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LACTIC ACID BACTERIA AS METAL QUENCHERS TO IMPROVE FOOD SAFETY AND QUALITY

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Abstract

Lactic acid bacteria (LAB) have long history of safe use in diverse fermented dairy and food products and offers health enlivening possessions as probiotics. Apart from this, recently, several Lactobacillus and Bifidobacterium spp. have been found to bind with metal ions through metabolism independent mechanism like surface binding by adsorption and ion exchange. The major contaminants encountered in milk and other food products chiefly include pesticide residues, heavy metals, and aflatoxin M1. Milk may get contaminated before milking from the cattle feed, sources/materials employed during the milk processing and inappropriate handling of milk during the pre- and post-processing period. It will ultimately lead to magnification of the specific metal or related contaminants in the finished product. The bioquenching/biosorption abilities of LAB will help to formulate natural treatments to decrease metal toxicity from drinking water, dairy and food products as well as within human body through development of functional foods and nutraceuticals.

***Key words**: heavy metals, lactobacilli, gut microbiota, bioquenching, biosorption, detoxification.*

INTRODUCTION

Heavy metals are widespread in nature. The rapid development of industrialization has resulted significant quantities of heavy metals release into the environment which is a serious hazard for mankind as well as animals. Cadmium (Cd^{+2}), copper (Cu^{+2}), lead (Pb^{+2}), zinc (Zn^{+2}), chromium (Cr^{+2}), arsenic (As^{+2}), mercury (Hg) and nickel (Ni^{+2}) are highly toxic metals ions to which people are exposed primarily through food and water. Heavy metal depositions are associated with a wide range of sources such as industries, vehicles and pesticides which can significantly affect the safety and quality of food (Fertmann et al., 2004). With rapid increase in the consumption of canned food products throughout the world, the possibility of food contamination with different metals is expected. Metals used in food packaging material or food processing equipment may contribute to contamination. Migration of compounds from packaging materials like metal cans into packaged food is well known (Mrvcic et al., 2012). Increased environmental metallic load is of serious concern. Prolonged consumption of unsafe concentrations of heavy metals in foodstuffs may disrupt the normal functioning of human body. Heavy metal accumulation gives rise to toxic concentrations in the body, while a number of elements (e.g. cadmium, chromium, arsenic) act as carcinogens and others (e.g. mercury and lead) are associated with developmental abnormalities in children (Canfield et al., 2003; Jarup and Alfven, 2004).

Methods for removing metal ions from aqueous solution mainly consist of physical, chemical and biological technologies. Conventional methods like ion exchange, precipitation, flocculation, membrane filtration etc., are employed for removal of metals from water at low concentrations. However, these available methods claimed to be expensive and inefficient, especially for developing nations where major pollution occurs. This has led researchers to seek alternative solutions for decontaminating heavy metals from surrounddings. Over past decade, a vast array of natural biological material, especially bacteria, algae, yeasts and fungi has gained much attention for resolving problem of heavy metal contamination. Bacterial species, such as *Bacillus, Pseudomonas*, *Escherichia, Micrococcus*, etc., have been tested for uptake of metals or organics. Bacteria may either possess the capacity for biosorption of many elements or, alternatively, may be element specific depending on the species (Wang and Chen, 2009).

Biosorption can be described as the process to remove metal or metalloid types, compounds and particulates from solution by biological material (Gadd, 1993). Use of inactivated microbial biomass as an adsorbent or biosorptive agent, has been suggested as an effective and cost-effective alternative for the exclusion of heavy metals from water, and the removal of a number of different minerals by altering microbiota have has been studied (Davis et al., 2003; Mehta and Gaur, 2005). These biosorbents possess metal-sequestering traits and can be employed to reduce the concentration of heavy metal ions in solution from ppm to ppb level. Biosorbent can efficiently sequester dissolved metal ions out of dilute complex solutions with high efficiency and swiftly, thus it can considered as an ideal candidate for the treatment of high volume and low concentration complex wastewaters (Wang and Chen, 2006). Many researchers have described the bioquenching or chelating property as an efficient and economical alternative in heavy metal removal from water and food (Bezawada and Rao, 2011). However, when it comes to food industry, it is of much concern regarding food safety and quality. Hence, approaches made to select metal ions induced stress resistant food grade bacterial group. In this context, one of the significant food grade bacterial groups known as lactic acid bacteria (LAB), appears a vital alternate.

LAB as starter culture in food industry must be suitable for large-scale industrial production and possesses the ability to survive in unfavourable processes and storage conditions. In current scenario, much attention is devoted to research of health aspects of LAB related to food fermentations or as probiotics. Above all, LAB has gained Generally Regarded as Safe (GRAS) status. It has been described that probiotic bacteria have the capacity to bind many toxic compounds like food-borne mutagens (Turbic et al., 2002), microbial toxins such as aflatoxins and microcystin-LR (Haskard et al., 2001; Meriluoto et al., 2005; Zoghi et al., 2014) in aqueous solution. Moreover, within the microenvironment of gastro-intestinal tract also several probiotic bacteria could bind with aflatoxin B1 (El-Nezami et al., 2000, 2006) and food-borne mutagen Trp-P-2 (Orrhage et al., 2002), thereby reducing their uptake. Few studies reported metal chelating properties of LAB. Probiotic LAB had proven to bind cadmium from water (Halttunen et al., 2003). On the other hand, the LAB interactions with metal ions and their possible applications have received less attention. Binding of heavy metals on the LAB could be a promising solution for the removal of toxic heavy metals from water, liquid food and from the body (Schut et al., 2011) while leaching of trace elements could enhances the value of fermented foods. The significance of food grade bacteria as metal quenchers in dairy and food industry is schematically represented in Figure 1.

Current review briefly discusses results demonstrating the new field of LAB application in detoxification processes as well as enhancing the nutritional value of food.

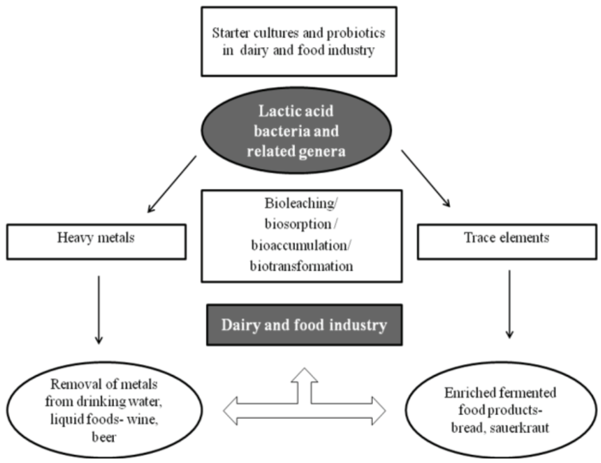

Figure 1. Functions of Food grade bacteria as metal quenchers to improve food safety and quality

MECHANISMS PROPOSED FOR BIOQUENCHING OF HEAVY METALS IN LACTIC ACID BACTERIA

The interaction of microorganism with metals to remove heavy metals from contaminated sites represents a unique process. Microorganisms have evolved several strategies to either transform the element to a less harmful form, or bind the metal intra- or extra-cellularly thereby preventing any harmful interactions in the host cell or by actively transporting the metal out of the cell cytosol. Metal ions quenching by LAB, as well as other microorganisms, is a complex process that depends on the two basic mechanisms; (i) biosorption—the passive no metabolically mediated binding process of metal ions to the LAB cell wall, and (ii) bioaccumulation—process associated with metabolism in which metal ions pass the cell membrane and accumulate inside the cell (Mrvcic et al., 2012). Usually Gram-positive bacteria have high adsorptive capacity, particularly due to high peptidoglycan and teichoic acid content in their cell walls. Certain Gram-positive gut microflora like lactobacilli used in food applications may potentially be an adjunct for reducing metal toxicity in individuals. Generally such bacteria have resistance mechanisms which are effective in preventing damage to cell itself and they can attach and sequester metal ions to cell surfaces (Sinha et al., 2011). Hydroxyl groups from the peptidoglycan and functional groups (mainly carboxyl group) of proteins have the important roles in metal ions binding by LAB, too (Mrvcic et al., 2012; Lin et al., 2005; Gerbino et al., 2011). Physical forces like electrostatic interactions are also responsible for passive binding of heavy metals on LAB surface (Salim et al., 2011). LAB metabolites including organic acids, exopolysaccharides, etc. also enhanced heavy metal biosorption efficacy of specific strains**.** Organic acids may chelate toxic metals (Gavin et al., 1998), producing metallo-organic molecules. Organic acids reduce the pH of environment, which lead to increases the solubilisation of metal compounds and leaching of metals from their surfaces whereas it is predicted that functional carboxyl groups found on microbial polymers can be biosorbed or complexed with heavy metals (Perpetuo et al., 2011).

Apart from the cell wall structure, some physicochemical parameters such as initial concentration of metal ions, pH, biosorbent concentration, contact time, and variation of temperature can also affect the biosorption process (Mrvcic et al., 2012). The detoxification of organic mercury in bacteria involves conversion of methylated mercury to inorganic mercury (Hg^{+2}), which does not absorbed well in the GI tract, and then to Hg^0, which is poorly absorbed (Monachese et al., 2012).

STUDIES REGARDING BIOQUENCHING OF HEAVY METALS IN LACTIC ACID BACTERIA

Urban and Kuthan, in 2004 reported xenobiotic detoxification therapy by probiotic. Authors assumed that probiotic bacteria could be used to decrease the heavy metals intake in humans directly in the lumen of intestine. Halttunen and co-workers (2003), have extensively investigated cadmium, lead and arsenic binding by different LAB and indicated that food and water are the primary sources of this metal ions exposure among the non-smoking population. Halttunen et al. (2007) carried out work on removal of cadmium and lead by specific LAB; *Lactobacillus rhamnosus* GG (ATCC 53103), *L. casei Shirota, L. fermentum* ME3, *Bifidobacterium longum* 2C, *B. longum* 46 and *B. lactis* Bb12, and two commercial starter cultures: FVDVS XT-303-eXact (*Lactococcus lactis* subsp. *cremoris, Lc. lactis* subsp. *lactis, Leuconostoc mesenteroides* subsp. *cremoris, Leuc. pseudomesenteroides* and *Lc. lactis* subsp. *lactis* biovar. *diacetylactis*) and YO-MIX 401 (*Streptococcus thermophilus* and L. *bulgaricus*) from water. They found significant removal which was found to be more specific to strain and metal, both. The removal was a rapid, metabolism-independent surface process. It was also strongly influenced by pH, indicative of involvement of ion exchange mechanisms. The most effective metal removers were *B. longum 46*, *L. fermentum* ME3 and *B. lactis* Bb12. The highest maximum cadmium and lead removal capacities obtained for strain *B. longum* 46 were 54.7 and 175.7 mg metal per g of dry biomass, respectively.

Kumar and Rao (2011) compared the efficiency of free and immobilized cells of *Saccharomyces cerevisiae* and *L. sporogenes* for the removal of heavy metals (Cu^{+2} and Pb^{+2} ions) from the aqueous system. The key factors such as pH, temperature, incubation time, and initial concentration of biomass were verified for process optimization. The best results were obtained for co-immobilized and immobilized cells than free cells. Selected micro-organisms exhibited maximum removal at an optimum concentration of 1 mg/mL, optimal time of 180 min, optimum temperature of 35^0C and pH 4-6.

In a recent study, Rayes (2012) examined the effect of probiotic lactic acid bacteria; *L. rhamnosus* GG, *L. fermentum* ME3, *L. bulgaricus* (Commercial strain) and *L. acidophilus* X 37 in removal of lead, cadmium and copper from cultured water in fish farming system for marine tilapia *T. spilurus*, and in addition he studied the effect of heavy metals lead and cadmium and copper on genotoxicity of tilapia fish as bio-indicator for heavy metal toxicity. The highest total concentration of removal obtained for *L. acidophilus* X37 (97.6) and subsequently followed by *L. rhamnosus* GG (74.8), *L. fermentum* ME3 (71.16) and *L. bulgaricus* (61.00). It was found that the optimal pH for *L. fermentum* ME3, *L. rhamnosus* GG, and *L. acidophilus* X37 was 6.0 while for *L. bulgaricus,* it was 5.0. The percentage removal of heavy metals depend on temperature where, the highest activity obtained at temperature between 25^oC and 37^oC then the activity was declined at 43^oC.

Bhakta and co-workers (2012) found out in their study that 26 probiotic LAB exhibited remarkable variations in their metal resistance as well as metal removal aptitudes. Of 26, seven strains (Cd54-2, Cd61-7, Cd69-12, Cd70-13, Pb82-8, Pb96-19 and Cd109-16) and four strains (Pb71-1, Pb73-2, Pb85-9 and Pb96-19) displayed relatively elevated cadmium- and lead-removal competences from water, respect-tively, in compared to the remaining strains. Strains Cd70-13 showed the highest cadmium (25%) and Pb71-1 showed the highest lead (59%) removal capacity from MRS (De Man, Rogosa and Sharpe) culture medium among all tested strains and demonstrated superior adhesive ability on fish mucus. A phylogenetic investigation of 16S rDNA sequences suggested that the two strains Cd70-13 and Pb71-1 were belonged to *L. reuteri*.

The surface molecules of LAB have been reported to bind with heavy metals passively mainly through electrostatic interactions. The supplementation of LAB to heavy metals treated group of rats, considerably enhanced the glutathione peroxidise activity together with the ratio of body weight and feed efficiency; and also lifted up liver and kidney functions (Salim et al., 2011). Beneficial health effects of LAB may be attributed to their aptitude to bind cadmium and lead that led to remove metal toxicity. On the other hand, Battikh et al. (2011) established a reduction in negative effects of cadmium on the iron distribution in tissues and organs of broiler chicken in the presence of LAB.

Copper bioquenching

In a study performed by Mrvcic et al. (2012), *L. brevis* was found to be the most resistant strain to copper; accumulated high concentration of Cu among all other tested strains. In previous study by the same researchers *L. brevis* was the most effective in copper binding with 26.5 mg/g followed by *Leuc. mesenteroides* (26 mg/g) and *L. plantarum* (15.5 mg/g) (Mrvcic et al., 2009a, b). On the other hand, Yilmaz et al., (2010) reported highest removal by *E. faecium* (106 mg/g). During cheese manufacturing, the influence of copper ions on LAB is highly significant. Swiss cheese Emmental is produced in particular vats made up of copper metal; thus, during cheese manufacturing process the copper ions can leach out in milk and influenced the growth of LAB due to acid production (Rodriguez and Alatossava, 2008).

While determining antioxidant potential of few LAB, Lee et al., (2005) revealed that *L. casei* strains showed a wide range of Fe^{+2} and Cu^{+2} chelating ability, from 1.1 to 10.6 ppm and from 1.35 to 21.8 ppm among the tested strains. In that, *L. casei* KCTC 3260 exhibited the higher metal ion chelating ability followed by *L. rhamnosus* GG. Conversely, strain *L. casei* KCTC 3109 showed lower chelating activity for both Fe^{+2} and Cu^{+2} metal ions at 1.1 ppm and 1.35 ppm, respectively.

Chang and Kikuchi, (2013) developed an environmental friendly process in that heavy metals like chromium, copper, and arsenic in the Chromated Copper Arsenate (CCA)-treated wood were able to be extracted through organic acids, in particular pyruvate and lactate produced by *Str. thermophilus* NBRC13957, *L. acidophilus* NBRC13951, *L. bulgaricus* NBRC13953, and *L. plantarum* NBRC15891.

Iron and Zinc bioquenching

LAB were considered to be a bacterial group having no iron requirement. LAB belonging to *Lactobacillus, Carnobacterium, Lactococcus,* *Leuconostoc,* and *Pediococcus* genera do not produce siderophores (Pandey et al., 1994). Also, there was no noteworthy growth stimulation when lactic cultures were grown in either iron supplemented or iron depleted media (Imbert and Blondeau, 1998).

Recently, genome sequences of *L. sakei* 23 K and *L. lactis* MG1363 have shown that LAB have genetic equipment involved in iron transport. The strain *L. sakei* 23 K had shown to possess some putative iron transport systems plus transcriptional regulators belonging to the *Fur* family. Iron is dispensable for the growth of *L. sakei*, but as suggested by Duhutrel et al., (2010) during sausage fermentation, iron sources present in the meat are highly beneficial for *L. sakei* sustaining long-term survival in such meat products.

Zinc is both vital in trace amounts and toxic at high concentrations like some other metals. The emergence of new knowledge about the beneficial effects of zinc on human health is also interesting (Stefanidou, 2006). A group of researchers observed that zinc can increase *L. plantarum* probiotic effect in the intestine as the number of pathogenic *E. coli* was significantly reduced if Zn-sulphate and Zn-propionate was added with *L. plantarum* in laboratory mice (Mudronova et al., 2004). In another study, *Leuc. mesenteroides* was the most effective in zinc binding with 27 mg/g as compared to *L. brevis* and *L. plantarum*, 20 and 10 mg/g respectively (Mrvcic et al., 2009a, b). In model lactic strain, *Lc. lactis* IL1403, ZitSQP is an ABC transporter supposed to be involved in high-affinity zinc uptake (Bolotin et al., 2001). However, very little information is available on systems for zinc uptake, storage and efflux in LAB.

In a recent experiment, Sofu et al., (2015) observed 90-100% Fe^{+2} and 70-90 % Zn^{+2} removal with all tested biomasses of LAB (*L. delbrueckii* ssp. *bulgaricus* Lb-12, *Str. thermophilus* STM-7) under optimal value of process parameters.

Arsenic, Cadmium and Lead bioquenching

When arsenic-contaminated water is used for irrigation, arsenic accumulates in crops prior to consumption and can reach dangerous levels in finished food. In contrast to arsenic, lead and cadmium are cationic. Although they are unique elements with different molecular weights, as well as difference in physiological effects and occurrences in nature, studies on lead and cadmium are often conducted together, as the elements seem to react alike with bacterial species.

Lead contamination of drinking water is frequently due to corrosion of lead-containing plumbing while food is the most important source of cadmium exposure among the non-smoking population. In particular, children are exposed to lead via ingestion of dust, soil and lead containing paints. Lead (Wyatt et al., 1998; Fertmann et al., 2004), but rarely cadmium, is found in drinking water at concentrations greater than WHO (2004) guidelines *viz.*10 μg/l for Pb and 3 μg/l for Cd. Both, cadmium and lead are also found in substantial amount as a result of point contamination, e.g. from industry. Oral exposure to Cd may have many consequences on human health, such as osteoporosis (Jarup and Alfven, 2004), renal damage (Satarug et al., 2011), renal cancer (Waalkes et al., 1999) and possibly prostate cancer (Waalkes et al., 1991). Chronic exposure to even very low cadmium level may perhaps also trigger adverse renal (Jarup et al., 2000) and negative bone effects (Jarup and Alfven, 2004). Alternatively, lead disturbs synthesis of hemoglobin, renal function and causes neurological and behavioural disorder in children (WHO, 1995). In children, very little blood lead concentrations have been linked with intellectual impairment (Canfield et al., 2003).

Few strains of *L. acidophilus* and *L. crispatus* DSM20584 are known to produce S-layer proteins, which may explain their activity against arsenic (Schar-Zammaretti and Ubbink, 2003). Singh and Sharma (2010) showed that *L. acidophilus* was competent to bind arsenic and reduce it from water at 50 to 1,000 ppb levels, and the maximum removal occurred within 4 h of exposure in a concentration-dependent manner. In a separate study, Bhakta et al. (2010) employed six strains of probiotic LAB (As99-1, As100-2, As101-3, As102-4, As105-7, and As112-9) for total removal of As, Cd, and Pb which was varied from 25.47 to 41 μg/l, 692.53 to 804.73 μg/l, and 2598 to 4609 μg/l, respectively. The As uptake efficiency of strain

As102-4 (0.006 μg/h/mg wet weight of cell) was higher (17 – 209%) compared to remaining LAB and 16S rDNA sequencing data revealed 97 to 99% (340 bp) homology to *Pediococcus dextrinicus* or *Pd. acidilactici* for these six isolates.

Ibrahim et al. (2006) also compared the abilities of *Propionibacterium freudenreichii* and *L. rhamnosus* LC-705 to bind and absorb cadmium and lead in aqueous solution. They reported a rapid effect of the bacteria to bind maximal amounts of metal after only 1 h of exposure; this was found to influence by pH.

Using electron microscopy and Fourier transform infrared spectroscopy (FTIR), for two strains of *L. kefir* CIDCA 8348 and JCM 5818, the metal precipitations in the cell S-layer and alteration in the secondary structure of the S-layer have been observed in terms of protein arrangement and structure after metal absorption (Gerbino et al., 2011). In a separate study, Zhai et al. (2013) investigated protective effects of *L. plantarum* CCFM8610 against acute cadmium toxicity in mice. Results revealed that CCFM8610 treatment can effectively decrease intestinal cadmium absorption, reduce accumulation of cadmium in tissues, alleviate renal and hepatic oxidative stress, and ameliorate hepatic histopathological changes as compared to the mice that received cadmium only. Administered living CCFM8610 cells after cadmium exposure offered the most significant protection.

Recently, a group of researchers investigated the capability of selected strains of LAB to remove cadmium, lead and arsenic as well as aflatoxin B1 (AFB1) from contaminated water (Elsanhoty et al., 2016). Among all tested LAB, *L. acidophilus* and *B. anglulatum* were detected as the most effective heavy metal removers.

Chromium, Mercury and Selenium bioquenching

Chromium exposure for longer period can generate resistant lactobacilli strains able to better tolerate metals (Upreti et al., 2011). Shrivastava et al. (2003) showed that lactobacilli and other gut-associated bacteria, along with some human immune cells, can transform chromium to its less-toxic form. Studies in mice revealed gut microbiota provided the first line of defence to the body by converting toxic Cr(VI) to a less-toxic Cr(III). In a recent study, exopolysaccharide obtained from *Lactobacillus* isolate showed 70% decrease in the concentration of Cr^{+2} after 20 day incubation period with the heavy metal from waste water (Khan and Dona, 2015).

Mercury has a long history of use in human applications, yet due to high toxicity its presence in many products has been phased out. Preliminary studies in laboratory have shown that certain strains of lactobacilli appear to sequester mercury (Monachese et al., 2012). However, no published scientific data on the ability of LAB or gut microbiota to bind and absorb mercury exist yet. Recently, the cell surface proteins of *Weissella viridescens* MYU 205 showed binding to mercury (Hg^{+2}) using the Hg (II) column assay and later authors identified, a ~14 kDa protein that might be acted as mercury binding proteins (Kinoshita et al.,2013). Moreover, *W. viridescens* MYU 205 showed high biosorption rate for Hg^{+2} (about 80%) followed by Cd^{+2} and very low for Zn^{+2} (Kinoshita et al., 2013; Kinoshita et al., 2015).

L. plantarum and *L. bulgaricus* seems to be the most promising bacteria for nutraceuticals relevance of selenium-enriched lactobacilli (Diowksz et al., 1999; Xia et al., 2007). LAB can accumulate selenium in elemental form owing to activate detoxification mechanism that includes reduction of tetravalent selenium to elemental selenium. In the food industry, the nutritional value of the food product can be enhanced through LAB mediated biotransformation of inorganic form of Se to the organic form (Alzate et al., 2008; Perez-Corona et al., 2011; Penas et al., 2012). During the growth and lactic acid fermentation process, inorganic selenium, which is potentially toxic and poorly bio-available, is transformed to the harmless and highly bio-accessible organic form. Yogurt, cheese, kefir, sauerkraut, bread and wine are the few examples of food products fruitfully enriched with organic selenium by LAB and yeast during the fermentation of Se-enriched raw material. The maximum Se concentration that allows kefir to be fermented was found to be 2.5 μg/g, much lower than the 50 μg/g found for yogurt (Alzate et al., 2008). As suggested by Famularo et al., (2005), the regular intake of probiotic preparations or LAB containing foods may serve to enhance the bioavailability of essential elements and proteins with simultaneous reduction of toxic metals in food.

CONCLUSIONS AND FUTURE PROSPECTS

Lactic acid bacteria have an established role as starter cultures and probiotics in the dairy and food industry. These eco-friendly food grade organisms can be used as an effective bioquenching/biosorptive agent to reduce the toxicity of heavy metals from foods and water and ultimately preventing their absorption in the human body. Further, their application in food may improve the bioavailability of trace elements. Though very limited information is available in this direction, the outcomes of different investigations done so far are encouraging and indicative of prospective use of LAB as detoxifying agent. Nevertheless, extensive investigation has to be done to make these processes suitable for industrial applications.

CONFLICTS OF INTEREST

All contributing authors declare no conflicts of interest.

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