Author cover page

The influence of Metabolic network structures and Energy metabolic pattern on *E. coli* K12 exposed to acoustic field: based on Gene Ontology and KEGG pathway enrichment analysis

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Abstracts Microorganism is an important part of ecosystems; it is very sensitive to environmental change. In order to study the effects of sound on organisms, it's meaningful to study mechanism of microbial exposure to sound. In our previous experiments, the effects of different sound intensity and frequency on the growth of *E. coli* K12 were studied. It was found that in stationary phase the bacteria concentration of sound expose group was higher than control. In this study, we aimed to understand the mechanisms of *E. coli* underlying sound expose by using Gene Ontology and KEGG Pathway Enrichment Analysis, and established a systematic pathway for the *E. coli* response to sound expose. At 6 hour, the chemotaxis pathway was significantly up-regulated which responses to the changes of the external environment and leads bacteria to favorable environment. At 12 hour, the glycerophospholipid pathway was significant up-regulated, it is related to the energy metabolism and cell division. At 24 hours, the energy metabolism, nucleotide synthesis and transcriptional translation pathways were significant differences. When bacteria entered the stationary phase (36 hour), in sound exposure group the pathways related to reduce the harmful substances were down-regulated and the pathways about degrade aromatic compounds provides energy were up-regulated, so that the *E. coli* K12 in sound exposure have a better ability to adapt poor living environment. Comparative transcriptome analysis of sound exposed *E. coli* K12 can not only reveal the behavior of *E. coli* K12 response to sound expose, but also lay a foundation for further study the mechanism of prokaryotes response to physical stimulus.

Keywords: *E. coli* K12; sound exposure; metabolic network structures; energy metabolic pattern; KEGG analysis

1. Introduction

Acoustic waves are widely present in nature and almost interact with all living organisms. In 1927, the German scientists Wood and Loomis have studied the biological effects of ultrasound (Wood and Loomis, 1927), to the 1960s, the infrasound and the interaction of organisms has gradually been concerned about (Broner, 1978; McKinlay, 2007). Over the years, on the audible sound's biological effects are being researched in-depth. In 1968, the Canadian scientist Weinberger and Measures found that audible sound waves can effectively promote the spring wheat and winter wheat germination and growth (Weinberger and Measures, 1968), Jiang and Huang found that music and crickets mixed sound can promote the growth of edible fungus, extend the harvesting period and improve the production of nutrients; and the same voice can also promote the growth of six kinds of vegetables and three kinds of open field crops, improve the disease resistance (Jiang and Huang, 2012). Japanese scientists Matsuhashi in the study of high salt and high temperature stress on the role of bacteria found that *Bacillus carboniphilus* can be stimulated by the external frequency of 6 ~ 10 kHz, 18 ~ 22 kHz and 28 ~ 38 kHz sonic, regardless of this sound waves from the nearby *Bacillus subtilis* or speakers , it seems that the stimulation effect of low frequency and intensity is more obvious (Matsuhashi et al, 1998). In 2000, Zaretsky, who worked at the Department of Industrial Microbiology and Fermentation at the Department of Biology of the Massachusetts Institute of Technology, discovered the "Humperdink effect". He played the repertoire of Hampeldink for *Escherichia coli* for 48

hours in a row and found the antibiotic production was increased (Wendy, 2009). Wang et al found that the appropriate intensity of sound stimulation can improve the content of ATP in kiwifruit callus, can increase the energy metabolism of cells; a certain frequency and intensity of the sound can improve the germination rate of rice seeds (Yang et al, 2002; Bochu et al, 2003). In our previous study, we also found that *E. coli* K12 showed different growth phenomenon at different sound intensities and frequencies, among which the most obvious difference showed under 8000 Hz and 85D. In addition, we detected the contents of intracellular protein and nucleic acid in different growth stages (Gu et al., 2016).

Most of these studies about sound waves were only detected the differences in growth, few intracellular products' change and so and. Neither establish a systematic intracellular metabolic network structure nor explore the differences in biological gene expression levels under sound exposure. This not only limits the development and utilization of sound waves, but also a lack for studying the mechanism of sound exposure. With the development of transcriptome sequencing technology, it has been applied to study the effects of different environmental factors on organisms. Anne et al. used transcriptome and proteomic analyze the ovary cells of Chinese hamster under low temperature and butyrate treatment, and the mechanism of increased productivity was reveal and the organelles associated with this effect was found (Kantardjieff et al, 2010). Yao et al. explored the biological processes of cotton root cells under salt stress and the key pathways associated with them by using transcriptome analysis (Yao et al, 2011). Vicky used the comparative transcriptome analysis reveals the senescence's differences in gene expression and signal pathway of Arabidopsis between the natural growth and dark/starvation-induced (Buchanan et al, 2005). Gene Ontology and KEGG Pathway Enrichment Analysis can explain the reason for the high cell concentration of *E. coli* K12 at stationary phase under acoustic exposure, and have the potential to reveal the bacteria's respond to sound stimulate. In this study, we made a transcriptome sequencing of *E. coli* K12 at different growth stages under acoustic exposure，established a metabolic network structure and energy metabolism pattern of *E. coli* K12 under the sound exposure. It had not only made great contribution to the study of the mechanism of sound, provided new ideas for the study of other environmental factors, but also laid a foundation for further study of sound wave. To our knowledge, this work is the first to evaluate the changes in gene expression of *E. coli* under sound exposure.

2. Materials and methods

2.1. Escherichia coli strains and growth conditions

E. coli strain K12 was purchased from CGSC（Coli Genetic Stock Center). Bacteria was routinely maintained and cultured in LB broth (1% Tryptone, 0.5% yeast extract, 1% NaCl, pH 7.2) at 37℃.

2.2. Sound stimulation

Sound exposure test were performed in the experimental installations (Fig. 1), and more details were described in Gu, Zhang & Wu (2016). *E.coli* K-12 was exposed to sound stimulation with frequency 8KHz and intensity 80dB. Sound frequency and intensity level

were controlled by computer. Samples without sound exposure served as a control group. The temperature within the sound waves load apparatus was maintained at 37± 1 ◦C. The sound exposure was performed continuously in the whole experiment.

2.3. Pretreatment the RNA of *E. coli* K12 and sequencing

The culture broth of *E. coli* K12 was collected at initial stage of exponential phase (6h), middle exponential phase (12h), late exponential phase (24h) and stationary phase (36h), respectively. Then, all samples were diluted to 1.0 (OD600). Total RNA was extracted using Hipure Bacterial RNA Kit (R4181-01, Magen, China) and quantified by a spectrophotometer (DS-11, DENOVIX, USA). Then samples were put into dry ice and sent to BerryGenomics for further transcriptome sequencing and analysis.

3. Results

3.1. Influence of acoustic exposure on growth of *E. coli* K12 in different time

The influence of acoustic exposure on growth of *E. coli* K12 was presented in Fig. 2. It could be seen from Fig. 2, there was no significant difference between the treatment and control groups at 0-12 h. With the prolongation of the exposure time, the cell concentration began to show a significant difference at 24 hour (increment 33%), reached the maximum at 36 hour (increment 36.2%) and declined slightly at 48 hour (increment 35.7%). There was not significant difference from the previous findings. We speculated that, although growth did not show significant differences in 0-12 hours, intracellular or membrane surface receptors should respond to acoustic stimuli. After 12 h, with the signal transduction, many intracellular organelles gradually to produce more specific differences including cascade signal amplification, material metabolism, and energy metabolism and so on. Thus, the following research was focused on the influence of metabolic network and energy metabolism based on Gene Ontology and KEGG pathway enrichment analysis derived from the transcriptome.

3.2. Influence of sound exposure on metabolic network structures and energy metabolic pattern in *E. coli K12* based on KEGG pathway enrichment analysis

3.2.1. Quality assessment of transcriptome data

Transcriptome analysis as an efficient research methods for the analysis the intracellular metabolic differences, it can help us better understand the response of *E. coli* K12 to sound exposure, which has important significance for further study of bacteria's sound exposure mechanism. In order to ensure the reliability of transcriptome data analysis, tight transcriptome data quality control was compulsory. A comparative transcriptome analysis between the treatment group and the control group was carried out. Table 1 and Table 2 showed the basic situation of the eight sample which used to transcriptome detection, total gene count expresses the total number of *E. coli* genes (4097), gene count indicates the number of gene expressed in samples and proportion of total genes, Reads>0, indicating that each gene has at least one read support; Reads>1, that each gene has at least 2 reads support,

followed by analogy to Reads>10. From the results shown in Table 1, the lowest gene expressed sample was sample s1 (90.65%), the largest was sample c4 (98.68%), and the number of expressed genes decreased slowly with the increase of read numbers. It indicated that the saturated amount was good and high reliability, which could be used for further analysis. In Table 2, Input means the number of read we got; Mapped represents the number of reads aligned to the genome, Mapped% represents the reads aligned to the genome as a percentage of all read number. Multiple alignments represent the read numbers that align to multiple genes, and multiple alignments% represents the percentage of multiple read. Uniq mapped indicates the number of readings for the unique gene, and Uniq mapped% indicates the percentage. It could be seen from the table, the percentage of multiple alignments for the 8 samples was very low, the largest was c2 (1.20%) and c4 (1.20%); the unique read percentage was really high, even the lowest value was reached 77.40% (s4). All of these proved that the credibility of the result for mapped was good, data coverage was broad and the further analysis results were accurate and reliable.

3.2.2. Transcriptome analysis of genes differentially expression

The correlation analysis of gene expression between the acoustic field exposure group and the control group was presented in Figure 3. For the scatter plots obtained between the stimulated and the control (Fig. 3), a good many probe sets felled either above or below the diagonal, the correlation coefficients were lower than 0.96, which indicated a significant change in the intensity of microarray expression after the stimulated. The correlation coefficients of stimulation group and control group were 0.905, 0.959, 0.94, 0923 at 6 hour, 12 hour, 24 hour and 36 hour respectively, which explained the difference in gene expression was greatest at 6 hour, followed by 36 hour, 24 hour and 12 hour. The results suggested that the effects of sound exposure on the gene expression of *E. coli* K12 were more extensive at initial stage of exponential phase (6h) and stationary phase (36h), and in the following experiments, we should pay more attention on these two time points.

Changes in expression level for the sample under the sound stimulus compared with the control using the logFC (logFC=log2case/ctrl), the logarithm of the ratio of the RPKM (Reads Per Kilobases per Millionreads) values of the treated and control groups. For a gene in which a sample's expression level is 0, the software will give it a small value (the value is calculated according to the algorithm), to prevent the denominator is 0. The differentially expressed genes are defined by FDR, which is called corrected-Pvalue. The statistical significance of the difference analysis is suitable for multiple hypothesis testing. In general, when the FDR <0.05 indicates that the gene is a differentially expressed gene. The effects of sound stimulation on gene expression in *E. coli* K12 were shown in Table 3. Analysis of gene expression revealed that the number of genes expressed in at least one of the two comparative samples was 3932, 3952, 4026, and 4048 at 6h, 12h, 24h and 36h, respectively. Compared with the control group, there were 289 genes with significant difference at the 6h, 110 up-regulated genes and 179 down-regulated genes. The number of the gene with significant difference at 12h was 80, 43 genes were up-regulated and 37 genes were down-regulated. At 24h, the total number of genes with significant difference was 201, including 108 up-regulated genes and 93 down-regulated genes. At 36 hours, 193 genes with significant difference, including up-regulated genes 87 and down-regulated genes 106. All results were

shown in Table 3. These genes existed in different metabolic pathways and their up-regulated or down-regulated affected the function of the pathway. Thus, the results indicated that during the different growth periods of *E. coli* K12, the sound exposure affected the metabolism of bacteria in many aspects, of which 12 hour was relatively small.

3.2.3. Influence of sound exposure on metabolic network structures in *E. coli* K12

Effects of sound exposure on metabolic network structures in *E. coli* K12 were presented in table 4. The early effect of sound exposure on the metabolic network happened on the *E. coli* K12's receptor, stimulated the bacterial chemotaxis, and gradually transferred from the receptor to the cell, causing differences in membrane metabolism. Cell membrane as an important cellular component, involving intracellular function, cell transcription, cell division and other aspects, the up-regulation of glycerophospholipid pathway explained the activation of cell function. In the middle stage of sound exposure, the differences in metabolism changed into the down-regulated of the cell secretory pathway and the up-regulated of the transcription and translation, which indicated that the metabolism had been changed from the passive feeling to the active response to the environment. In the later stage of bacterial growth, the accumulation of sound exposure resulted in the down-regulated of the pathways about the production of harmful secondary metabolites and the up-regulated pathways of using aromatic compounds for energy. The down-regulated pathways of harmful secondary metabolites caused the down-regulated of flagellar assembly, which is associated with draw to the advantages and avoid disadvantages. The differences in these pathways could be used to explain the phenomenon that the control group began to decline and the sound exposure group was still growing slowly. All these changes constituted a system of metabolic networks.

In the early stage of stimulation, the main effects of acoustic exposure were membrane surface receptors, and some metabolic changes associated with sensing were significantly different, such as bacterial chemotaxis. At 6 hour, there was a significant difference up-regulated pathway between the stimulated group and the control group, bacterial chemotaxis (ko02030). Chemotaxis research describes the cells' movement processes that control the organisms to the favorable environment. Involved in bacteria and archaea, motility by sensing the environment of histidine kinases and the response regulator controled by two-component system, this type of signal transduction described a very common reaction regulator in prokaryotes (Szurmant and Ordal, 2004). Microbiological ability to rapidly sense and adapt to changes in the environment plays an important role in building microbial communities, affecting microbial activity, and interacting with various micro-organisms in the surrounding environment. The plasma membrane senses the change of environment first, because it is the outer layer of the cell. Therefore, we believed that, in the early stage of the experiment, the sound as a physical signal of the external environment stimulated the *E. coli* K12. *E. coli* K12 responded by using the bacterial chemotaxis system, then regulated the metabolism, made itself in a more favorable living environment.

With the continuous accumulation of sound exposure time, the difference of metabolism gradually evolved into the function or composition of membrane. At 12 hour, as the only pathway with significant difference, glycerophospholipid metabolism pathway has been reported related to the metabolism. Glycerophospholipids is one of the most abundant

phospholipids in the body. It is one of the components of biofilm, bile and membrane surface active substances. It is also involved in the recognition and signal transduction of proteins (Farooqui, Horrocks and Farooqui, 2000). From the KEGG pathway map of Glycerophospholipid metabolism, it can be seen that the metabolism of Glycerophospholipids involves a very wide range metabolic networks, the most direct relationship is cell division, signal transduction and energy metabolism. As an up-regulated pathway of the treatment group, it was demonstrated that at 12 hour, the response of bacteria to acoustic exposure had evolved from external to internal metabolic, and the energy metabolism, cell division and signal transduction were enhanced in the stimulated group.

At 24 hour, the down-regulated pathway Bacterial secretion system is associated with cell membrane transport and environmental regulation. It is widely expressed in Gram-negative bacteria. The functions of the pathway include the organogenesis of organelles such as cilia and flagellum, nutrient availability, efflux toxicity drugs and other toxins (Gauthier and Finlay, 2001). In up-regulated pathways, flagellum assembly is related to cell movement and cellular processes. As a widespread cellular device in prokaryote, it has a chemical tendency to help the bacteria move toward the nutrient and escape the harmful substance (Szurmant and Ordal, 2004). Pyrimidine metabolism pathway and alanine, aspartate and glutamic acid metabolic pathway are closely related DNA transcription and translation. In addition, the most directly pathway associated with the transcription and translation was the ribosome. In this experiment, the growth difference between the experimental group and the control group showed at about 22-28 hour. The transcriptome analysis showed that the difference between the treatment group and the control group had changed from the intracellular metabolic to the gene expression.

At 36 hour, the control group entered the stationary phase and gradually began to decline, while the treatment group still slowly growth. When the bacteria growth into stationary phase, the nutrient consumption in the medium and the accumulation of harmful metabolites resulting in the bacterial growth rate gradually decreased, the number of bacterial deaths increased slowly. Some signal factors inhibit the division of bacteria increases and cause some bacteria lysis. Therefore, we hypothesized that the pathway's down-regulated in sound exposure group like flagellar assembly which used to avoid disadvantages, made the ability of the treatment group to feel the unfavorable environment became weak, so that it could better adapt to harsh living environment. In addition, geraniol degradation pathway, valine, leucine and isoleucine metabolic pathways, TCA cycle and fatty acid degradation pathways were also reduced. The pathway of geraniol degradation is related to the metabolism of terpenoids and polyketides. Polyketides is a class of secondary metabolites produced by organisms that are not essential for the growth and development of organisms but can be used to defense and cell-to-cell communication. Polyketide may have the function for antibiotics, antifungal, cell stability or natural pesticides (Wolken and Van, 2001). Terpenoids is a class of secondary metabolites of organisms. Biological functions include photosynthesis, respiration, regulation of metabolic hormones, regulation of growth and development, biological defense, intracellular signaling, effects cell membrane's structure and function. It's widely used in fragrance compounds, antimalarial drugs, anti-cancer drugs, with a biological function for anti-microbial growth (Martin, 2003). We speculated that the treatment group had a weaker sense to the harsh environment, therefore the related metabolic pathways were also reduced.

The pathway of geraniol degradation is related to leucine metabolism, so that the valine, leucine and isoleucine metabolic pathway was also down-regulated. The metabolism of terpenoids and polyketide are both need the CoA (Teufel, 2010). The degradation of fatty acids is orthologous associated with CoA, so the fatty acid degradation pathway and TCA cycle were correspondingly down-regulated. The down-regulation of these pathways indicated that the response of the treatment group to the secondary metabolites was slower than control group, the reason may be the receptors of the treatment group to harsh environments were passivation or in treatment group the secretion of the signal factors which activating the receptor were less than control group.

3.2.4. Influence of sound exposure on energy metabolic pattern in *E. coli* K12

Energy metabolism as an important indicator to study bacteria response to sound exposure, it can not only help to understand the metabolic in differences growth stage, but also explain the slow growth of the treatment group in stability stage, lay a foundation for study the similar physiological phenomena. Influence of sound exposure on energy metabolic pattern in *E. coli* K12 was shown in Table 5.

At the early stages of sound exposure, the bacterial chemotaxis and glycerophospholipid metabolism pathway as main significant difference pathway, they were not only associated with cell sensitization and stress, but also accompanied with some energy metabolism pathway. For example, the bacterial chemotaxis requires ATP provide energy to the flagellum, so that the flagella rotate can cause cell movement; the relationship between the production/metabolism of glycerol phospholipids and the energy metabolism are inseparable; the role and function of cell membrane is directly related to energy metabolism. But as a multiple alignment, these energy-related metabolic pathway's P-value didn't reach significant. With the prolongation and accumulation of time, energy metabolism pathways gradually became the main difference pathways and formed a pattern.

At 24 hours, there were 2 down-regulated pathways, except the Bacterial secretion system, the Propanoate metabolism pathway is mainly related to the metabolism of carbohydrates. It has direct orthology relationship with many pathways related to CoA. In addition, it has some correlation with many carbohydrate metabolism related diseases (Huang et al, 2006). There were seven up-regulated pathways. Pathways related to energy metabolism were photosynthesis, oxidative phosphorylation and sulfur metabolism. The oxidizing capacity of sulfur is very common in bacteria and archae, including phototrophic and chemical autotrophic types. Sulfate reduction can occur in the energy consumption assimilation pathway and the energy-producing alienation pathway, both of them began to activate the sulfate by reacting adenosine sulfate (APS) with ATP (Frigaard and Dahl, 2008). Oxidative phosphorylation is the most important energy metabolic pathway. In bacteria, when material oxidation it is a coupled reaction for providing the energy for ADP and inorganic phosphorus synthesis of ATP. The process of oxidative phosphorylation involves a complex electron transport chain process, in which the electron transport of prokaryotes uses the energy released by the oxidized substrate to pump the ion transmembrane to produce an electrochemical gradient. Pyrimidine metabolism and alanine, aspartate and glutamic acid metabolic pathways are not only associated with DNA transcription and translation, but also closely related energy metabolism. These two approaches involve the provision of material,

energy supply, synthesis of precursor material and the regulation of nucleic acid (Wu, 1998). The down-regulated of the disease associated carbon metabolic pathway is associated with down-regulated of cell secretory pathway. The differences in bacterial growth began to appear at about 24 hour, and the bacterial transcription and translation were significantly increased. Both the enhancement of bacteria division and the increased of the transcription and translation require a large amount of energy, which explained the up-regulated of the pathways of energy supply.

At 36 hour, the bacteria growth into stationary phase, according to our hypothesis, the treatment group's ability to perceive external factors became weak. Because treatment groups were less sensitive to harsh environment, there was no need to spend a lot of energy to combat the environmental change. Such as the down-regulated of the flagellar assembly pathway, it also explained the down-regulated of Alanine, aspartate and glutamate metabolism and Oxidative phosphorylation. In the up-regulation pathways, benzoic acid metabolism, phenylalanine metabolism and aromatic degradation are closely linked (Chen et al, 2012). 2-oxocarboxylic acid, also known as α-keto acid, is the intermediate of the three major nutrient metabolisms. Phenylpropionic acid can be metabolized to produce 2-oxocarboxylic acid, so that the aromatic compounds can be degraded to provide energy. Aromatic compounds are widely founded in nature, is the primary or secondary metabolites of many organisms. As microorganism, *E. coli* to a certain extent, can also synthesized aromatic compounds, but the production is very few, and the accumulation capacity is weak (Koma et al, 2012). Aromatic compounds can provide energy to microorganisms, but *E. coli* use aromatic compounds only in the undernutrition, and when the branched chain of aromatic benzene ring is very long, *E. coli* will directly decompose branched chain to provide energy without degradation the benzene ring. In this condition, the metabolism will not enter the phenylalanine metabolism and benzoic acid metabolic pathway (Koma et al, 2012; Díaz, 2010). The result showed that the degree of treatment group’s phenylalanine metabolism was significantly higher than the control group. It indicated that at this period, the bacteria’s living conditions became very hard and they began to use aromatic compounds to provide energy. When *E. coli* K12 in acoustic exposure, the efficiency of degrading aromatic compounds for energy was significantly improve. In the stationary phase, the ability of treatment group provides energy by using aromatic compounds and adapt to the harsh environment was stronger than control group. It also explained the phenomenon that the control group had a declining trend in latter, while the treatment group was still slowly growing. Because of the greater ability to adapt to harsh environments, the concentration of bacteria in the treatment group was higher than the control group. It also proved that it’s feasible for some countries play music for microbes to improve their effluent treatment efficiency. Phenylalanine metabolism is not only used to degrade aromatic compounds, but also produce the intermediates which used to synthesis the non-essential amino acids. So 2-oxocarboxylic acid metabolism, xylene metabolism, C5 branched-chain dicarboxylic acid metabolism, cysteine and methionine metabolism and amino acid synthesis are all associated with the degradation of aromatic compounds and the phenylalanine metabolics(Morasch et al, 2004; Berger et al, 2003), all of them were significant up-regulated at 36 h .

4. Discussion

Based on the results of the KEGG pathway analysis, we constructed a network of KEGG pathways (Figure 4) that *E. coli* K12 responded under acoustic exposure, which basically contained all the pathways with significant differences. As a kind of mechanical wave, we thought that the sound wave acts on the cell membrane by mechanical vibration. This mechanical vibration could cause the changes of cell membrane tension and intracellular cytoskeleton. The study on sound exposure of chrysanthemum callus showed that under certain frequency and sound intensity, the hydrophobicity of cell membrane is decreased, the fluidity is enhanced, the synthesis of membrane lipid is increased, and the catabolism is decreased, which means that the physical state and metabolism of membrane lipid is sensitive to sound stimulation. In addition, the sound stimulus may also cause cell membrane protein secondary structure changes (Hongbo et al, 2008). Cell-associated forces including the osmotic pressure and the relevant force when skeleton push and pull the plasma membrane and intracellular organelles which generated by the cytoskeleton. Cells respond to stimulation by altering division, cell death and differentiation, motility, signal transduction, gene expression, secretion and swallowing function (Apodaca, 2002). The receptors of cell chemotaxis are located on the cell membrane, the small phosphorylation response regulator can be combined with the rotating flagellar motor to cause the switching, and it's concentration can be alter by the transduction of the sensory signal. This simple approach has provided an example for the sensory system in general (Wadhams and Armitage, 2004). When intensity and frequency in a certain range, the stress significantly reduce the phase transition temperature, and the high intensity or frequency stress can lead to phase transition temperature increase. The low phase transition temperature and enhanced cell wall and membrane fluidity make cell growth and division faster and easier (Hongbo et al, 2008). In our study, bacterial chemotaxis, glycerol phospholipid metabolism, bacterial secretion, flagellar assembly and other ways were directly linked with the cell structure.

Since 2002, Professor Wang Boxuchu of Chongqing University and his research group have conducted a systematic study on the acoustic effect and mechanism of auditory stimulated plants. In the study of sonic stimulating chrysanthemum callus, sonic wave stimulation can cause changes in cell membrane structure and secondary structure of membrane proteins. Sonic stimulation can increase the activities of some intracellular and growth metabolic enzymes, and enhance intracellular RNA, soluble sugar and protease. Acoustic wave can affect the cell cycle and increase the number of S phase cells. Sonic wave stimulation may affect the uptake of extracellular Ca^{2+} by cell wall, and cell wall calcium may mediate the phosphorylation of PMH^{+}-ATPase. At the same time, it is also demonstrated that acoustic stimulation can lead to differences in gene expression levels (Zhao et al, 2002; Hongbo et al, 2008). R.B.M. Aggio and V. Obolonkin used to study the metabolomics of yeast under high frequency, low frequency and music. The results show that the sound frequency can not only increase the growth rate of yeast in liquid culture, but also affect the metabolism of yeast, and different metabolic pathways are affected differently by different sound frequency. Besides, different sound has different effects on the permeability of yeast membrane (Aggio, Obolonkin and Villas-Bôas, 2012). Specific frequency of sound waves can stimulate the human intestinal contraction and the passage of barium in the duodenum, changes of the mitochondrial transmembrane ATPase, affecting the production of ATP (POLOUS and Kurko, 1991). These research confirm our transcriptome analysis results in

different aspects, our research interpreted these phenomena caused by acoustic exposures at the transcriptome level and showed the specific pathways leading to the change. The analysis at different time explained the reason of acoustic exposure increase the cells number at S phase and made the mechanism of sound stress effect more clearly. The analysis of the differentially pathway revealed the specific process lead to the membrane structure change, the increase of enzyme activity, the enhancement of metabolic activity and the elevation of RNA and cAMP content. In our study, pathways such as oxidative phosphorylation, thiol metabolism, and aromatic compound degradation were closely related to energy metabolism. The up-regulated or down-regulated of these pathways, indicated that acoustic exposure enhances intracellular energy metabolism by different pathways, and explained the phenomena in previous studies.

It could be seen from the Figure 4, the pathways of *E. coli* K12 which played a role in acoustic exposure could be divided into four aspects: cell secretory pathway, energy metabolism, bacterial secretion system and transcriptional translation, while other pathways were mostly belonging to the downstream of these four aspects. These four aspects could construct a network map of metabolic network structures and energy metabolic pattern when *E. coli* under acoustic exposure. These four aspects linked to each other, mutual support, common interpretation the apparent change when *E. coli* in sound exposure. This not only made a significant contribution to the study of the mechanism of exposure, but also provided a new way to study the mechanism. The study of sound exposure at different stages could be specific to a pathway or a metabolic process or even a gene, laid a foundation for the further study of cell mechanical signal transmission and acoustic exposure to other species.

5. Concluding remarks

According to the above results, we could conclude that in the early stage of *E. coli* K12 exposure to acoustic, intracellular gene changes are more complex, but the most significant was the cell chemotaxis pathway, the up-regulation of this pathway meant that bacteria have a movement trend to favorable environment. When the acoustic exposure lasted for 12 hours, the most significant difference was the glycerophospholipid pathway, which meant that the ability of bacteria to division and energy metabolism had increased. When the sound exposure lasted for 24 hours, the secretion of extracellular products by bacteria was lower than the normal level, while the transcriptional translation and nucleic acid synthesis was higher than that of normal bacteria. At 36 hour, the bacteria of the treatment group were better at using the complex compounds to provide energy than the common bacteria, at the same time the degradability of the terpenoids and ketones stimulated metabolites were decreased. This may be the passivation of the receptor for secondary metabolites, and may also be the decrease in the secretion of secondary metabolites in treatment group. According to the analysis of the 24-hour down-regulation pathway, it is more likely the secretion of secondary metabolites was reduced, but the fact need to further experiments.

Acknowledgements

This work was supported by National Natural Science Foundation of China (Grant No.

U1304307) and the Young Core Instructor Foundation from the Education Commission of Henan Province, China (Grant No. 2014GGJS-056).

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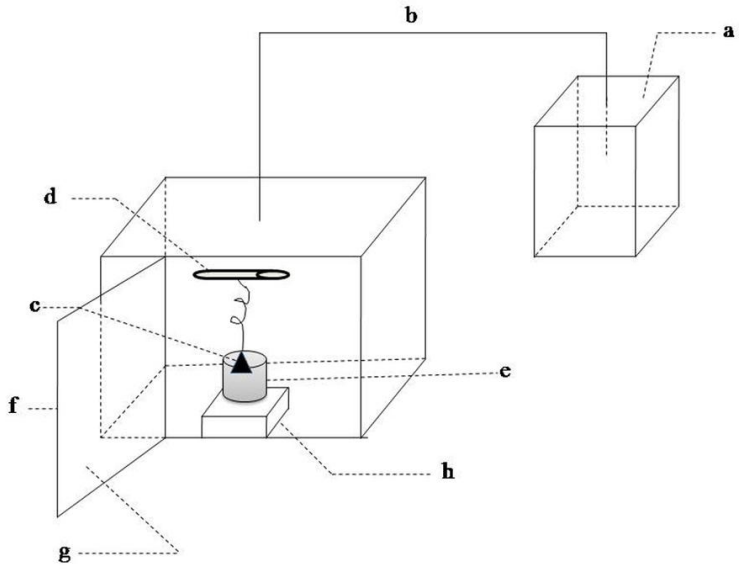
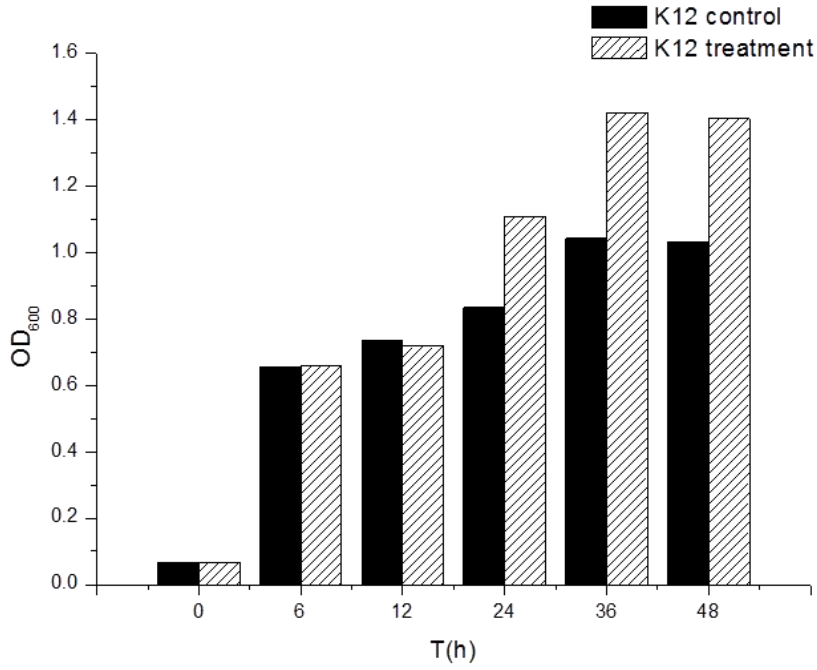
Figure Legends

Figure 1. Schematic of sound waves load apparatus



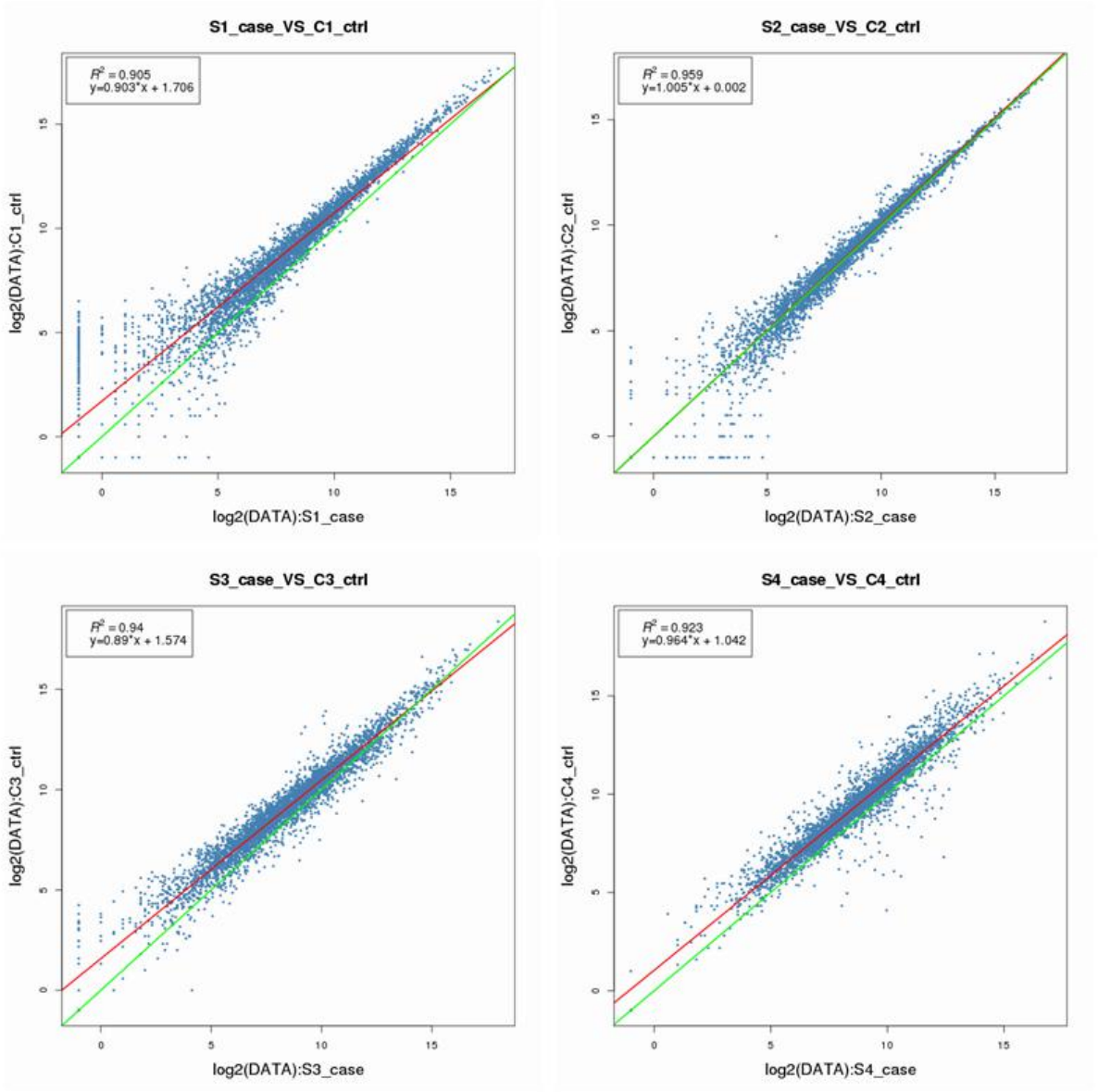
Note: (a) sound waves source; (b) sound waves transmission conductor; (c) speaker; (d) ultraviolet light; (e) beaker; (f) metal case; (g) sound-absorbing material; (h) magnetic stirrer.

Figure 2. Influence of acoustic exposure on growth of *E. coli* K12 during different growth stages



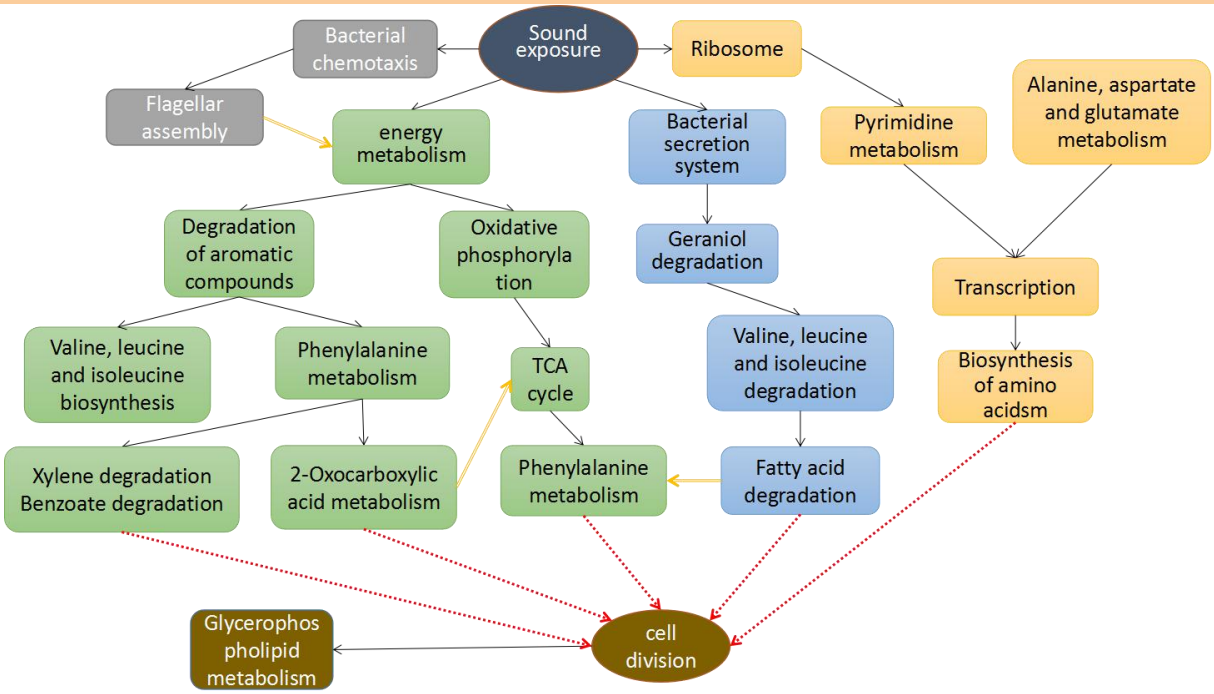
Note: *E. coli* K-12 exposed to acoustic field with 8 kHz frequency and 85 dB intensity level acted as treatment group. Samples without sound exposure served as a control group.

Figure 3. Correlation analysis of two samples' expressed genes, based on the reads' number of per gene



Note: the abscissa represents the log2 fold-change of the reads' number in the treatment group, and the ordinate represents the $\log_2$ fold-change of reads' number in the control group. Correlation coefficients (R^2) are 0.905, 0.959, 0.94 and 0.923, respectively.

Figure 4. The hypothesis model of acoustic stress response in *E. coli* based on metabolic networks and energy metabolism



566

Table 1. The data quality of transcriptome sequencing for gene expression

Sample	Total_gene_count	gene_count(Reads>0)	gene_count(Reads>1)	gene_count(Reads>2)	gene_count(Reads>5)	gene_count(Reads>10)
C1	4,097	4,039(98.58%)	4,030(98.36%)	4,008(97.83%)	3,968(96.85%)	3,897(95.12%)
C2	4,097	4,026(98.27%)	4,010(97.88%)	3,995(97.51%)	3,964(96.75%)	3,898(95.14%)
C3	4,097	4,058(99.05%)	4,054(98.95%)	4,052(98.90%)	4,040(98.61%)	4,008(97.83%)
C4	4,097	4,058(99.05%)	4,057(99.02%)	4,056(99.00%)	4,050(98.85%)	4,043(98.68%)
S1	4,097	3,933(96.00%)	3,914(95.53%)	3,881(94.73%)	3,820(93.24%)	3,714(90.65%)
S2	4,097	4,044(98.71%)	4,036(98.51%)	4,017(98.05%)	3,984(97.24%)	3,906(95.34%)
S3	4,097	4,047(98.78%)	4,037(98.54%)	4,027(98.29%)	3,988(97.34%)	3,915(95.56%)
S4	4,097	4,057(99.02%)	4,057(99.02%)	4,051(98.88%)	4,036(98.51%)	4,014(97.97%)

Note: C1 represents 6 hours sample without acoustic stimulation, C2 represents 12 hours sample without acoustic stimulation, C3 represents 24 hours sample without acoustic stimulation, C4 represents 36 hours sample without acoustic stimulation, S1 represents 6 hours sample exposed to acoustic field with 8 kHz frequency and 85 dB intensity level, S2 represents 12 hours sample exposed to acoustic field, S3 represents 24 hours sample exposed to acoustic field, S4 represents 6 hours sample exposed to acoustic field. Total gene count is the total gene number of this species. Gene_count is the number of genes expressed in the sample and the proportion to the total gene (reads>0, indicating that each gene has at least 1 read support, and so on).

Table 2. The data quality of transcriptome sequencing for mapping

Sample	Input	Mapped	Mapped(%)	Multiple_alignments	Multiple_alignments(%)	Uniq_mapped	Uniq_mapped(%)
C1	25,712,838	24,124,019	93.80%	189,177	0.70%	23,934,842	93.10%
C2	20,517,122	19,455,650	94.80%	239,493	1.20%	19,216,157	93.70%
C3	21,243,230	20,222,226	95.20%	183,059	0.90%	20,039,167	94.30%
C4	22,120,548	21,374,225	96.60%	255,590	1.20%	21,118,635	95.50%
S1	15,510,048	14,495,295	93.50%	112,857	0.70%	14,382,438	92.70%
S2	18,916,864	18,007,091	95.20%	182,066	1.00%	17,825,025	94.20%
S3	16,089,770	15,105,299	93.90%	141,484	0.90%	14,963,815	93.00%
S4	18,384,248	14,389,892	78.30%	154,622	0.80%	14,235,270	77.40%

Note: C1 represents 6 hours sample without acoustic stimulation, C2 represents 12 hours sample without acoustic stimulation, C3 represents 24 hours sample without acoustic stimulation, C4 represents 36 hours sample without acoustic stimulation, S1 represents 6 hours sample exposed to acoustic field with 8 kHz frequency and 85 dB intensity level, S2 represents 12 hours sample exposed to acoustic field, S3 represents 24 hours sample exposed to acoustic field, S4 represents 6 hours sample exposed to acoustic field. Input is the number of read. Mapped is the number of Reads match to genome. Multiple_alignments is the Reads number of multiple alignments. Uniq_mapped is the Reads number of uniq mapped.

583 **Table 3.** The effects of acoustic exposure on *E. coli* K12 gene expression during different growth stages

Time of acoustic exposure	Total number of expressed genes	Data of differentially expressed genes		
		Number of Up-regulated gene	Number of Down-regulated gene	Total genes of differentially expressed genes
6h	3932	110	179	289
12h	3953	43	37	80
24h	4026	108	93	201
36h	4048	87	106	193

584

585 Note*: E.coli* K-12 exposed to acoustic field with 8 kHz frequency and 85 dB intensity level acted as treatment
586 group. Samples without sound exposure served as a control group.

Table 4. The effects of sound exposure on metabolic network structures in *E. coli* K12

Time of sound exposure	up-regulated pathway		down-regulated pathway	
	Term of pathway	genes differentially expressed in the pathway	Term of pathway	genes differentially expressed in this pathway
6h	Bacterial chemotaxis (ID ko02030)	motB; cheZ; tar; cheY		
12h	Glycerophospholipid metabolism (ID ko00564)	glpA; glpC; glpB		
24h	Flagellar assembly (ID ko02040)	flgC;flgH;flgE;flhC;fliM;flgB;fliO; fliN;flgG;fliH;flhD;flgD;fliG;fliF;fliK;flgF	Bacterial secretion system (ID ko03070)	gspC;gspI;gspK
	Ribosome (ID ko03010)	rplC;rpsC;rplV;rplW;rpsJ;rplP; rplQ;rpsS;rplD;rplB;rpmD;rplA		
	Alanine, aspartate and glutamate metabolism (ID ko00250)	glnA;purB;purF;pyrI;carB;carA;pyrB		
	Pyrimidine metabolism (ID ko00240)	ndk;pyrD;codA;pyrI;carB;carA;pyrC;pyrB;upp		
36h			Flagellar assembly (ID ko02040)	flgC;fliM;flgB;fliO;fliN;flgG;fliH;flgE; flgD;fliG;fliF;flgF
			Geraniol degradation (ID ko00281)	fadB;fadI;fadA;fadJ
			Valine, leucine and isoleucine degradation (ID ko00280)	fadB;fadI;lpd;fadA;fadJ
			Citrate cycle (TCA cycle) (ID ko00020)	lpd;sucA;sucC;sucD;sdhA;sdhB;sdhD
			Fatty acid degradation (ID ko00071)	fadB;fadI;fadA;fadE;fadJ

Note*: E.coli* K-12 exposed to acoustic field with 8 kHz frequency and 85 dB intensity level acted as treatment group. Samples without sound exposure served as a control group. The metabolic pathway presented in this table arrived to the significantly differences (corrected-Pvalue<0.05) , which below the level is not shown. There are 179 down-regulated genes at 6 hour and 37 down-regulated genes at 12 hour, some of them are found in the pathways without significant differences(corrected-Pvalue>0.05), while others do not involve in these pathways. Term is the functional description of this pathway in KEGG database. ID is the unique number of Pathway in KEGG database.

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593 **Table 5.** The effects of sound exposure on the energy metabolic pattern in *E. coli* K12

Time of sound exposure	up-regulated pathway		down-regulated pathway	
	Term of pathway	genes differentially expressed in the pathway	Term of pathway	genes differentially expressed in this pathway
6h	Bacterial chemotaxis (ID ko02030)	motB; cheZ; tar; cheY		
12h	Glycerophospholipid metabolism (ID ko00564)	glpA; glpC; glpB		
24h	Photosynthesis (ID ko00195)	atpA;atpH;atpG;atpF;atpD	Propanoate metabolism (ID ko00640)	prpD;prpE;prpB;prpC
	Oxidative phosphorylation (ID ko00190)	ppa;atpH;atpA;cyoB;atpG;atpF;atpD		
	Sulfur metabolism (ID ko00920)	tauB;sbp;cysN;cysJ;cysD;tauA		
36h	Phenylalanine metabolism (ID ko00360)	paaB;paaD;hcaE;paaZ;paaC;paaE;hcaF;mhpC; mhpB;mhpA;paaA;paaF;paaG;mhpE;hcaC; paaH;paaI;hcaB;hcaD;mhpD	Alanine, aspartate and glutamate metabolism (ID ko00250)	glnA;purB;purF;pyrI;carB; carA;asnB;pyrB;asnA
	Degradation of aromatic compounds (ID ko01220)	hcaD;hcaB;hcaE;mhpD;hcaF;mhpC;mhpB ;mhpA;mhpE;hcaC	Oxidative phosphorylation (ID ko00190)	sdhB;cyoA;sdhA;atpA;cyoB;atpG;sdhD;atpD
	Valine, leucine and isoleucine biosynthesis (ID ko00290)	leuD;leuB;le uC;leuA;ilvN;ilvE;ilvB		
	Xylene degradation (ID ko00622)	hcaD;mhpD;mhpF;mhpE;hcaC		
	2-Oxocarboxylic acid metabolism(ID ko01210)	leuD;leuB;leuC;leuA;ilvN;ilvE;ilvB		
	Benzoate degradation (ID ko00362)	paaH;paaF;mhpF;mhpE;mhpD		
	C5-Branched dibasic acid metabolism (ID ko00660)	leuD;ilvN;leuC;ilvB		
	Cysteine and methionine metabolism (ID ko00270)	thrA;metB;metC;metA;metK;metL		
	Dioxin degradation (ID ko00621)	mhpF;mhpE;mhpD		
	Biosynthesis of amino acids (ID ko01230)	leuD;leuB;leuC;leuA;ilvN;thrA;metB;metC; metA;ilvE;metK;metL;ilvB		

594 Note*: E.coli* K-12 exposed to acoustic field with 8 kHz frequency and 85 dB intensity level acted as treatment group. Samples without sound exposure served as a control

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