J Therm Anal Calorim (2012) 108:177–183
DOI 10.1007/s10973-011-1897-7

Can curcumin food and *Bacillus subtilis* drug be taken simultaneously?

Da Zhang · Cheng Jin · Jiaoyang Luo · Xiaoping Dong · Xiaohe Xiao

Received: 1 August 2011 / Accepted: 30 August 2011 / Published online: 7 October 2011

Abstract Curcumin (CUR), a frequently-used food additive and flavorings, has been reported to be safe at a wide dose range. *Bacillus subtilis* (*B. subtilis*) is commonly found in soil and decomposing organic matter, and it was reported beneficial for humans when ingested. Up to now, there have been no contraindication of *B. subtilis* except for the avoidance of the drug combination with antibiotics, and the interaction of food and *B. subtilis* drug is blank. In this study, the interaction of CUR and *B. subtilis* was investigated. Microcalorimetry was applied to evaluate the effect of CUR on *B. subtilis* growth. By analyzing the main parameters extracted from the heat-flow power–time curves, it was concluded that CUR could inhibit the growth of *B. subtilis*, and the 50% inhibiting concentration (IC_{50}) valued 109.9 μg mL^{-1}. The results revealed that it is unreasonable to take CUR and *B. subtilis* at the same time, and it also provided a new way for the investigation of the interaction between food and drug. Meanwhile, this study indicated that the safety of CUR should be re-evaluated.

Keywords Curcumin food · *Bacillus subtilis* drug · Microcalorimetry · Safe medication

D. Zhang · C. Jin (✉) · J. Luo · X. Xiao (✉)
Integrative Medicine Center of the 302nd Military Hospital, China Military Institute of Chinese Materia Medica, Beijing 100039, People's Republic of China
e-mail: pharmasci@126.com

D. Zhang · J. Luo · X. Dong
College of Pharmacy, Chengdu University of Chinese Traditional Medicine, Chengdu 610075, People's Republic of China

Introduction

Curcumin (CUR) is extensively distributed in curcuma genus, and it attracts many people in the world by virtue of its special color and taste [1, 2]. CUR has been introduced as a food additive in India, China, and Japan for a long time [3, 4]. As one of the most popular spices, curry includes CUR as an essential component [5]. Along with its increasing role in our daily life, CUR has been reported with many bioactivities, e.g., antibacterial [6], antifungal [7, 8], antioxidant, and anti-tumor activities [9, 10]. More importantly, no side effects or toxic reaction has been tested even at large doses [11, 12], making it possible for CUR to be exploited as a promising drug or applied in food therapy. Then, if there is no need to pay attention to its safety while taking CUR or if there is any clause that we have to follow when it comes to drug combination?

Bacillus subtilis (*B. subtilis*), which is commonly found in soil and decomposing organic matter, is a "friendly" micro-organism which can create tremendous benefits for humans when ingested [13]. This bacteria, it turned out, is so strong that it practically cannibalizes all harmful micro-organisms in the human body [14, 15]. It has been proved that *B. subtilis* can secrete some active compounds during its process of growth, such as subtilin, bacillosporin, anticandine, and gramicidin [16–18], which can inhibit pathogenic bacteria or opportunist caused by autoinfection. In addition, *B. subtilis* can stimulate the growth and development of the immune organs of animals, activate T and B lymphocyte, enhance the immune globulin and antibody level, and reinforce the cell immunity and humoral immune function [19–22]. Furthermore, *B. subtilis* can synthesize vitamins B group including vitamin B1, B2, B6, and nicotinamide, enhancing the activity of interferon and macrophage in vivo [23–26]. For many years, cultures of *B.*

subtilis were sold worldwide as a medicinal product (sold in the US and Mexico, for example, under the brand name Bacti-Subtil); rapidly becoming the world's leading treatment for dysentery and other intestinal problems [27]. *B. subtilis* is still used widely today in Germany, France, and Israel, where safe, effective all-natural therapeutic products are more highly esteemed by the health-prescient public than the more expensive synthetic drugs espoused by the orthodox medical establishment with all of their dangerous side effects [28–30].

However, apart from the avoidance of the drug combination of *B. subtilis* and antibiotics, we can hardly notice any contraindication of *B. subtilis*. Shall we pay attention to food and drink while taking *B. subtilis*? To answer this question, we chose CUR and *B. subtilis*, for example, to investigate their interaction. Microcalorimetric technique [31–33], which is non-destructive, online, and sensitive, was applied in this study to investigate the effects of CUR on *B. subtilis*. By analyzing the heat-flow power (HFP)–time curves of the bacteria growth in the presence of CUR and the quantitative thermo-kinetic parameters obtained from the growth curves with multiple analytical methods, the effects of CUR on *B. subtilis* growth were characterized. Furthermore, the potential mechanism for antibacterial effects of CUR was discussed. The results of this study would not only provide reference for whether it is rational to take CUR and *B. subtilis* at the same time, but also helpful for the criterion of choosing food during the period of taking medicine. Meanwhile, it indicated that the safety of CUR should be re-evaluated rather than be utilized without announcements.

Experimental

Materials

CUR was purchased from Beijing Yasser Co. Ltd. (Beijing, PR China). The purity of CUR was determined to be over 95% by UPLC analysis. The structure of CUR was given in Fig. 1. Dimethyl sulfoxide (DMSO) was chosen as a solvent for preparing the original solution of CUR. All the other chemicals used were of analytical grade.

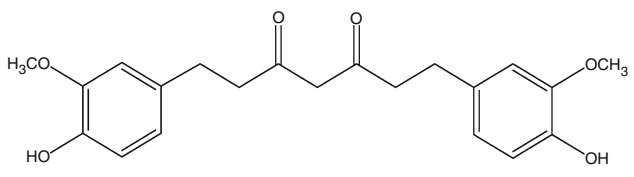

Fig. 1 Chemical structure of CUR

Broth culture

B. subtilis (ACCC 11060) was provided by China Center for Type Culture Collection, Wuhan University, Wuhan, PR China. The broth culture medium contained per 1,000 mL (pH 7.0–7.2): peptone (10 g), beef extract (6 g), and NaCl (5 g). The volume of the container was 100 mL, and the volume of the culture medium was 25 mL. The culture medium was sterilized in high pressure steam at 121 °C for 30 min. *B. subtilis* was inoculated in conical flask with 25 mL broth culture medium, and then incubated in the shaker at 37 °C for 8 h. The rotation speed of incubator shaker was 120 rpm. The conical flask was enveloped with a cotton plug, so that there was enough oxygen for bacteria.

Luria–Bertani (LB) medium

B. subtilis was grown in the LB culture medium (pH 7.0–7.2) of 1,000 mL prepared from peptone (10 g), yeast extract (5 g), and NaCl (5 g). The medium was sterilized by autoclaving at 121 °C for 30 min, and stored in a refrigerator at 4 °C.

Microcalorimetric studies

The experiments were performed at 37 °C using TAM III isothermal microcalorimeter (Thermometric AB, Sweden) with ampoule method [34, 35]. *B. subtilis* was inoculated in 100 mL LB medium; initially with the density of 1×10^6 colony forming units (CFU/mL). 2 mL of the bacterial suspension was added into each sterilized 4 mL glass ampoule. CUR was diluted in 2 mL DMSO, then, the solution at different concentrations was introduced into this suspension. Eventually, each ampoule containing different concentration of CUR and *B. subtilis* was sealed up and put into the equilibrium position of the calorimeter block. After about 15 min (the ampoules reached equilibrium in the air), the ampoules were lowered into the measuring position of the calorimeter block. After another 45 min (the temperature of the ampoules reached 37 °C), the HFP–time curves were recorded until the recorder returned to the baseline. All data was continuously collected using the dedicated software package (PicoLog TC-80, TA Corporation, USA).

Similarity analysis (SA)

To learn from the SA for HPLC chromatographic fingerprints of traditional Chinese medicine from different sources [36]. The thermogenic curves of *B. subtilis* growth affected by different concentrations of CUR were investigated by their similarities to intuitively and quickly find the

influence of the compounds on the bacterial growth. In this study, the correlation coefficients of similarity among the thermogenic curves of *B. subtilis* growth with and without CUR were calculated using the cosine method [37].

Canonical correlation analysis (CCA)

The typical use for CCA in the experimental context is to take two sets of variables and see what is common among the two sets. By seeing how one set of variance relates to another, we could gain insight into what dimensions are common between the tests and how much variance is shared. In this study, CCA was used for the relationship between the values of the concentrations and the nine thermo-kinetic parameters extracted from the power–time curves using SAS statistical software (SAS for Windows 8.0, SAS Inc., USA).

Results and discussion

Consideration of the concentration of chaotropic agent

In this study, CUR was first dissolved in DMSO, and then diluted with the LB culture. Different concentrations of DMSO, i.e., 0.1, 0.2, 0.3, 0.4, 0.5% (v/v), were investigated, which indicated that CUR can be well-distributed in the final solution containing more than 0.3% DMSO. Meanwhile, the effect of different concentration of DMSO on *B. subtilis* growth was investigated to eliminate the influence of the solvent. The results showed that with the increase of the concentration of DMSO, especially above 0.3% (v/v), all the peak height and the appearance time of second peak declined gradually. When the concentration was less than 0.3% (v/v), the influence could be neglected. By repeat experiments, the concentration of DMSO was defined within 0.3% during the experiments.

HFP–time curves of *B. subtilis* growth

The growth thermogenic curves of *B. subtilis* at 37 °C in the absence of any substance were shown in Fig. 2. The HFP–time curve shows the total metabolism profile of *B. subtilis*, and each could be divided into two stages (stages I and II) and the following five phases, i.e., a lag phase (A–B), the first exponential growth phase (B–C), a transition phase (C–D), the second exponential growth phase (D–E), and a decline phase (E–F).

Similarly, the HFP–time curves of *B. subtilis* growth in the presence of different concentrations of CUR were recorded and the corresponding curves were shown in Fig. 3. We can easily conclude from Fig. 3 that the shapes of the HFP–time curves changed regularly along with the increase of the concentration of CUR.

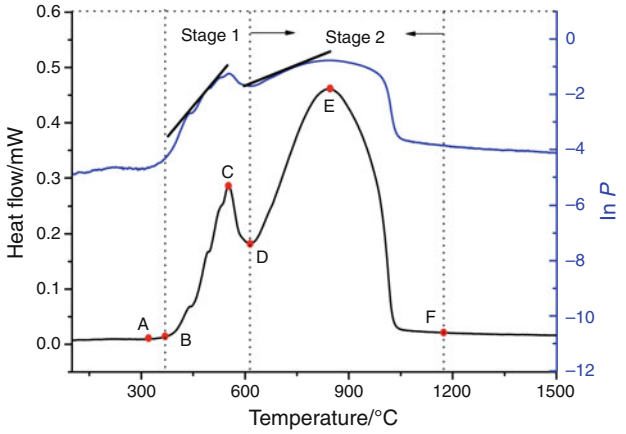

Fig. 2 HFP–time curves of *B. subtilis* in the absence of any substance. This is a typical metabolic profile of the bacteria culturing in LB culture medium supplemented without any substance monitored by the microcalorimeter at 37 °C. The profile could be divided into two stages (stages I and II) and five phases, i.e., a lag phase (*a–b*), the first exponential growth phase (*b–c*), a transition phase (*c–d*), the second exponential growth phase (*d–e*), and a decline phase (*e–f*)

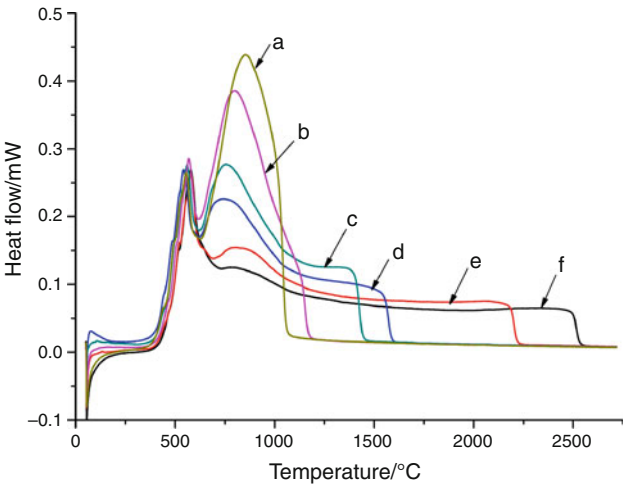

Fig. 3 The HFP–time curves of *B. subtilis* growth at 37 °C in the presence of different concentrations of CUR. The concentrations of CUR of *a–f* in this profile was increasing by orders, and the concrete concentrations for all the experiments were shown in Table 1

Quantitative thermo-kinetic parameters for *B. subtilis* growth

The HFP–time curves of *B. subtilis* growth could be delineated with the following equation [38]: $P_t = P_0 \exp(kt)$ or $\ln P_t = \ln P_0 + kt$, where P_0 and P_t represented the HFP at time zero and t (min), respectively. Using this equation, the growth rate constants (k_1 and k_2) of the first

and second exponential phase for *B. subtilis* growth at 37 °C in the absence of any substance were calculated by analyzing the data of the first and second highest peak. Each experiment was repeated for eight times for the bacteria in the absence of any substance so as to test the reliability, and good reproducibility was obtained. Thereafter, the quantitative thermo-kinetic parameters, such as the HFP of the first and second highest peak (P_1 and P_2), the appearance time of the first and second highest peak (t_1 and t_2), the heat output in stage 1 and stage 2 ($Q_{sta,1}$ and $Q_{sta,2}$), and total heat output (Q_t) were obtained from the HFP–time curve of the bacteria growth affected by different concentrations of CUR and shown in Table 1.

Similarity analysis

The similarities among the HFP–time curves of *B. subtilis* growth with different concentrations of CUR were calculated on the correlative coefficient of original data in Table 1 with cosine method using software of Microsoft Excel 2003 [39]. The thermogenic curves which showed the growth of *B. subtilis* in the absence of any substance were regarded as the reference, and the thermogenic curves in the presence of different concentrations of CUR were compared accordingly with them. The corresponding dataset of similarity was shown in Fig. 4.

It can be illustrated from Fig. 4 that different concentrations of CUR had varied effects on *B. subtilis* growth. The decrease of the correlation coefficient indicated that the antibacterial effects were enhanced with the increase of the concentration of CUR. However, by analyzing the multivariate variables in Table 1, we might notice that the nine parameters showed different change trends (increasing and decreasing) with the increase of the concentrations of CUR, making it difficult to accurately compare the antibacterial effects of CUR on *B. subtilis* growth. Therefore, it was necessary to extract the main parameter(s) that played the most relevant role in evaluating the antibacterial effect of CUR on *B. subtilis* growth. CCA was consequently introduced afterwards.

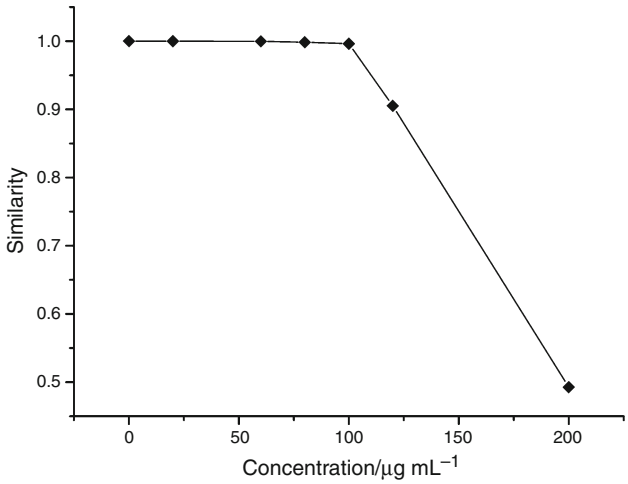

Fig. 4 Delineation of similarities of thermogenic curves

Canonical correlation analysis

CCA was used for the relationship between the values of the concentrations of CUR and the nine thermo-kinetic parameters extracted from the power–time curves. The results were shown in Table 2. The correlation coefficient between the concentrations and thermo-kinetic parameters in Table 2 showed that the growth rate constant of the second exponential phase k_2 and the HFP of the second highest peak P_2 had a close correlation with the concentrations of CUR. These results indicated that the canonical variable was mainly influenced by parameters k_2 and P_2, which played more important role in evaluating and comparing the antibacterial effect of CUR, thus these parameters could be applied for evaluating the antibacterial activities of *B. subtilis*.

Evaluation of the antibacterial activity and potential mechanisms

The CCA results showed that k_2 was the main parameter of the HFP–time curves of *B. subtilis* growth. The inhibition ratios (I) of CUR on *B. subtilis* were obtained from k_2, which could describe the change tendency of the

Table 1 Quantitative thermo-kinetic parameters for *B. subtilis* growth at 37 °C affected by CUR

c/μg mL^{-1}	k_1/min^{-1}	R^a	t_1/min	P_1/mW	k_2/min^{-1}	R^a	t_2/min	P_2/mW	$Q_{sta,1}$/J	$Q_{sta,2}$/J	Q_t/J
0	0.0090	0.9720	551.9	0.2861	0.0063	0.9992	845.4	0.4612	2.24	9.82	12.06
20	0.0094	0.9817	554.1	0.2646	0.0056	0.9949	854.4	0.4287	1.92	9.60	11.52
60	0.0099	0.9894	568.1	0.2855	0.0049	0.9990	801.6	0.3454	2.07	9.51	11.58
80	0.0083	0.9835	559.6	0.2758	0.0043	0.9837	752.3	0.2769	2.32	9.51	11.83
100	0.0102	0.9869	542.5	0.2691	0.0038	0.9955	744.0	0.2259	2.44	8.92	11.36
120	0.0093	0.9890	579.0	0.2639	0.0025	0.9804	808.0	0.1844	2.28	8.88	11.16
200	0.0120	0.9903	578.8	0.2690	0.0003	0.9159	776.1	0.0753	2.35	8.38	10.73

[a] Correlation coefficient

Table 2 Standardized canonical coefficients for the nine variables extracted from the HFP–time curves

	k_1	t_1	P_1	k_2	t_2	P_2	$Q_{sta,1}$	$Q_{sta,2}$	Q_t
c	0.1318	0.0685	−0.1315	−1.5131	−0.7567	1.1502	0	0	0

antibacterial efficacy among different concentrations of CUR. Finally, the 50% inhibiting concentration (IC_{50}) of CUR was calculated and valued 109.9 μg mL^{-1}. Good linear correlation between k_2, P_2, I and the concentration (c) were obtained (Fig. 5).

In exponential phases, the metabolism of bacteria is productive, the growth of bacteria is fast, and the reproductive cycle is stationary, which makes the bacteria good materials in these phases for the investigation of analytic metabolism of microbes [40]. Moreover, the shapes, color, and bioactivity of bacteria are typical, and it is sensitive to environmental impact, so that these stages of bacteria are the best object for the research of bacterial characters [41, 42]. Therefore, in this study, the atlas and data within the exponential stages were selected as the main index.

By analyzing the HFP–time curves of *B. subtilis* growth in Table 1, we found out that CUR scarcely inhibited the lag phase, first exponential growth phase, and transition phase of *B. subtilis*, but significantly inhibited the second exponential growth phase, decreased the maximum concentration of the cells (the second highest peak P_2) and extended the time of declined phase. Based on the above regularity, we deduce that the mechanism of antibacterial activity of CUR on *B. subtilis* is possibly that CUR can suppressor cell activity and inhibit the cell proliferation within the two stages of *B. subtilis* growth.

Significance of this study

This study gave us a warning that the external factors, such as food and gastrointestinal bacterial flora, should be included when investigating the side effect and toxicity of a drug. These interactions or contraindications should be denoted unequivocally in the dispensatory to refrain from such side effect as anaphylaxis or the decrease of curative effect. Meanwhile, the safety of CUR should be re-evaluated rather than be utilized without announcements. This viewpoint agrees with the statement of European Food Safety Authority (EFSA) in 2010 that Food Additives and Nutrient Sources added to Food (ANS) re-evaluate the safety of CUR (E100) that is serviced as edible pigment [43].

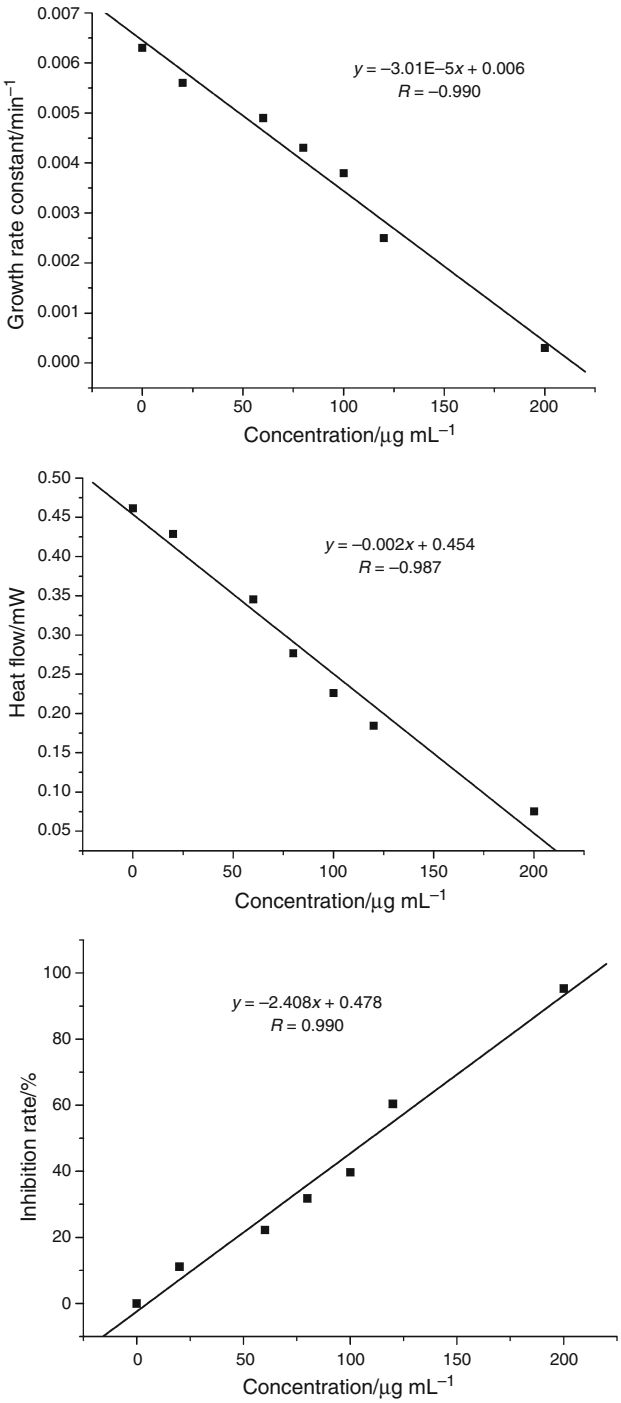

Fig. 5 Relationship between k_2, P_2, I and the concentration (c) of CUR

Conclusions

The interaction of CUR and *B. subtilis* was first investigated in this study, which indicated that CUR could inhibit the growth of *B. subtilis*, showing rigorous dose–effect relationship. The IC_{50} of CUR was 109.9 μg mL^{-1}. The HFP–time curves and main thermo-kinetic parameters extracted from these curves of *B. subtilis* growth in the presence of CUR were selected as the quantitative indicators for their interaction. The results reveal that it is unreasonable to take CUR and *B. subtilis* at the same time. Meanwhile, this study presents a new mode for the investigation of the interactions between food and drug.

Acknowledgements The authors are grateful to the support of Scientific and Technological Major Special Project for the "Significant New Drugs of Creation of China" (2009ZXJ09004-057, 2009ZX09502-022), National Science Foundation of China (81073043), and the Open Research Fund of State Key Laboratory Breeding Base of Systematic Research, Development and Utilization of Chinese Medicinal Resource.

References

1. Scotter MJ. Synthesis and chemical characterisation of curcuminoid colouring principles for their potential use as HPLC standards for the determination of curcumin colour in foods. LWT Food Sci Technol. 2009;42:1345–51.
2. Tsatsaroni E, Liakopoulou-Kyriakides M, Eleftheriadis I. Comparative study of dyeing properties of two yellow natural pigments-effect of enzymes and proteins. Dyes Pigments. 1998;37:307–15.
3. Hanif R, Qiao L, Shiff SJ, Rigas B. Curcumin, a natural plant phenolic food additive, inhibits cell proliferation and induces cell cycle changes in colon adenocarcinoma cell lines by a prostaglandin-independent pathway. J Lab Clin Med. 1997;130:576–84.
4. Hashem MM, Atta AH, Arbid MS, Nada SA, Asaad GF. Immunological studies on Amaranth, Sunset Yellow and Curcuminas food colouring agents in albino rats. Food Chem Toxicol. 2010;48:1581–6.
5. Sikora E, Bielak-Zmijewska A, Piwocka K, Skierski J, Radziszewska E. Inhibition of proliferation and apoptosis of human and rat T lymphocytes by curcumin, a curry pigment. Biochem Pharmacol. 1997;54:899–907.
6. Shankar TNB, Murthy VS. Effect of turmeric (*Curcuma longa*) fractions on the growth of some intestinal and pathogenic bacteria in vitro. Indian J Exp Biol. 1979;17:1363–6.
7. Lee SH, Chang KS, Su MS, Huang YS, Jang HD. Effects of some Chinese medicinal plant extracts on five different fungi. Food Control. 2007;18:1547–54.
8. Reddy KRN, Reddya CS, Muralidharan K. Potential of botanicals and biocontrol agents on growth and aflatoxin production by *Aspergillus flavus* infecting rice grains. Food Control. 2009;20:173–8.
9. Toda S, Miyase T, Arichi H, Tanizawa H, Takino Y. Natural antioxidants. III. Antioxidative components isolated from rhizome of *Curcuma longa* L. Chem Pharm Bull. 1985;33:725–8.
10. Banerji A, Chakrabarti J, Mitra A, Chatterjee A. Effect of curcumin on gelatinase A (MMP-2) activity in B16F10 melanoma cells. Cancer Lett. 2004;211:235–42.
11. Shankar TNB, Shantha NV, Ramesh HP, Murthy IAS, Murthy VS. Toxicity studies on turmeric (*Curcuma longa*): acute toxicity studies in rats, guinea pigs and monkeys. Indian J Exp Biol. 1980;18:73–5.
12. Sambaiah K, Ratankumar S, Kamanna VS, Satyanarayana MN, Rao MVL. Influence of turmeric and curcumin on growth, blood constituents and serum enzymes in rats. J Food Sci Technol. 1982;19:187–90.
13. Kinsella K, Schulthess CP, Morris TF, Stuart JD. Rapid quantification of *Bacillus subtilis* antibiotics in the rhizosphere. Soil Biol Biochem. 2008;41:374–9.
14. Carrillo C, Teruel JA, Aranda FJ, Ortiz A. Molecular mechanism of membrane permeabilization by the peptide antibiotic surfactin. Biochim Biophys Acta Biomembr. 2003;1611:91–7.
15. Asaka O, Shoda M. Biocontrol of *Rhizoctonia solani* damping-off of tomato with *Bacillus subtilis* RB14. Appl Environ Microbiol. 1996;62:4081–5.
16. Nagorska K, Bikowski M, Obuchowski M. Multicellular behavior and production of a wide variety of toxic substances support usage of *Bacillus subtilis* as a powerful biocontrol agent. Acta Biochim Pol. 2007;54:495–508.
17. Ongena M, Jacques P. *Bacillus* lipopeptides: versatile weapons for plant disease biocontrol. Trends Microbiol. 2008;16:115–25.
18. Williams V, Fletcher M. *Pseudomonas fluorescens* adhesion and transport trough porous media are affected by lipopolysaccharide composition. App Environ Microbiol. 1996;62:100–4.
19. Caruso A, Flamminio G, Folghera S, Peroni L, Foresti I, Balsari A, Turano A. Expression of activation markers on peripheral-blood lymphocytes following oral administration of *Bacillus subtilis* spores. Int J Immunopharmacol. 1993;15:87–92.
20. Hu B, Li C, Lu HJ, Zhu ZB, Du SW, Ye M, Tan L, Ren D, Han J, Kan S, Wang J, Jin N. Immune responses to the oral administration of recombinant *Bacillus subtilis* expressing multi-epitopes of foot-and-mouth disease virus and a cholera toxin B subunit. J Virol Methods. 2011;171:272–9.
21. Sun P, Wang JQ, Zhang HT. Effects of *Bacillus subtilis* natto on performance and immune function of preweaning calves. J Dairy Sci. 2010;93:5851–5.
22. Tseng DY, Ho PL, Huang SY, Cheng SC, Shiu YL, Chiu CS, Liu CH. Enhancement of immunity and disease resistance in the white shrimp, *Litopenaeus vannamei*, by the probiotic, *Bacillus subtilis* E20. Fish Shellfish Immunol. 2009;26:339–44.
23. Sakai A, Kita M, Katsuragi T, Ogasawara N, Tani Y. yaaD and yaaE are involved in vitamin B6 biosynthesis in *Bacillus subtilis*. J Biosci Bioeng. 2002;93:309–12.
24. Sakai A, Kita M, Katsuragi T, Tani Y. serC Is involved in vitamin B6 biosynthesis in *Escherichia coli* but not in *Bacillus subtilis*. J Biosci Bioeng. 2002;93:334–7.
25. Muscettola M, Grasso G, Blach-Olszewska Z, Migliaccio P, Borghesi Nicoletti C, Giarratana M, Gallo VC. Effects of *Bacillus subtilis* spores on interferon production. Pharmacol Res. 1992;26:176–7.
26. Kosaka T, Maeda T, Nakada Y, Yukawa M, Tanaka S. Effect of *Bacillus subtilis* spore administration on activation of macrophages and natural killer cells in mice. Vet Microbiol. 1998;60:215–25.
27. Smith TJ, Foster SJ. Autolysins during sporulation of *Bacillus subtilis* 168. FEMS Microbiol Lett. 1997;157:141–7.
28. Morimoto I, Watanabe F, Osawa T, Okitsu T, Kada T. Mutagenicity screening of crude drugs with *Bacillus subtilis* rec-assay and Salmonella/microsome reversion assay. Mutat Res Environ Mutagen Relat Subj. 1982;97:81–102.
29. Hong HA, Khaneja R, Tam NMK, Cazzato A, Tan S, Urdaci M, Brisson A, Gasbarrini A, Barnes I, Cutting SM. *Bacillus subtilis* isolated from the human gastrointestinal tract. Res Microbiol. 2009;160:134–43.

30. Salinas I, Cuesta A, Esteban MÁ, Meseguer J. Dietary administration of *Lactobacillus delbrüeckii* and *Bacillus subtilis*, single or combined, on gilthead seabream cellular innate immune responses. Fish Shellfish Immunol. 2005;19:67–77.
31. Phipps MA, Mackin LA. Application of isothermal microcalorimetry in solid state drug development. Pharm Sci Technol Today. 2000;3:9–17.
32. Medina S, Raviv M, Saadi I, Laor Y. Methodological aspects of microcalorimetry used to assess the dynamics of microbial activity during composting. Bioresour Technol. 2009;100:4814–20.
33. Yan D, Jin C, Xiao XH, Dong XP. Antimicrobial properties of berberines alkaloids in *Coptis chinensis* Franch by microcalorimetry. J Biochem Biophys Methods. 2008;70:845–9.
34. Kong WJ, Li ZL, Xiao XH, Zhao YL, Zhang P. Activity of berberine on *Shigella dysenteriae* investigated by microcalorimetry and multivariate analysis. J Therm Anal Calorim. 2010; 102:331–6.
35. Lago N, Legido JL, Paz Andrade MI, Arias I, Casás LM. Microcalorimetric study on the growth and metabolism of *Pseudomonas aeruginosa*. J Therm Anal Calorim. 2010;105: 651–5.
36. Chen Y, Zhu SB, Xie MY, Nie SP, Liu W, Li C, Gong XF, Wang YX. Quality control and original discrimination of *Ganoderma lucidum* based on high-performance liquid chromatographic fingerprints and combined chemometrics methods. Anal Chim Acta. 2008;623:146–56.
37. Xie PS. Chromatographic fingerprints of traditional Chinese medicine (in Chinese). 1st ed. Beijing: People's Medical Press; 2005.
38. Xie CL, Tang HK, Song ZH, Qu SS, Liao YT, Liu HS. Microcalorimetric study of bacterial growth. Thermochim Acta. 1988;123: 33–41.
39. Gao XH, Guo LH, Li H. Discrimination between natural and cultured *Gastrodia elata* blumes by X-ray diffraction fingerprint patterns and similarity degree of different fingerprint patterns. Chem Res Appl. 2005;17:58–60.
40. Dakna M, Opatrný T, Welsch DG. Homodyne measurement of exponential phase moments. Opt Commun. 1998;148:355–75.
41. Brunner B, Yu JY, Mielke RE, MacAskill JA, Madzunkov S, McGenity TJ, Coleman M. Different isotope and chemical patterns of pyrite oxidation related to lag and exponential growth phases of *Acidithiobacillus ferrooxidans* reveal a microbial growth strategy. Earth Planet Sci Lett. 2008;270:63–72.
42. Wang HW, Cheng HR, Wei DZ, Wang FQ. Comparison of methods for measuring viable *E. coli* cells during cultivation: great differences in the early and late exponential growth phases. J Microbiol Methods. 2011;84:140–3.
43. EFSA Panel on Food Additives and Nutrient Sources added to Food (ANS). Scientific Opinion on the re-evaluation of curcumin (E 100) as a food additive. EFSA J. 2010;8:1679–1724.