EGYPTIAN JOURNAL OF BOTANY (EJBO)

Print ISSN: 0375-9237
Online ISSN: 2357-0350

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PUBLISHED BY
THE EGYPTIAN
BOTANICAL SOCIETY

Chromium (II), Cobalt (II) and Tungesten (VI) mixture increments the nutritive value of *Candida utilis* (Torula) as a promising mineral-enriched fodder and food

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Single-cell protein such as Torula represents a promising solution for the future food shortage crisis. Cr^{3+}, Sr^{2+}, Ni^{2+}, Co^{2+}, W^{6+}, Mo^{6+}, Li^{+}, Sn^{2+}, $B^{+,}$ and Se^{4+} at (10, 50, 100, 150, 200 µM) were screened for their effect on *Candida utilis* AUMC10703 (Torula) biomass, all metal ions stimulated biomass production except Co^{2+}. Se^{6+} increased biomass by 137.5 % at 200 µM, Cr^{3+} and Li^{+} increased biomass by 145% and 130% at 150 µM respectively, Sr^{2+} increased it by 114 % at 100 µM. W^{6+}, $Mo^{6+,}$ and Ni^{2+} increased it by 112%, 117 %, and 114% at 10 µM respectively. B^{+} and Sn^{2+} increased biomass by 137% and 117% at 50 µM, respectively. Co^{2+}, $Cr^{3+}$and W^{6+} mixture (Co^{2+} 100µM, Cr^{3+} 150µM, W^{6+} 50µM) increased amino acids, protein, DNA, RNA, and organic acids content of *C. utilis* biomass by 184%, 141.5%,148.5%,145%, and 329%, respectively. Only carbohydrate content decreased to 23%. Raffinose, glucose, and maltose production were induced. All essential amino acids increased except cysteine and glycine. SDS –PAGE analysis of *C. utilis* biomass revealed that the mixture induced two new intracellular protein production with sizes of 75 and 72 KDa and increased the expression of an intracellular protein with a size of 25 KDa. Cr^{3+} decreased organic acids production, but Co^{+2} increased their production. The 100µM Co^{2+} and 50µM W^{6+} mixture increased organic acids by 157% and induced citric, pyruvic, and malic acid production in the culture media.

Keywords: *Candida utilis*, Chromium (II), Cobalt (II) and Tungesten (VI), Single cell protein, organic acids

ARTICLE HISTORY
Submitted: August 08, 2024
Accepted: December 09, 2024

CORRESPONDENCE TO
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DOI: 10.21608/ejbo.2024.310886.2961

EDITED BY: N. Khalil

INTRODUCTION

Fodder yeast *Candida utilis* is referred to often as Torula yeast, a name derived from its former name *Torulopsis utilis. Candida utilis* Synonyms include *Pichia jadnii*, *Saccharomyces jadinii, Torula utilis,* and *Cyberlindnera jadinii* (Tomita et al., 2012), it was first described by Henneberg 1926, and its industrial utilization started in World War. *Candida utilis* is a popular microorganism and it has been used to produce several biologically useful materials, such as amino acids, RNA, biotin, glutathione, citric acid, ethanol, and different enzymes (TamaKawa et al., 2010). *Candida utilis* are also used for fodder production and the food industry as they have been approved by the FDA (Food and Drug Administration) as safe for consumption and have been added to the American GRAS list (Akkani et al., 2015). The single-cell protein (SCP) is a dehydrated cell consisting of a mixture of proteins, lipids, carbohydrates, nucleic acids, inorganic compounds, anda variety of other non-protein nitrogenous compounds such as vitamins. SCP is a cheap protein source used asanimal feed to ensure the adequate nutritive intake of vitamins and minerals for better growth of animals which may play an important role in overcoming the deficiency of protein (Somda et al., 2017).

Candida utilis biomass as a good source of protein consumed by humans as it contains a high protein content of up to 50% of the dry weight and a high value of essential amino acids, particularly lysine. Cell biomass of *candida utilis* yeasts also contains vitamins B complex, riboflavin, folic acid, and nicotinic acids and it could bind metal ions from the culture medium often in amounts exceeding the natural demand, so this fodder yeast may be used for minerals supplementation. The role of different types and amounts of microelements in the improvement of the growth, development, and metabolism of microorganisms has recently become a very interesting field of research work on an industrial scale (Zhai et al., 2019). Some metals play an important role in biological processes at low concentrations, acting as co-factors of enzymes, activating metalloregulators and trace element-dependent proteins forming functional complexes with secondary metabolites, and promoting the detoxification of reactive oxygen species (ROS) (Wintsche et al., 2016, Locatelli and Goo, 2016). Mineral deficiency represents one of the challenging problems of the developing and the underdeveloped regions. Several mineral-enriched yeasts are already being used as food supplements such as Chromium and selenium-enriched yeast (Meena et al., 2020). *Candida utilis* could accumulate large amounts of trace elements such as selenium, and transform inorganic form (low bioavailability, potentially toxic) into organic form (safer and highly bioactive) such as selenomethionine (Schrauzer, 2000). In June 2000, selenium-enriched yeast was approvedby the FDA as a source for feed- feed-supplemented organic selenium for chickens (Federal, 2000, Kamel, 2013).

Chromium-rich yeasts have a good impact on glucose tolerance and blood lipids (Offenbacher and Pi-Sunyer, 1980).

An expert in the World Health Organization (WHO) classified the essential trace elements into three categories, selenium, chromium, cobalt, and molybdenum were involved in the first category, nickel and boron were in the second category and the last class included toxic ones that may be needed for human body functions at low doses. Stannous and lithium were among them (Squitti et al., 2019, Jie et al., 2022). However, strains of the laboratory and brewing yeast *Saccharomyces cerevisiae* were found to be able to uptake Strontium Sr^{2+} (Avery and Tobin, 1992). Many yeast species may be in use commercially to produce organic acid. Citric acid and its production are very sensitive to the concentration of trace elements in the fermentation media (Lopez-Garcia, 2002). The study aims to investigate how metal ions such as Cr^{3+}, Sr^{2+}, Ni^{2+}, Co^{2+}, W^{6+},Mo^{6+}, Li^{+}, Sn^{2+}, $B^{+,}$ and Se^{4+}can affect the nutritive value and organic acids production by *Candida utilis* AUMC 10703.

MATERIALS AND METHODS

Microorganisms and cultural conditions

The molecularly and biochemically identified *Candida utilis* AUMC 10703 was supplied from Assiut University, Moubasher Mycological Center, Assiut, Egypt. It was reactivated and maintained on the YPD medium as described by (Sirilun et al., 2016).

Effect of metal ions on *Candida utilis* biomass

5% of pre−cultured *Candida utilis* inoculum was inoculated in 50 ml liquid media with the following composition: (2%) glucose, (1%) yeast extract, (0.5%) $(NH_4)_2So_4$, K_2HPO_4 (0.25%), KCl (0.05%), $CaCl_2$. 2H2O (0.025%), $MgSO_4.7H_2O$ (0.0025%), pH of the medium was adjusted to 5.5 and incubated at 150 rpm and 30°C for 96 hrs. Ten metal ions were investigated for maximum biomass production: selenium (Se^{4+}), tungsten (W^{6+}), nickel (Ni^{+2}), chromium (Cr^{3+}), Lithium (Li^{+}) cobalt (Co^{2+}), Boron (B^{+}), stannous (Sn^{2+}), Strontium (Sr^{2+}) and molybdenum (Mo^{6+}). Each one was prepared in five different concentrations to obtain final concentrations of 10, 50, 50, 50,100,150, and 200 µM in the culture medium. Three metal ions: Cr^{3+} (high biomass), W^{6+} (mild biomass), and Co^{2+} (low biomass) were chosen for further experiments. Seven treatments were tested for yield experiments: Co^{2+} at 100 µM, Cr^{3+} at 150 µM and W^{6+} at 50 µM, mixture at the same concentrations of (Co^{2+}, W^{6+}), (Co^{2+}, Cr^{3+}), (Cr^{3+}, W^{6+}) and finally a mixture of (Cr^{3+} + Co^{2+} and W^{6+}). After 4 days, *Candida utilis* biomass was harvested by centrifugation at 10,000 rpm for 15 minutes, the pellet was washed in 0.5 ml Phosphate Buffer Saline (PBS) and was centrifuged at 10,000 rpm for 10 minutes**,** the pellet was dissolved in distilled water and was sonicated for 30 seconds and 10 cycles keeping the amplitude on 100 and the cycle as 150rpm (biologic type UP 400S) (Mirzaei et al., 2015). Cell debris was removed by centrifugation at 10000 rpm for 10 minutes and crude cell was stored at -5°C.

Analysis of the nutritional value of *Candida utilis* biomass

Spectrophotometric analysis: Protein concentration was determined by the method of (Dawoud and Mowgoud, 2008). The total carbohydrate was estimated by the Anthrone method (Ouédraogoa et al., 2012). DNA estimation was done by the Diphenylamine method and RNA by the Orcinol method (Zhao et al., 2013). Total organic acids of samples were determined according to (Kola and Goli, 2012). Total amino acid content was measured by the ninhydrin method (Agbadi et al., 2017). Protein content, amino acid content, total carbohydrate, and total organic acids were estimated in culture media and cell-free extract. DNA and RNA content were only determined in biomass.

Amino acids analyzer: Amino acid profile for biomass for control and treatment with low and high total amino acids content were estimated as follows, 1g dried cell sample was hydrolyzed with 50 mL 1 N HCl in a boiling water bath. The hydrolyzate was recovered by centrifugation at 10,000 rpm. A 100 µL aliquot of this mixture was injected into an automatic amino acid analyzer (Biochrom 30) and quantified according to the method described by (Rajoka et al., 2006). The chemical score of SCP was calculated following the method of (AOAC, 1984).

HPLC analysis of organic acids and carbohydrate profile: Analysis of organic acids and carbohydrate profile of *Candida utilis* biomass for control and treatment of low and high total organic acid content was performed by (HPLC) method according to (Lapena et al., 2020) with some modifications. Chromatographic separation was carried out at 65 °C with a mobile phase of 0.005M H_2SO_4 at a flow rate of 0.6 mL.min−1 on a Rezex®column for organic acid analysis (7.8x300 mm) equipped with a cation H+ micro guard cartridge. Knauer® HPLC equipment consisted of a quaternary pump, an online degasser,

a column oven, a UV–visible detector (all Series 200), and a refractive index detector thermostat at 35 °C (Series Flexar) (Germany). The UV detector was set at 214 nm for the detection of organic acids. Briefly, 5 ml of fermentation media were diluted with 0.005 M H_2SO_4 to 50 ml. The suspension was homogenized and centrifuged at 15,000 xg/20 min/4 °C. The supernatant was filtered through a 0.45µm membrane (Millex, Millipore, Braziland) injected into the chromatograph, using a loop of 60 µl. Quantification was achieved using the peak areas from external calibration with standard solutions (Sigma Aldrich, USA). The column used for carbohydrates was Waters® Sphrisorb® NH_2 250x4.6mm column temperature kept constant at 40° C, mobile phase was acetonitrile: HPLC grade water 80:20 (v/v), detection by RI detector. Data was collected and processed on a computer with the software (clarity-chrome) for organic acids and carbohydrates.

SDS-PAGE protein analysis

Protein precipitation was performed using Chloroform by the method (Wessel and Flugge, 1984) with some modification. Sonicated cells of *C. utilis* and culture supernatants (centrifugation 10 minutes, 10,000 rpm) were used to precipitate protein. 500µl methanol, 200µl chloroform, and 300µl distilled water were added to over 500µl of sample then the mixture was centrifuged for 3 minutes at 1000 rpm. The supernatant was removed and 300µl methanol was added to the pellet. The mixture was stirred and centrifuged again at 1000 rpm for 3 minutes. The precipitated proteins were air-dried. 25µl SDS buffer was added to samples, and they were boiled for 5 minutes. SDS-PAGE was performed as (Dawoud, 2012). Comparative protein profile for five protein samples was carried out using Ruler™ unstained protein ladder Ferment as containing a mixture of 14 highly purified proteins ranging in size between 10kDa to 200kDa and molecular weights of proteins present in the sample were determined.

Statistical analysis

All experiments were carried out in triplicate except HPLC and amino acid analyzer samples were analyzed with only one replicate. The presented results are the means of replicates, and the standard deviations are shown as error bars in the figures. Data handling and statistics were performed using the Excel software package (Microsoft Excel 2010). The data were subjected to analysis of variance (ANOVA) (Sugimoto, 2004) and comparing all pairs using Tukey's test by (Mastate program). The least significant difference was used to compare means of treatment at 5% probability (α=0.05) (Walter and Duncan, 2003).

RESULTS AND DISCUSSION

Effect of metals ions on *Candida utilis* biomass

After 4 days of fermentation, all metal ions resulted in a significant increase of *Candida utilis* AUMC 10703 biomass, only Co^{2+} decreased the dry weight compared to the control as illustrated in Figure 1 (p<0.05). W^{6+}, $Mo^{6+,}$ and Ni^{2+} stimulated *C. utilis* biomass production at10 µM with 112%, 117 %, and 114% respectively. B^{+} and Sn^{2+} increased *C. utilis* biomass by 137% and 117% at 50 µM respectively.

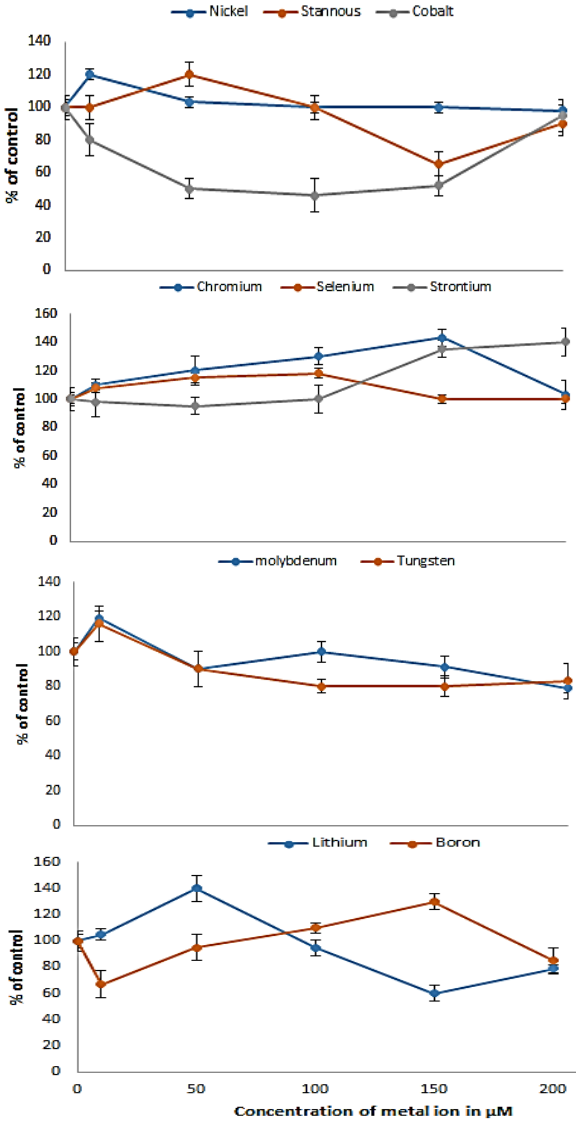

Figure 1. Effect of different metal ions concentrations (10, 50, 100, 150, 200 µM) on dry weight gain (g/L) of *Candida utilis* (p<0.05).

For these metal ions, *C. utilis* biomass was negatively affected at higher concentrations (100, 150, 200 µM). These results agree with (Zhu and Tan, 2009) who reported that some enzymes as hydrogenases catalyzing the synthesis of acetyl-CoA require low concentrations of nickel, nickel is only required as a trace element and may be toxic to cells when it is used in high concentration (Kim et al., 2015). Molybdenum is a bio-essential for microbes' metabolism and it acts as a co-factor for several enzymes (Mendel and Bittne, 2006). No evidence of genotoxicity of molybdenum substances while toxic effects seem to be due to the accumulation of the elements and metabolic unused or alteration of membrane permeability due to toxic effects (Burzlaff et al., 2017, Mitchell, 2009). Tungsten is known to be a biological antagonist of molybdenum and the addition of tungsten to a medium result in the formation of inactive analogs of Mo^{6+} containing enzymes (Morozkina et al., 2005). The ability of the Rh. glutinis yeast to grow in a tungsten-containing medium suggests either synthesis of alternative enzymes or tolerance of the existing enzymes to tungsten. Increasing tungstate concentration enhanced conversion efficiency for both butyrate and propionate. Gene expression analysis suggested that tungsten-containing formate dehydrogenase (FDH) and tungsten-containing aldehyde ferredoxin oxidoreductases (AORs) were involved in the improved biosynthesis of acetate, ethanol, 1-propanol, and 1-butanol. AORs and FDH contribute to the fatty acids' re-assimilation pathway (Ammam et al., 2016). Boron is a growth stimulator for Saccharomyces cerevisiae at optimal concentration, but excess boron can cause toxicity in yeast cells (Bennett et al., 1999, Uluisik et al., 2011). Sn^{2+} is both toxic and mutagenic to yeast forming DNA lesions at high concentrations (Silva et al., 1994). Yeast cells of exponentially growing cultures were killed to about the same extent at 0.1% of $SnCl_2$ as respective cells in the stationary phase, suggesting that a major involvement of physiological parameters of post-diauxic shift oxidative stress resistance to enhanced stannous tolerance (Pungartnik et al., 2005).

On the other hand, Cr^{3+}, Se^{4+}, Li^{+}, and Sr^{2+} had a significant enhancement at higher concentrations, $Cr^{3+}$and Li^{+} achieved maximum biomass increase with 145% and 130% at 150 µM respectively. Se^{6+} increased the biomass by 137.5 % at 200µM, while Sr^{2+} increased it by 114 % at 100 µM. The results suggest that *C. utilis* biomass was sensitive to Co^{2+} even under low concentration 10 µM. Metal-induced toxicity towards yeast is expressed at the levels of both cytotoxicity and genotoxicity through damage inflicted on cellular proteins andDNA. The inhibitory effect of Co^{2+} may be the result of the high production of vitamin B12 in yeasts which is a coenzyme for the methyl transferase mediating the terminal step in the biosynthesis of methionine (Kruszewski, 2003). Also, Co^{2+} reduced microbial growth by inducing oxidative stress or due to its competition with nutritional cations (Thorgersen and Downs, 2009). Resistance to chromate in yeasts can be caused by deficient sulphate transport and by impaired chromium uptake by the yeast cells, the chelation of Cr^{3+} increases the survival of the cells by the formation of less toxic organo-Cr^{3+} complexes (Ksheminska et al., 2006). Microbial respiration rates decreased linearly with increasing lithium concentrations and as lithium concentrations increased, less nitrogen was utilized relative to carbon (Porter and Bernot, 2010). *C. utilis* characterized by high resistanceto oxidative stress associated with a higher concentration of selenium in the culture medium than that of *S. cerevisiae* (Infante et al., 2014). Yeasts are characterized by high cell biomass production and a relatively high capacity for accumulation of selenium in a culture medium supplemented with a high concentration of selenium (Kieliszek et al., 2019). Selenium inhibited the growth of yeast *Candida utilis* ATCC 995 and increased the activity of antioxidative enzymes (glutathione S-transferase, glutathione reductase, glutathione peroxidase, and thioredoxin reductase (Kieliszek et al., 2020).

Analysis of the nutritional value of *Candida utilis* biomass

Spectrophotometric analysis: Protein content, amino acid content, total carbohydrate and total organic acids were estimated in culture media and cell-free extract of *Candida utilis* AUMC 10703 using seven treatments Co^{2+} at 100 µM, Cr^{3+} at 150 µM and W^{6+} at 50 µM, mixture at the same concentrations of (Co^{2+}, W^{6+}), (Co^{2+}, Cr^{3+}), (Cr^{3+}, W^{6+}) and finally mixture of (Cr^{3+} + Co^{2+} and W^{6+}). DNA and RNA content were only determined in biomass. Cr^{+3} had negative impact on organic acid production by *C. utilis* o n intracellular or extracellular levels, while CO^{2+} increased the production extracellularly and intracellularly compared to the control. W^{6+} increased its production only inside the biomass. Maximum enhancement of organic acids production was achieved using a mixture Co^{2+}, W^{6+}, and Cr^{3+} with 329% in the biomass compared to the control. On the

other hand, the highest extracellular production of organic acids was achieved using (Co^{2+}, W^{+6}) mixture by 157%, followed by using either mixture of (Co^{2+}, W^{6+}, Cr^{3+}) that increased organic acids extracellularly with 146% as demonstrated in Figures 2 and 3. Also, (Co^{2+}, Cr^{3+}) mixture achieved 146% enhancement extracellularly. Sayer et al. (2001) revealed the binding ability of cobalt by organic acids in biomass *Aspergillus niger* and culture filtrates if grown in the presence of insoluble cobalt phosphate so we can conclude that the presence of cobalt in culture media induces the production of organic acid in biomass and culture filtrate. A mixture of (Co^{2+}+ Cr $^{3+}$ + W^{6+}) induced the highest enhancement in the nutritive value of *C. utilis* biomass. They increased protein content, free amino acid, DNA content, RNA content, and organic acid content to (184%, 141.5%,148.5%,145%, and 329%), respectively. On the other hand, residual carbohydrate content decreased to 23% compared to control using the mixture of (Co^{2+}+ Cr^{3+} + W^{6+}) as illustrated in Figure 3. However, all metal ion treatments hurt carbohydrate content. It was suggested that the presence of chromate reductase activity in a medium supplemented with chromate ions was possibly associated with an increase in the cytosolic or membrane proteins (Martorell et al., 2012).

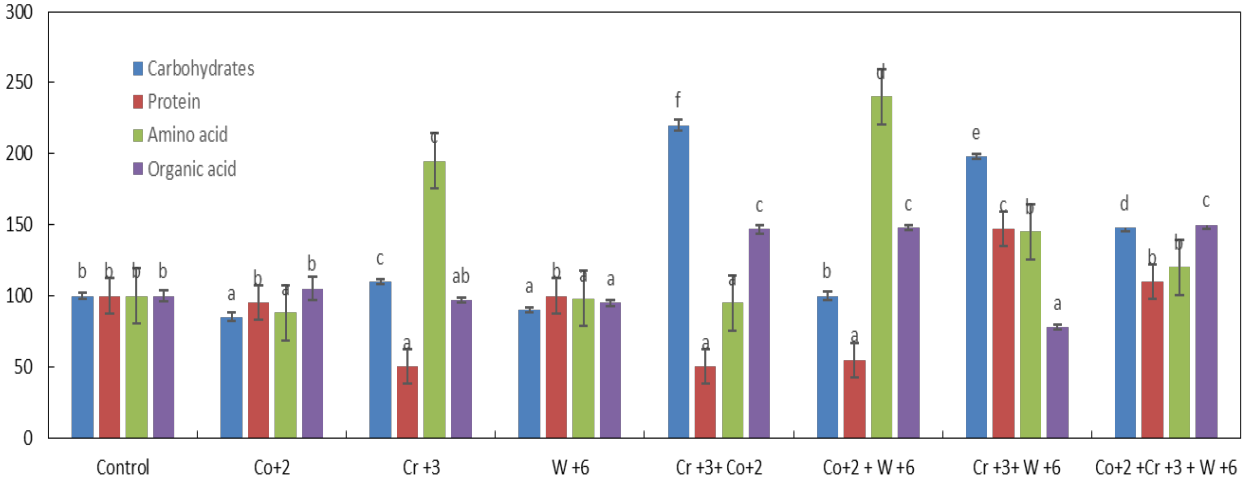

Figure 2. Effect of metal ions treatment (Co^{2+}, Cr^{3+}, W^{6+}, Co^{2+} + Cr^{3+}, Co^{2+} + W^{6+}, Cr^{3+}+ W^{6+} and Co^{2+} + Cr^{3+}+ W^{6+}) on *Candida utilis* AUMC 10703 productivity of carbon and nitrogen compounds in culture filtrate compared with control (p=<0.05).

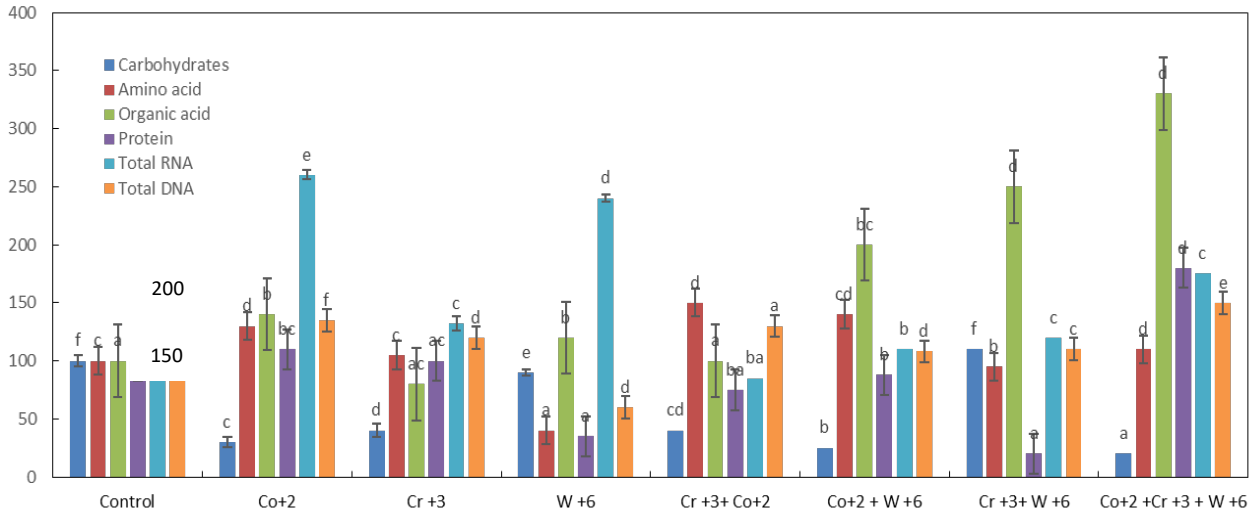

Figure 3. Effect of metal ions (Co^{2+}, Cr^{3+}, W^{6+}, Co^{2+} + Cr^{+3}, Co^{2+} + W^{6+}, Cr^{3+}+ W^{6+} and Co^{2+} + Cr^{3+}+ W^{6+}) on *Candida utilis* AUMC 10703 productivity of carbon, nitrogen and nucleic acid compounds by cell biomass compared with control, (p=<0.05).

C. neoforman, *C. albicans,* and *S. cerevisiae* respond to metal stress by the formation of molecules to which elements are bound; these molecules are divided into proteinaceous and non-proteinaceous forms. Proteinaceous may be metal stress proteins, metal transport proteins, and metalloenzymes, while non-proteinaceous molecules are such as organic acids and polysaccharides (Wehmeier et al., 2020). Cobalt is an essential trace element for the formation of vitamin B12 in microorganisms that is an essential part of two enzymatic systems involved in multiple metabolic reactions, such as in the metabolism of carbohydrates, amino acids, and DNA (González-Montaña et al., 2020). This contrasts with the finding of (Robins et al., 2013) stated that Cr^{6+} can be reduced indirectly by increasing the formation of redox intermediate organic compounds such as amino acids, nucleotides, sugars, and organic acids. Giner-Lamia et al. (2014) reported that cytoplasmic tungsten molecules in microorganisms can cause serious damage to DNA, lipids, and other proteins such as enzymes. While Roy and Adams (2020) indicated that the growth of *P. furiosus* is dependent upon tungsten, three tungsten-containing enzymes were isolated from this organism which oxidizes aldehydes of various types to organic acid the enzymes are termed aldehyde. ferredoxin oxidoreductase (AOR) glyceraldehyde-3- phosphate ferredoxin oxidoreductase (GAPOR) and formaldehyde ferredoxin oxidoreductase (FOR), these enzymes have broad substrate specificity but are most active with aldehydes-derived from amino acids (via transamination and decarboxylation) this also means that tungsten increasing production of organic acid in culture medium by reducing amino acid content.

Amino acids analyzer analysis: The amino acids profile of *Candida utilis* AUMC 10703 biomass and was analyzed for control and two metal ions treatment: W^{6+} (the lowest amino acids content in the biomass) and Co^{3+}, $Co^{2+,}$ and W^{6+} mixture (the highest amino acids content in the biomass). As demonstrated in Table 1, comparing the amino acids profile of *C. utilis* control with that of food and agriculture organization (FAO) indicated that glutamic acid was the most abundant amino acid followed by alanine and aspartic acids and all amino acids concentrations were higher than FAO reference except for isoleucine, tyrosine, phenylalanine, histidine, proline, cysteine, and methionine. For the treatments, (CO^{2+} + Cr^{3+}+ W^{6+}) mixture increased the amount of all essential amino acids except cysteine and glycine, while W^{6+} decreased nearly all the amino acids content in biomass except tyrosine and arginine concerning the control. So, Cr^{3+} + Co^{2+} and W^{6+} mixture treatment is promising for SCP production. Glutamic, alanine, and aspartic acid were the most abundant amino acids of *Candida* biomass either in the presence or absence of a metal ion mixture. The SCP resulted from *C. utilis* containing all the essential amino acids for human nutrition (Rajoka et al., 2004). The highest concentrations of aspartate and glutamate (10.4 and 18.09 % respectively) were also present in marine yeast *Candida* biomass while cysteine, methionine, histidine, and arginine had the lowest concentrations (1.14-2.87%) (Joseph, 2009). Alanine, histidine, and leucine were observed to be the highest (8-10.4 %) amino acids content of two marine yeasts *Debaryomyces hansenii* Yeast-14 and *Candida austromarina* Yeast-16, and found, while cysteine, serine, proline, and methionine were lowest (0.4 - 3.9%) (Kang et al., 2006).

HPLC analysis of organic acids and carbohydrate profile: The organic acids content of cell-free extract for control and metal ions treatments with low organic acids content (Cr^{3+} and W^{6+}) and high total organic acids content (Co^{2+} and W^{6+}) were estimated by using HPLC. CO^{2+} and W^{6+} mixture increased organic acid content extracellularly to 1249.24 mg/L but Cr^{3+}+ W^{6+} mixture gave the least amount (228.738 mg/L) confirming the previous analysis as illustrated in Table 2. CO^{2+} and W^{6+} mixture inhibited all the organic acids that were present in the control (oxalic, succinic, acetic, propionic acid) and induced the production of new organic acids that were not present in the control (citric, pyruvic, and malic acid).Cr^{3+}+ W^{6+} mixture inhibited oxalic and succinic acid production, but the same mixture increased acetic and propionic acid production (139%, and 202% respectively) concerning the control. Succinic acid (390.94 mg/L) was the most abundant in the control treatment followed by oxalic acid (115.941 mg/L), succinic acid and oxalic acid were the most abundant ones also using Cr^{3+}+ W^{6+} but with much lesser amounts.

Succinic acid production by yeasts is significantly increased if fermentation is conducted in the presence of oxygen (Rosenfeld and Shaeffer, 2004). Oxalic acid is produced by a variety of fungi and *A. niger* is a very efficient oxalic acid producer (Dutton and Evans, 1996). On the other hand, citric acid (653.037mg /L) was the most abundant organic acid using (CO^{2+}+ W^{6+}) mixture treatment followed by

malic acid (437.688 mg/L). Citric, pyruvic, and malic acids production was only induced using metal ions treatment either (CO^{2+}+ W^{6+}) mixture or (Cr^{3+}+ W^{6+}) mixture, but they never were detected in the control treatment, Citric, pyruvic, and malic acids were far higher using (CO^{2+}+ W^{6+}) mixture. Propionic acid was absent using CO^{2+}+W^{6+} mixture. *Ralstonia metallidurans* exposed to chromate showed increased expression in genes related to glyoxylate and dicarboxylate cycle, pyruvate metabolism, and butanoate metabolism and this explains a variety of organic acid profiles in culture medium supplemented with Cr^{+} (Monsieurs et al., 2011). Carbohydrate profile for biomass of control and metal ions treatments with low total carbohydrate content (Co^{2+} + Cr^{3+} and W^{6+}) and high total carbohydrate content (Cr^{3+} and W^{6+}) were estimated using HPLC as demonstrated in Table 3. The result indicated that xylose and fructose were the most abundant sugar in cellular carbohydrate control biomass, both metal ions treatment had a high negative impact on carbohydrate content. Metal ions treatment was able to induce the production of certain sugars that were not present in the control. Cr^{3+} and W^{6+} mixture induced raffinose and glucose production, while in $Co^{2+,}$ $Cr^{3+,}$ and W^{6+} mixture induced raffinose, glucose, and maltose production.

SDS –PAGE analysis

SDS–PAGE of proteins in culture media and biomass of *C. utilis* AUMC10703 under metal ions treatments with low protein content and high total protein content were performed as demonstrated in Figure 4 and Table 4. Comparative analysis of lanes for culture media revealed the presence of five extracellular proteins with the following size (45, 40, 35, 25, and 10 KDa) in the control. Using Cr^{+3} or $Cr^{+3}$and W^{+6}mixture induces the production of three extracellular proteins that were not present in control with size 50, 56, and 60 KDa. Five extracellular proteins were produced in control and their production level increased using Cr^{3+} or $Cr^{3+}$and W^{6+} mixture with sizes 35, 40, and 45 KDa. Protein pattern analysis of *C. utilis* AUMC10703 biomass revealed the presence of five intracellular proteins with the following size (60, 56, 50, 48, and 25 KDa). Using W^{6+} only decreased the production level of the intracellular proteins with size (60, 56, and 50 KDa) compared to the control. While Cr^{3+}+Co^{2+}+W^{6+} mixture induced the production of the two new intracellular proteins with size 75 and 72 KDa that were not present in the control. Also, the production of the intracellular protein with size 25KDa increased using Cr^{+3}+C^{+2}+ W^{+6} mixture. Intracellular protein with size 48 KDa that was present in the control treatment was produced using W^{6+} treatment.

Table 1. Effect of metal ions (Co^{2+} + Cr^{3+} + W^{6+}) and (W^{6+}) on amino acid profile of *Candida utilis* AUMC 10703 represented as (mg/g) and %control.

Amino acid	Control (% mg/g)	W^{6+}	% Control	Co^{2+}+W^{6+}+Cr^{3+}	% Control	FAO/WHO reference
Aspartic	34.1	31.1	91%	39.5	116%	18.5
Threonine	21.1	20.6	98%	26.2	124%	10.0
Serine	19.5	18.6	95%	30.3	155%	18.0
Glutamic	45.6	43.3	95%	47.5	104%	18.2
Glycine	19.5	17.8	91%	18.6	95%	18.5
Alanine	36.4	35.2	97%	37.6	103%	18.1
Valine	21.6	20.4	94%	27.7	128%	16.0
Isoleucine	19.2	19.0	99%	24.3	127%	22.0
Leucine	27.9	26.8	96%	32.5	117%	22.0
Tyrosine	14.5	14.9	103%	17.3	119%	28.0
Phenylalanine	17.1	16.3	95%	22.5	132%	22.0
Histidine	8.40	8.10	96%	9.50	113%	18.5
Lysine	28.6	27.3	95%	32.4	133%	16.0
Argnine	19.7	20.2	103%	24.6	125%	17.8
Proline	15.9	14.5	91%	26.4	166%	18.4
Cysteine	8.10	6.50	80%	4.00	49%	22.0
Methionine	5.20	5.70	100%	5.70	108%	22.0

Table 2. Effect of Co^{2+}+ W^{6+} and Cr^{3+}+W^{6+}treatment on organic acid profile of culture filtrate of *Candida utilis* AUMC 10703 represented as mg/L.

Organic acid	Control (mg/L)	Co^{2+}+W^{6+}	%Control	Cr^{3+}+W^{6+}	% Control
Oxalic acid	115.941	91.029	78. 5%	84.65	72%
Citric acid	–	653..037	*	21.68	*
Pyruvic acid	–	20.056	*	0.016	*
Malic acid	–	437.688	*	18.78	*
Succinic acid	390.99	44.229	11%	103.48	27%
Acetic acid	0.323	0.0202	6.25%	0.45	139%
Propionic acid	0.043	0.00	0.00%	0.087	202%
Total	507.296	1249.24	246%	228.738	46%

Table 3. Effect of (Cr^{3+}+W^{6+}+ Co^{2+}) and (W^{6+}+ Cr^{3+}) mixture on intracellular carbohydrate ofCandida *utilis* AUMC 10703 biomass represented as mg/g. *These sugars were only induced under the treatment and were not present in the control.

Carbohydrate mg/g	Control	Cr^{3+}+W^{6+}	% Control	Co^{2+}+W^{6+}+ Cr^{3+}	% Control
Xylose	12.49	3.92	31%	2.42	21%
Fructose	11.76	4.47	38%	1.80	14%
Trehalose	3.78	0.46	12%	1.16	31%
Lactose	5.95	1.79	3%	–	0.00%
Glucose	–	4.24	*	3.94	*
raffinose	–	16.58	*	1.94	*
Maltose	–	–	–	1.95	*
Total	33.98	31.45	63%	13.23	39%

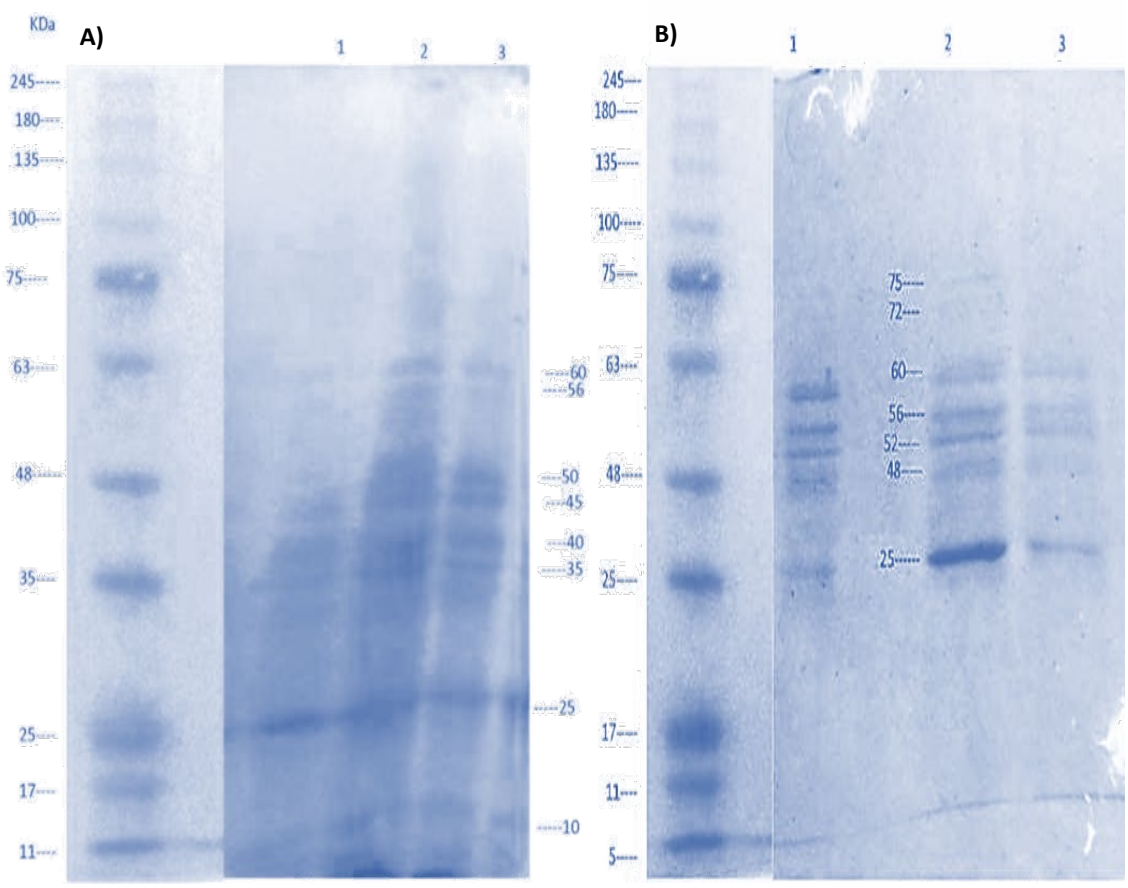

Figure 4. SDS-PAGE showing protein profile for *Candida utilis* AUMC 10703: A) for culture filtrate lane1: control, lane 2: medium supplemented with Cr^{3+} + W^{6+}, lane 3: culture medium supplemented with Cr^{3+}, B) for biomass lane 1: control, lane to 2: (Cr^{3+}+ Co^{2+} +W^{6+}) treatment, lane 3: W^{6+} treatment supplemented with Cr^{3+}.

Table 4. Protein molecular mass of culture filtrate and biomass of *Candida utilis* AUMC 10703 using metal ions compared with untreated control, intensity of protein bands (-): absence of band, (+): light, (++): moderate intensity, (+++):high intensity.

Molecular mass (KDa)	Culture filtrate			Biomass SCP		
	Control	Cr^{3+}+W^{6+}	Cr^{3+}	Control	Cr^{3+} +Co^{2+}+ W^{6+}	W^{6+}
75	-	-	-	-	+	-
72	-	-	-	-	+	-
60	-	+	+	++	++	+
56	-	+	+	++	++	+
52	-	-	-	++	++	+
50	-	+++	++	-	-	-
48	-	-	-	+	+	-
45	+	+++	++	-	-	-
40	+	+++	++	-	-	-
35	+	+++	++	-	-	-
25	++	+++	++	+	+++	+
10	+	+	+			
Total band	5	8	8	5	7	4

CONCLUSION

It was concluded that the mixture of (Co^{2+}+ Cr^{3+}+ W^{6+}) is promising for enhancement of *Candida utilis* AUMC 10703 biomass nutritive value as SCP as it contains high concentration of essential amino acids for food and feed as aspartic, glutamic, alanine and lysine, while (Co^{2+}+ W^{6+}) is promising for industrial use for organic acids production especially citric, malic and pyruvic acids. No previous literature on the use of Sr^{2+}, W^{6+}, Li^{+}, $Sn^{2+,}$ and B^{+} for the improvement of *Candida utilis* biomass. In future studies, agricultural waste should be investigated to be studied in combination with metal ions treatments as a cheap and eco-friendly method to produce Torula. Also, other metal treatment combinations and their interactions should be harnessed.

ACKNOWLEDGMENT

The authors would like to thank all members of the Botany and Microbiology Department, Faculty of Science, Cairo University.

DATA AVAILABILITY

The data used and analyzed in this study are available from the corresponding author upon reasonable request.

FUNDING

No funding was received

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Applicable.

CONSENT FOR PUBLICATION

Applicable.

CONFLICT OF INTEREST

The authors declare no competing interests.

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