http://www.pjbs.org

PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Pakistan Journal of Biological Sciences 16 (18): 969-975, 2013
ISSN 1028-8880 / DOI: 10.3923/pjbs.2013.969.975

Growth and Physiological Characteristics of *E. coli* in Response to the Exposure of Sound Field

[1]Shao-Bin Gu, [1]Bin Yang, [1,2]Ying Wu, [1]Shi-Chang Li, [1]Wen Liu, [1]Xiao-Fei Duan and [1]Meng-Wei Li
[1]College of Food and Bioengineering, Henan University of Science and Technology, Postcode 471003, Luoyang, People's Republic of China
[2]Hefei Institutes of Physical Science, Chinese Academy of Sciences, Postcode 230031, Hefei, People's Republic of China

Abstract: It is undeniable that environmental sonic vibration can affect our emotions and mood, but so far the study of physical stimuli provoked by audible wave on single cells has been rarely concerned. To investigate the response of *E. coli* to audible wave exposure, the growth status and alterations in antioxidant enzyme activity were studied in liquid culture. The data showed that the growth of *E. coli* was promoted in the treatments of different frequencies sound wave. The most significant effect on growth promotion appeared when sound wave was maintained at 100 dB and 5000 Hz. Simultaneously, sonic vibration evoked significantly increases the level of total protein content contents. And the changes of activities of Super Oxide Dismutase (SOD) and catalase (CAT) were observed obviously. The results suggested that the growth promotion effect of audible sound may be non-linear and shows obvious frequency and intensity peculiarities. Moreover, the increase in activity of antioxidant enzymes implied that a number of active oxygen species generated in bacterial cell under the exposure of audible sound. We speculate that the audible sound may cause a secondary oxidative stress. Further studies are needed to elucidate the mechanisms of active oxygen species generation induced by audible sound.

Key words: Sound field, *E. coli*, response, active oxygen species

INTRODUCTION

Acoustic wave is a kind of pressure wave caused by the vibration of an object in a conductive medium such as air, water and solids. Thus, a sound wave is characterized as a typical mechanical wave (Danet, 2005). In the natural world almost all living organisms are "Aimmersed" in a variety of sound waves and interact with them (Danet, 2005; Davies, 2009). In terms of the sound frequency, there are roughly three regimes: infrasound (10^{-4}~20 Hz), audible sound (20~2H10^4 Hz) and ultrasound (2H10^4~10^{12} Hz). In the past few decades, studies of biological effects and mechanisms concerned with ultrasound and infrasound were performed in many extensive fields (Leighton, 2007; Leventhall, 2007). As for audible sound, more attention has been drawn toward mechanism of voice forming, broadcasting, reception of sound, sound-processing and recognition. And humanity has puzzled over the effect of sound on animal behaviors and physiology for centuries; we have limited knowledge of the role of sound on single cells. However, the interaction between audible sound and single-cell organisms is usually neglected in the field of biological research. There is little attention paid to the relation of organisms and audible sound.

Recently the biological effects induced by audible sound wave in plants were investigated. To explain the potential mechanism, a hypothesis on plant meridian system was put forward (Hou and Mooneyham, 1999a, 1999b). However, the plant meridian system based on the human body meridian theory is complicated and imperfect. It is not effortless to understand the mechanism of biological effects induced by audible sound waves. Furthermore, the complexity of multicellular organisms has also brought great difficulties to the study of the mechanism of audible sound waves biological effects.

Matsuhashi *et al*. (1996) reported that acoustic wave with specific frequency can evidently improve the colony formation of B. *carbophilus* grown on non-permissive media. Joanna observed that audible sound with the frequency of 1,000 Hz, 5,000 Hz, 15,000 Hz, separately was able to promote the growth of *E. coli*. The significant

Corresponding Author: Shao-Bin Gu, College of Food and Bioengineering, Henan University of Science and Technology, No.263, Av. Kaiyuan, Luoyang, People's Republic of Chin Tel: +86-379-64283928 Fax: +86-379-64282342

increase in cell number of *E. coli* for the growth culture appeared when the frequency of audible sound was maintained at 5 KHz (Ying *et al*., 2009). A report recently published in the Metabolomics suggested that sonic waves can affect *Saccharomyces cerevisiae* strain VIN13 metabolism when growing in liquid culture (Aggio *et al*., 2011). Previous studies from our laboratory have indicated that audible sound field stimulation can significantly affect *E.coli* growth under the normal condition or environmental stresses (Gu *et al*. 2010). However, the dose-response relationship between the acoustic stimulus and cell growth, physiological characteristics and potential mechanism still remains unclear. In this study, we investigated the influence of audible sound waves stimulation on bacterial cell *E. coli* growth, energy metabolism level, synthesis of protein and activities of anti-oxidant enzymes. This work provides us a powerful model to test the role of audible sound waves on bacterial cell growth and metabolism. At the same time, it has also positive significance for us to get more biological effects information concerned with exposure to audible sound wave and reveal its underlying mechanism.

MATERIALS AND METHODS

Bacterial strains and growth conditions: *E. coli* N43 was presented by Professor A. Jacq of France (Institute of Genetics and Microbiology UMR 8621, University Paris-Sud 11). Cells were first cultured in slant agar medium (1% tryptone, 0.5% yeast extract, 0.5% NaCl and agar 1.5%, pH 7.2) at 37°C for 18 h. Then cells expanded in a 250 mL Erlenmeyer flask containing 100 mL of liquid medium (1% tryptone, 0.5% yeast extract and 0.5%, pH 7.2) with agitation of 200 rpm on a rotary shaking incubator at 37°C for 12 h to logarithmic phase.

Sound wave load apparatus: To investigate the bioeffects of biological material exposed to the acoustic environment, the sound wave load apparatus was designed (Fig. 1). This equipment is composed of two parts. One is the sound wave generating unit. The other is sound wave load chamber. The former contain a waveform generator and the amplifying circuit. The signals produced by the waveform generator are amplified and then send to a speaker. The speaker along with UV lamp is installed in the sound load chamber whose inner walls are made with sound absorbing material. The outer walls of chamber are wrapped by a metal shell in order to reduce influence of environmental noise on the experimental result. When the experiments were performed, the desired audible sound wave was generated

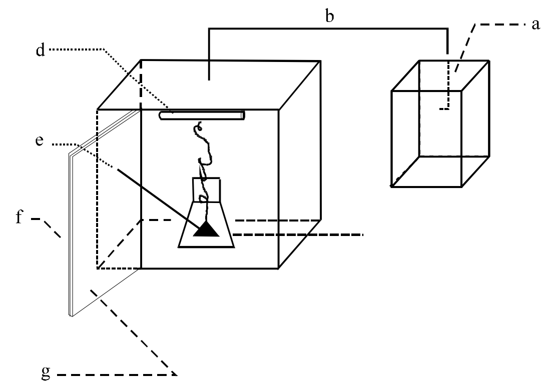

Fig. 1: Schematic of sound wave load apparatus. Notes: a: Wave generation unit; b: Sound wave transfer wire; c: Speaker; d: UV-lamp (it is used for sterilization before the start of sound stimulation experiments); e: Erlenmeyer flask; f: Outer wall of chamber (metal shell); g: Inner wall of chamber (made with sound absorbing material)

by the apparatus and the frequency is precisely adjusted from 1 to 20 kHz. The sound intensity is set according to the experiment design.

Sound stimuli: After 12 h of growth the culture was inoculated to be 1% (v/v) into fresh liquid medium. Then the samples were put into the sound load chamber and turn on the power. The sound stimuli were played continuously by for the duration of the experiment. The parameters of frequency and intensity of sound were adjusted according to the experiment design. Samples without audible sound wave treatment served as a control group. The temperature within the sound wave load apparatus was maintained at 371°C.

Measurement of cell densit: At the end of sound stimuli, Optical Density (OD) of the treatments and controls were measured at 600 nm using a Hitachi (model U-1100) spectrophotometer.

Assay the content of total cellular proteins: Bacterial cells were collected in a sample tube by centrifugation (2200 g, 5 min), washed with a 1% (w/v, g/ 100 mL) aqueous NaCl solution, centrifuged again, freeze-dried and disrupted by ultrasonication at high power for 10-min using a commercial sonic bath (Ultrasons, J.P. Selecta, Barcelona, Spain) in lysis buffer (5 mL L^{-1} of Triton X-100, 0.3722 g L^{-1} of ethylenediaminetetraacetic acid disodium salt, 0.0348 g L^{-1} of phenyl methyl sulfonyl fluoride).The coomassie light blue method was used to measure protein content (Bradford, 1976).

Enzyme extraction and Determination of enzyme activity: To measure activity of bacteria intercellular superoxide dismutase and catalase, bacteria were harvested from liquid samples into 0.1 M Tris buffer (pH 8.0). Harvested cells suspended in Tris buffer were centrifuged at 15,000 g for 10 min at 4EC. The resulting pellet was washed twice and resuspended in the same buffer. Cells were lysed by twice freezing in liquid nitrogen and thawing the suspensions which were allowed to thaw completely before re-freezing. To obtain crude extracts, the lysates were centrifuged at 25,000 g for 10 min at 4EC and the supernatant carefully separated from the pellet made up of cell-envelope debris. SOD activity was measured through the pyrogallol autoxidation method (Marklund and Marklund, 1974) and CAT activity was measured through hydrogen peroxide method (Bailly *et al*., 1996). One unit of SOD activity was defined as the amount of enzyme required to inhibit the rate of pyrogallol autoxidation by 50% at 25°C. One unit of catalase was defined as the amount of lysate that decomposed 1 μmol of H_2O_2 per mg of protein at pH 7.0 and 25°C in 1 min.

Statistical analysis: Statistical analysis was performed using AVOVA. All measurements were repeated in six independent experiments. All results were represented as means±S.E. from three independent series of experiments. Significant differences from control values were determined at $p<0.05$ according to Duncan's multiple range tests.

RESULTS AND DISCUSSION

The influence of audible sound wave stimuli on the growth of E. coli: Figure 2 shows effects of audible sound waves stimuli on *E.coli* growth. It was found that all treatments were able to promote the growth of *E. coli*. The growth promotion of bacterial cells had a rapid increase with the increasing of sound frequency (or intensity) and then a slow decrease appeared when frequency was up to 10000 Hz and intensity up to 110 dB. The optical density of bacteria cell reached 0.53 after stimuli by 100 dB sound wave with the frequency 5000 Hz, corresponding to more 2 times than that of the control. The results suggested that the growth promotion effect of audible sound may be non-linear and shows obvious frequency and intensity peculiarities. In spite of underlying mechanisms of frequency- and intensity-dependent effects have still remained unknown, more and more investigations still showed that mechanical stress may play an important role in cell cycle, growth and proliferation (Altman *et al*., 2002; Mitchell and Myers, 1995; Wang *et al*., 2003). As a mechanical wave, audible sound would produce a mechanical stress to *E.coli* cells, similar to an alternate pull and press forces. Consequently, concerning to cells, responding to audible sound forces may be the main reason to create biological effects, though it is not very clear that how the audible sound signal is taken over and then transferred to intracellular by the cells to bring about a further series of biological effects.

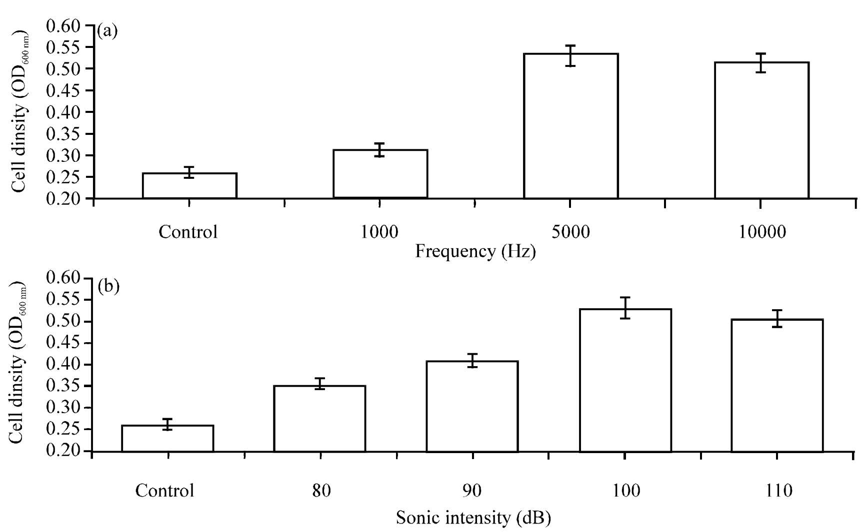

Fig. 2: Influence of audible sound wave stimulation on the growth of *E. coli*. (a) Experimental result when intensity of audible sound was maintained a constant (100dB) and variable frequency and (b) Experimental result when frequency of audible sound was maintain a constant (5 kHz) and variable intensity

The influence of audible sound stimuli on the total protein content: The influence of audible sound stimuli on the content of total protein was shown in Fig. 3. The results indicated that, no matter what kinds of combination of sound frequency and intensity, sound stimulation could obviously increase the total protein level of E. coli. Fig. 3a shows that the total protein content reached 32, 67 and 76 mg per g wet weight after treatment by the 100 dB sound wave with frequency of 1, 5 and 10 kHz, separately, corresponding to more 1.2, 2.5 and 3 times than that of the control. The similar results were observed in Fig. 3b when the sound frequency was constant at 5 kHz. The total protein content of treated group by 110 dB audible sound with frequency of 5 kHz was approximately 3-fold higher than that of the control.

The above results implied that protein synthesis has been enhanced obviously. Some stress-induced genes might be switched on under sound stimulation and the level of transcription increased. Wang *et al.* (2003) reported that the contents of RNA and protein changed greatly under audible sound exposure. Moreover the content of protein had a very close relationship with that of RNA. Shao *et al.* (2008) observed SG7B1 gene was preferably expressed in Chrysanthemum under the mode of sound-stress stimulation. Expression of Clone-SA8 gene of *Dendrobium candidium* was regulated by sound wave stimuli (Wang *et al.*, 2010). These results indicated that expression of some genes was positively regulated under sound wave exposure.

The influence of audible sound stimuli on the activity of antioxidant enzyme: As well known, exposure of organisms to abiotic stress such as temperature extremes, heavy metals, mechanical stimuli, nutrient deficiency, or salt stress can increase the production of ROS. To protect themselves against these toxic oxygen intermediates, cells employ antioxidant defense systems. The components of antioxidant defense system are enzymatic and non-enzymatic antioxidants. SOD and CAT were found in almost all organisms and are known as important antioxidant enzymes. To investigate whether acoustic stimuli leads to the increased production of reactive oxygen species, the activities of SOD and CAT were determined. Fig. 4 shows the relationship between SOD activity and sound stimulation dose. The results indicated that, no matter what kinds of combination of sound frequency and intensity, sound stimulation could obviously enhance SOD activity of *E. coli*. The SOD activity of treated group by 100dB audible sound with frequency of 5 kHz was approximately 1.8 -fold higher than that of the control. When the frequency of 100dB sound was up to 5000 Hz, the enzyme activity increased slowly (Fig. 4a). The similar phenomenon was noted in Fig. 4b when the sound frequency was constant at 5 kHz. The influence of sonic wave stimuli on CAT activity of *E. coli* was shown in Fig. 5. Compared with SOD, there was no doubt that the activity of CAT was more strongly affected by sound wave stimuli. The CAT activity of bacterial cells a rapid increase with the increasing of sound frequency

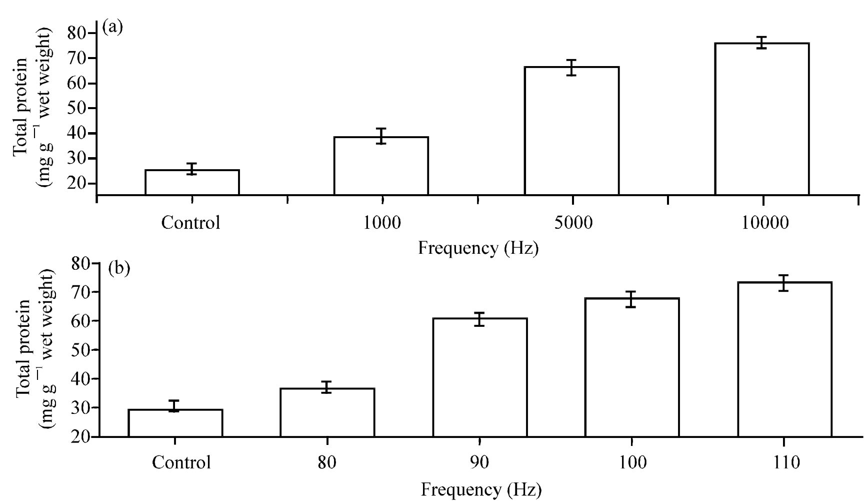

Fig. 3(a-b): Influence of sound stimulation on the total protein content of *E. coli.* (A) Experimental result when intensity of audible sound was maintained a constant (100dB) and variable frequency and (b) Experimental result when frequency of audible sound was maintain a constant (5 kHz) and variable intensity

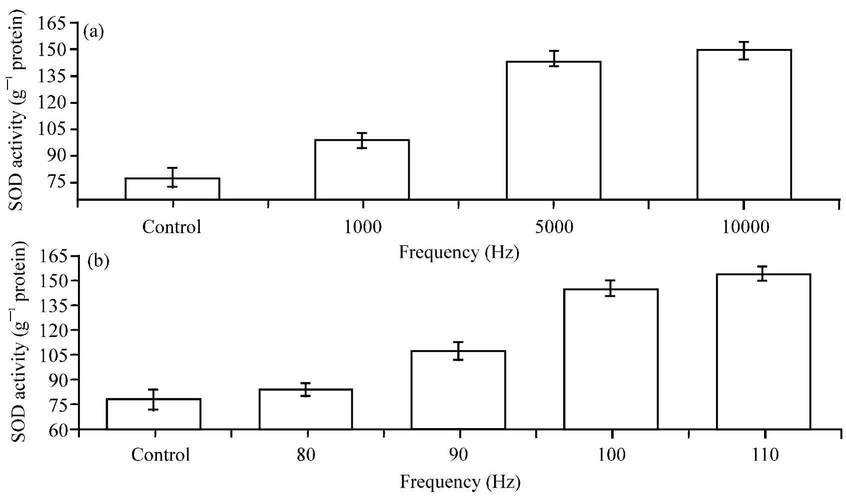

Fig. 4(a-b): Influence of sound stimuli on the SOD enzyme activity of *E. coli*. (a) Experimental result when intensity of audible sound was maintained a constant (100dB) and variable frequency and (b) experimental result when frequency of audible sound was maintain a constant (5 kHz) and variable intensity

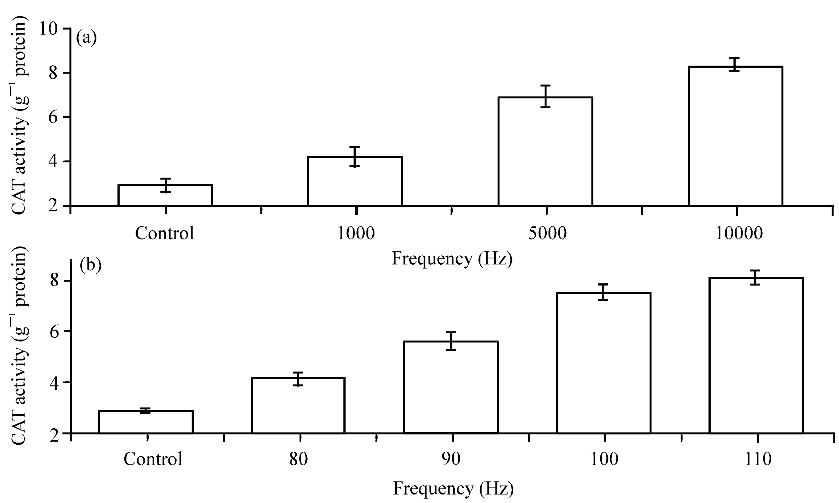

Fig. 5: Influence of sound stimulation on the CAT enzyme activity of *E. coli* (a) Experimental result when intensity of audible sound was maintained a constant (100dB) and variable frequency and (b) Experimental result when frequency of audible sound was maintain a constant (5 kHz) and variable intensity

(or intensity). The enzyme activity of treated group by 100dB audible sound with frequency of 10 kHz was 2.8 times than that of the control.

These results suggested that audible sound wave exposure reliably lead to in increase the production of ROS. Similar results were obtained when *Dendrobium candidum* was exposed sound waves environment (Li *et al*., 2008). The activities of SOD, CAT and peroxidase (POD) raised significantly that after the treatment of sound stimulation. In Wei *et al*. (2008) found that sound stimulation might activate on the POD isozymes gene expression in *Dendrobium candidum* (Wei *et al*., 2008). It is believed that these changes should be an adaptation to physical stress stimuli.

CONCLUSION

Using a single cell organism as a model, we have exposed *E.coli* to audible sound, to estimate whether sonic waves of different doses (frequency or intensity) affect bacteria cell metabolism and growth. The results not only showed that audible sound stimuli can remarkably enhance the content of total protein but significantly increase the activities of antioxidant enzymes. Simultaneously, growth of *E. coli* was stimulated in the treatments of different doses (frequency or intensity) sound wave. The most significant effect on growth promotion was observed when sound wave was maintained at 100 dB and 5000 Hz. The results suggested that the growth promotion effect of audible sound may be non-linear and shows obvious frequency and intensity peculiarities. On the other hand, the increase in activity of antioxidant enzymes implied that a number of active oxygen species generated in bacterial cell under the exposure of audible sound. Hence, further studies are needed to elucidate the mechanisms of active oxygen species generation induced by audible sound.

ACKNOWLEDGMENTS

This investigation was supported by the National Natural Science Foundation of China (31000017), Fund for Creative Research Talents of Henan University of Science and Technology (2012ZCX009) and the Startup Foundation for Advanced Talents of Henan University of Science and Technology (06-021).

REFERENCES

Aggio, R., V. Obolonkin and S.G. Villas-Boas, 2011. Sonic vibration affects the metabolism of yeast cells growing in liquid culture: A metabolomic study. Metabolomics, 8: 670-678.

Altman, G., R. Horan, I. Martin, J. Farhadi, P. Stark and V. Volloch *et al.*, 2002. Cell differentiation by mechanical stress. FASEB J., 16: 270-272.

Bailly, C., A. Benamar, F. Corbineau and D. Come, 1996. Changes in malondialdehyde content and in superoxide dismutase, catalase and glutathione reductase activities in sunflower seeds as related to deterioration during accelerated ageing. Physiol. Plant., 97: 104-110.

Bradford, M.M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem., 72: 248-254.

Danet, A.F., 2005. Environmental pollution monitoring: pollution, analysis, legislation, quality assurance and managing. S.C. Pro Act Birotic S.R.L.. Bucharest, pp: 56-64.

Davies, A., 2009. Acoustic trauma: Bioeffects of sound. Alex Davies BFA Honours.

Gu, S.B., Y. Wu, K.W. Li, S.C. Li, S.Y. Ma, Q.N. Wang and R. Wang, 2010. A pilot study of the effect of audible sound on the growth of *Escherichia coli*. Colloids Surf. B Biointerfaces, 78: 367-371.

Hou, T.Z. and R.E. Mooneyham, 1999a. Applied studies of plant meridian system: I. The effect of agri-wave technology on yield and quality of tomato. Am. J. Chin. Med., 27: 1-10.

Hou, T.Z. and R.E. Mooneyham, 1999b. Applied studies of the plant meridian system: II. Agri-wave technology increases the yield and quality of spinach and lettuce and enhances the disease resistant properties of spinach. Am. J. Chin. Med., 27: 131-141.

Leighton, T.G., 2007. What is ultrasound. Prog. Biophys. Mol. Biol., 93: 3-83.

Leventhall, G., 2007. What is infrasound? Progr. Biophys. Mol. Biol., 93: 130-137.

Li, B., J.M. Wei, X.L. Wei, K. Tang, Y.L. Liang, K.X. Shu and B.C. Wang, 2008. Effect of sound wave stress on antioxidant enzyme activities and lipid peroxidation of *Dendrobium candidum*. Colloids Surf. B Biointerfaces, 63: 269-275.

Marklund, S. and G. Marklund, 1974. Involvement of superoxide anion radical in the autoxidation of pyrogallol and a convenient assay for superoxide dismutase. Eur. J. Biochem., 47: 469-474.

Matsuhashi, M., A.N. Pankrushina, K. Endoh, H. Watanabe and H. Ohshima *et al.*, 1996. *Bacillus carboniphilus* cells respond to growth-promoting physical signals from cells of homologous and heterologous bacteria. J. Gen. Applied Microbiol., 42: 315-323.

Mitchell, C.A. and P.N. Myers, 1995. Mechanical stress regulation of plant growth and development. Hortic. Rev., 17: 1-42.

Shao, H.B., B. Li, B.C. Wang, K. Tang and Y.L.Liang *et al.*, 2008. A study on differentially expressed gene screening of *Chrysanthemum* plants under sound stress. Comptes Rendus Biol., 331: 329-333.

Wang, B.C., L.Q. Zhu, B. Li and W. Gong, 2010. Clone-SA8, regulated in expression by sound wave stimulation in *Dendrobium Candidium*. Proceedings of the World Congress on Medical Physics and Biomedical Engineering, September 7-12, 2009, Munich, Germany, pp: 1789-1791.

Wang, X.J., B.C. Wang, Y. Jia, D.Q. Huo and C.R. Duan, 2003. Effect of sound stimulation on cell cycle of *chrysanthemum (Gerbera jamesonii)*. Colloids Surf. B Biointerfaces, 29: 103-107.

Wei, JM., B. Li, B.C. Wang, K. Tang, Y.L. Liang and K.X. Shu, 2008. Effect of sound wave stimulation on peroxidase isozyme gene expression in *Dendrobium candidum*. Applied Acoust., 27: 462-468.

Ying, J.C.L., J. Dayou and C.K. Phin, 2009. Experimental investigation on the effects of audible sound to the growth of *Escherichia coli*. Modern Applied Sci., 3: 124-127.