in (w/v) %: Glucose, 2; Yeast extract, 1 and Peptone, 2, otherwise stated agar, 2 %, Liu *et al*. (1997).

2- Inoculum preparation:

A loop full of (YPD) slant yeast cells was cultivated in 50 ml liquid YPD medium in 250 Erlenmeyer flask at 30 °C on a rotary shaker for 24h at 200 rpm **Pearce and Sherman (1999)**

Factory effluents:

The present study was undertaken using the wastewaters resulted from ammonia unit of Fertilizer Factory (FF).

Preparation of the heavy metal solutions:

The different metal ions were sterilized by filtration through a pore filter of 0.22 um and were added to achieve final concentrations of 25, 50, 75, and 100 % in YPD media, Liu *et al.* (1997). At the end of incubation period, cultures were harvested, centrifuged at 10,000 x g , final metals concentration were determined and metal removal(%) was calculated .

Antibiotics and Heavy Metals Used in Genetic Marking:

Sensitivity of *S cerevisiae* strains toward antibiotics and heavy metals were tested in this study to be used as a genetic markers in conjugation. Different concentrations of antibiotics and heavy metals were used as shown in Tables 2 and 3.

Table 2. Antibiotics and their concentration used for genetic marking against *Saccharomyces cerevisiae* strains.

Antibiotics	Concentration (µg/ml)	Designation
Cefadroxil	100	*CF*
Ampicillin	100	*AP*
|Cephradine	25	*CP*
Amoxycillin and flucloxacillin	100	*Am-Fluc*
Amoxycillin	80	*Am*
Erythrocin	100	*EY*
Nystain	100	*NY*
Pencillin	100	*PE*
Genetamycin	100	*GE*
Chloramphenicol	100	*Ch*

Table 3: Heavy metals used in genetic marking of *Saccharomyces cerevisiae* strains with 100µg/ml concentration

Heavy metals salt	Designation
$CdSO_4$	*Cd*
$CoSO_4$	*Co*
FeCl	*Fe*
$MnCL_2.4H2O$	*Mn*
$NiCl_2.6H_2O$	*Ni*
$ZnCL_3$	*Zn*
$CuSo_4$	*Cu*

II. Methodology:

Genetic Markers Test:

It was measured by plate diffusion method, according to Collins and Lyne (1985) with cultures grown to logarithmic growth phase in nutrient broth for each strain. The plates were incubated overnight at 30°C and the diameter of resulting zones of inhibition was measured according to Toda *et al*. (1989).

Conjugation:

Matings were conducted using YPD broth cultures, in the late-exponential growth phase were used. Qualitative spot matings were carried out by inoculating 10-l1 samples of serial dilutions of the donor cultures onto the surface of selective medium previously seeded with 100 RlI of the recipient culture. Quantitative filter mating were performed according to Lessel *et al.* (1992). Conjugation was carried out between strains carrying the opposite genetic markers as shown in Table 4. Five single colonies from that appeared in each conjugation were picked up and transferring to a YPD slant, each colony may differ than other ones on the same plate resulted from the same mating in harboring genetic background. This because these are recombinations, each recombination resulted from the mating between two yeast cells.

Uptake experiments:

In the heavy metals uptake test, overnight cultures form yeast grown in YPD for yeast were harvested, washed twice with distilled water, and resuspended in 250 ml conical flasks each containing 150 ml factory effluents supplemented with 1 mg glucose/ 10 ml wastewater, glucose was used as a sole source of carbon. The flasks were incubated under a static conditions at 30^{o}C for 48 h. Thereafter, the cells were collected by filtration on membrane filter (pore size 0.45 mm). After the cells were removed the filtrate was used to determine the amount of heavy metals using atomic absorption spectrophotometry. Amounts of metals taken up by the cells were determined according to Nakajima and Sakaguchi (1986).

Metal biosorption:

Metal biosorption experiments were carried out in a 250 ml flask at 30^{o}C without shaking. The flask was filled with 150 ml of previously prepared media containing factory effluents without any dilution. Each experiment was conducted for 48 h, which was enough time to achieve steady state biosorption. The pH was uncontrolled throughout the experiment.

Dry cell weight:

Dry cell weight measurements were carried out by passing a volume of 50 ml cell culture through a previously weighted Millipore filters (Watman No.1). Cell pellets were also washed twice with filtered deionized/distilled water to remove non-biomass ash. Filtered and collected cells were dried in an oven set at temperature 110 ^{0}C and weight for every 24 h until constant weight was obtained.

Determination of heavy metals concentration:

The samples were collected and filtered using Millipore filters of 0.22 ⁻m. The filtrate was collected for heavy metals analysis. The concentration of heavy metals in solution was determined using atomic absorption spectrophotometer at the Atomic Absorption Unit, Soil, Water and Environmental institute, Agriculture research Center . Heavy metals under investigation in this study included heavy metals ions, which as follows; Lead, Cadmium, Nickel, Copper, Cobalt, Iron, Manganese, Molybdenum and Zinc.

Data evaluation (Langmuir isotherms):

The uptake of the metals (in mg of metal/g of dry cell weight) was calculated according to Liu *et al*. (2004).

3. Results and Discussion:

Horizontal Gene Transfer:

One of the most important interactions between microorganisms is the transfer of genetic material or horizontal gene transfer. Horizontal gene transfer is

the direct transfer of genetic material from one organism to another. Conjugation was discovered in 1946 by Joshua Lederberg and Edward Tatum, as a mechanism of horizontal gene transfer - as are transformation and transduction - although these mechanisms do not involve cell-to-cell contact (Griffiths *et al*., 1999). The genetic information transferred is often beneficial to the recipient cell. Benefits may include; antibiotic resistance, heavy metals uptake, other xenobiotic tolerance, or the ability to utilize a new metabolite (Holmes and Jobling, 1996). Matings were performed using five *Saccharomyces cerevisiae* strains. All matings between *Saccharomyces cerevisiae* strains were successes. Two isolates were selected at random from each successed matings to be tested. Yeasts matings was carried out in this study to obtain recombinants may have higher uptake of pollutants (Table 4).

Table 4: Mating between bacterial strains that having the opposite genetic markers.

No. of mating	Mating	Revelant geneotype of mating
1	Y-566X Y-154	*Am-Fluc*$^+$, *PE*$^+$, *Ni* $^-$ x *Am-Fluc*$^-$, *PE*$^-$, *Ni* $^+$
2	Y-566X YB-399	*Ni* $^-$, *GE* $^+$x *Ni* $^+$, *GE* $^-$
3	Y-572X Y-154	*Am-Fluc*$^+$, *PE*$^+$,*Co*$^-$x *Am-Fluc*$^-$, *PE*$^-$,*Co*$^+$
4	Y-564X Y-154	*Am-Fluc*$^+$, *PE*$^+$,*Ch*$^-$x *Am-Fluc*$^-$, *PE*$^-$,*Ch*$^+$
5	Y-572 X Y-564	*Ch*$^+$, *Co*$^-$ x *Ch*$^-$, *Co*$^+$

Uptake of Heavy Metals by *S Cerevisiae* Using YPD Medium Supplemented with wastewaters:

Heavy metal pollution occurs directly by effluent outfalls from industries, refineries and waste treatment plants and indirectly by the contaminants that enter the water supply from soils/ground water systems and from the atmosphere via rain water. (Vijayaraghavan and Yun, 2008). Modern industry is, to a large degree, responsible for contamination of the environment. Lakes, rivers and oceans are being overwhelmed with many toxic contaminants. Among toxic substances reaching hazardous levels are heavy metals. (Vieira and Volesky, 2000).

As shown from the results presents in Table 5. Most of yeast hybrids appeared higher levels for all heavy metals uptake than the parental strains. The hybrid strain no 2 resulted from mating between the parental yeast strains (Y-566 X Y-154) was more efficient in copper uptake than all strains whereas, the hybrid strain no 9 resulted from mating between the parental yeast strains (Y-572 X Y-564) was higher increase in recovery of iron above all strains. This is agreement with Volesky and May-Phillips, (1995) who investigated that living *S. cerevisiae* can sequester three times as much Cu^{2+}as the dead cells, and in the living strains, UO_2^{+2} was adsorbed continuously because of its favorable deposit within the cell. This indicated that the yeast *Saccharomyces cerevisiae* has been used to remove heavy metals such as Cr(VI), Fe(III) , etc (Goyal *et al.*, 2003), Cd (II) (Liu *et al*., 1997),Cu(II) (Jianlong, 2002), zinc and nickel (Zouboulis *et al*., 2001) from aqueous solutions. Moreover, It can distinguish different metal species based on their toxicity, such as selenium, antimony and mercury. *Saccharomyces cerevisiae* can remove toxic metals, recover precious metals and clean radio-nuclides from aqueous solutions to various extents. *S. cerevisiae* is not only a by-product of established fermentation processes, but also can be easily obtained in considerably substantial quantities at low costs (Goksungur *et al*., 2005). Often, the economics of the process can be improved by using waste biosorbent instead of cultured biosorbent (Marques *et al*., 2000). The application of *S. cerevisiae* as a biosorbent not only removes metals from wastewaters but also eases the burden of disposal costs associated with the waste (Ting and Sun, 2000). These are in agreement with Machado *et al.* (2009) who reported that strains of *S cerevisiae*, with high potential as bioremediation effectors.

Biosorption of heavy metals by *Saccharomyces cerevisiae* and their hybrids using wastewaters supplemented with 0.01% glucose as a carbon source:-

The use of microbial cells as biosorbents for heavy metals offers a potential alternative to existing methods for decontamination or recovery or both of heavy metals from a variety of industrial aqueous process streams Shumate *et al*., (1978)

The role of the microbial cell wall in the biosorption process is to adsorb metal ions in the cell wall itself or pass through the cell membrane into the vacuoles. To balance the stimulatory or inhibitory effects of essential ions and to counteract the toxicity of nonessential metals, all organisms possess homeostatic mechanisms that properly control the cellular accumulation, distribution, and detoxification of metals, .*S. cerevisiae* provides an ideal system. Gadd (1990) stated that microorganisms can take up nickel intracellular or the presence of chelating ligands that may be present on the cell surface in trace amount even after washing the biomass thoroughly and before using in biosorption experiments. Hence, both type of the heavy metal and its concentration affect behavior of

Saccaromyces biosorption.

Table 5: Percentage of heavy metals uptake by parental strains of *Saccharomyces cerevisiae* and their hybrids growing on YPD medium supplemented with wastewater.

Strains	Cells Dry Weight (mg)	Heavy metals uptake percentage (ppm)				
		Cu	Co	Fe	Cd	Pb
Y-566	0.314	88	87	54	16	45
Y-154	0.415	75	74	35	55	18
M.P	0.365	81.5	80.5	44.5	35.5	31.5
Hybrid No. 1	0.316	85	75	66	87	55
Hybrid No. 2	0.401	90	77	64	65	44
Y-566	0.314	88	87	54	16	45
Y-399	0.311	54	94	66	68	77
M.P	0.313	71	90.5	60	42	61
Hybrid No. 3	0.345	79	91	14	79	95
Hybrid No. 4	0.134	77	87	20	87	91
Y-572	0.154	81	85	20	77	35
Y-154	0.415	75	74	35	55	18
M.P	0.285	78	79.5	27.5	66	26.5
Hybrid No. 5	0.351	66	86	57	79	86
Hybrid No. 6	0.254	66	91	18	89	95
Y-564	0.411	67	65	77	88	74
Y-154	0.415	75	74	35	55	18
M.P	0.413	71	69.5	56	71.5	46
Hybrid No. 7	0.411	75	87	16	86	97
Hybrid No. 8	0.511	66	87	18	86	89
Y-572	0.154	81	85	66	77	35
Y-564	0.411	67	65	77	88	74
M.P	0.260	74	75	71.5	82.5	54.5
Hybrid No.9	0.383	75	70	98	88	89
Hybrid No. 10	0.454	88	55	88	54	88

Strains	Cells Dry Weight (mg)	Heavy metals uptake percentage (ppm)			
		Ni	Zn	Mo	Cr
Y-566	0.314	73	96	88	61
Y-154	0.415	61	82	62	35
M.P	0.365	67	89	75	48
Hybrid No. 1	0.316	69	76	38	17
Hybrid No. 2	0.401	73	85	76	43
Y-566	0.314	96	88	61	73
Y-399	0.311	96	82	39	58
M.P	0.313	96	85	50	65.5
Hybrid No. 3	0.345	68	90	48	58
Hybrid No. 4	0.134	61	58	65	78
Y-572	0.154	61	82	62	35
Y-154	0.415	89	43	91	65
M.P	0.285	75	62.5	76.5	50
Hybrid No. 5	0.351	77	85	79	43
Hybrid No. 6	0.254	75	85	80	43
Y-564	0.411	95	83	39	78
Y-154	0.415	96	82	39	58
M.P	0.413	95.5	82.5	39	68
Hybrid No. 7	0.411	96	80	43	38
Hybrid No. 8	0.511	97	86	65	50
Y-572	0.154	82	39	58	83
Y-564	0.411	91	65	73	89
M.P	0.260	86.5	52	65.5	86
Hybrid No.9	0.383	81	52	78	61
Hybrid No. 10	0.454	67	87	95	76

The results presented in Table 6 appeared the biosorption of heavy metal ions by*Scerevisiae* strains and their hybrids. It can be found that Hybrid No. 3 appeared a good uptake of heavy metal ions than other *S cerevisiae* strains and the hybrids obtained. On the other hand, the potential role of yeast hybrid No. 6 that resulted from mating between (Y-572 x Y-154) in uptake of copper ions is very high than other *Saccharomyces cerevisiae* strains. The results indicated that bioremediation of heavy metal pollution remains a major challenge in environmental biotechnology. The mechanism of metal sorption by yeast cells gave good fits for Freundlich and Langmuir models. Characteristic of a good and useful biosorbent is its ability to be utilized as a fixed or expanded bed for continuous system. This yeast biomass was shown to be suitable for use in column reactor. This are in agreement with Brierley *et al*., (1986), who has suggested that a metal loading capacity greater than 15% of biomass could be used as an economic threshold for practical applications of biosorption as compared with alternative techniques. Saccharomyces cerevisiae is the most popular biomass investigated as a useful biosorbent as seen in this study. This also are in agreement with Jung *et al* .,1998, who reported that on the basis of the above results and discussions, a reliable mechanism of Pb^{2+} accumulation in *S.cerevisiae* has been produced. The first step of this mechanism is a rapid binding to the cell wall and a passive transport of Pb^{2+} through the cell wall for a short time within 3, 5 min, and this process is metabolism-independent. The second step is the penetration through the cell membrane and into the cytoplasm, but this step cannot be clearly labeled as metabolism-dependent or independent. Cationic ion exchange between Pb^{2+} and potassium-magnesium occurred through the first and second steps. A much slower process that is obviously independent of metabolism and cation exchange follows the first and second steps. The third step is the Pb^{2+} accumulation into the cell cytoplasm even though the cells have already entered a dead phase after 24 h. It can be concluded that, because the mode of Pb^{2+} accumulation is closely related to the cell dry weight and initial Pb^{2+} concentration, careful consideration should be taken to determine the time needed to reach an equilibrium state.

Table 6: Biosorption percentage of heavy metals by *Saccharomyces cerevisiae* and their hybrids using wastewaters supplemented with 0.01% glucose as a carbon source:

Strains	Cells Dry Weight (mg)	Heavy metals uptake percentage(ppm)				
		Cu	Co	Fe	Cd	Pb
Y-566	0.986	85	86	75	59	78
Y-154	0.35	86	77	75	63	71
M.P	0.668	85	82	75	61	74
Hybrid No. 1	1.301	79	98	75	61	78
Hybrid No. 2	1.032	78	78	68	56	71
Y-566	0.986	85	86	75	59	78
Y-399	1.046	91	73	68	56	78
M.P	1.016	88	80	72	58	78
Hybrid No. 3	1.21	95	85	78	63	89
Hybrid No. 4	1.045	74	84	73	41	78
Y-572	0.824	83	91	84	42	71
Y-154	0.35	80	89	78	56	78
M.P	0.587	81	90	81	49	74
Hybrid No. 5	1.178	70	82	75	54	78
Hybrid No. 6	1.531	97	83	28	51	71
Y-564	0.89	83	91	84	42	71
Y-154	0.35	82	82	76	54	89
M.P	0.62	82	87	80	48	80
Hybrid No. 7	1.31	81	85	73	49	89
Hybrid No. 8	0.874	83	88	72	48	78
Y-572	0.824	73	88	83	37	89
Y-564	1.305	86	77	75	63	71
M.P	1.065	80	83	79	50	80
Hybrid No.9	1.541	84	86	72	45	78
Hybrid No. 10	1.301	89	77	68	25	78

Table 6: Continued

Strains	Cells Dry Weight	PPb			
		Ni	Zn	Mo	Cr
Y-566	0.986	64	65	58	74
Y-154	0.35	87	38	42	50
M.P	0.668	75	51	50	62
Hybrid No. 1	1.301	75	65	58	73
Hybrid No. 2	1.032	78	41	54	68
Y-566	0.986	81	87	58	25
Y-399	1.046	83	0	21	46
M.P	1.016	82	44	40	35
Hybrid No. 3	1.21	87	48	61	87
Hybrid No. 4	1.045	70	94	64	60
Y-572	0.824	76	75	58	64
Y-154	0.35	85	42	25	29
M.P	0.587	81	59	42	47
Hybrid No. 5	1.178	69	55	77	68
Hybrid No. 6	1.531	78	49	75	65
Y-564	0.89	91	24	58	54
Y-154	0.35	85	42	50	29
M.P	0.62	88	33	54	42
Hybrid No. 7	1.31	50	41	63	63
Hybrid No. 8	0.874	40	65	58	60
Y-572	0.824	91	44	42	58
Y-564	1.305	85	42	54	29
M.P	1.065	88	43	48	44
Hybrid No.9	1.541	94	75	63	54
Hybrid No. 10	1.301	47	72	81	50

Conclusions:-

In conclusion, biosorption is being demonstrated as a useful alternative to conventional systems for the removal of toxic metals from industrial effluents. The development of the biosorption processes requires further investigation in the direction of modeling, of regeneration of biosorbent material with industrial effluents. Due to the extensive research and significant economic benefits of biosorption, some new biosorbent materials are poised for commercial exploitation. Our results showed that yeast strains and their hybrids have biosorption capability, by being able to sequester substantial amounts of heavy metals.

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