Spoilage Bacteria Identification and Food Safety Risk Assessment of Whole Soybean Curd

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Abstract As a highly hydrated gel-type food, soybean curd is perishable and the development of spoilage bacteria has been described. Whole soybean curd (WSC), an innovative soy product retains the most nutrients in raw ingredients and exhibits more nutritive value compared with conventional soybean curd (CSC). However, the risk assessment of WSC is not well evaluated up to now. In this study, the same species of spoilage microorganism were separated from WSC and CSC. Two main spoilage strains were separated and identified as *B. subtilis* and *B. cereus*. The risk ranking scores of WSC was higher than that of CSC but still within medium risk range. In summary, we reported the presence of *B. subtilis* and *B. cereus* in WSC for the first time. Further, application of the risk ranger tool confirmed that the risk profile of WSC was medium and acceptable.

Keywords Whole soybean curd · Risk ranking · Predictive models · Spoilage bacteria

Chenzhi Wang and Qinling Du have contributed equally to this work.

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The high nutritional value and cholesterol-free property make them the best source of plant protein for poor people suffering from malnutrition problems. Soybean curd, a highly hydrated gel-type food chemically composed of protein and lipid, is a fundamental part of food cultures in Asian countries [1]. Currently, its consumption grows rapidly in western countries due to its rich content in bioactive compounds, such as isoflavones and soyasaponin [2, 3].

Being a gelatinous product with relatively high moisture content (80–88%), moderate pH (5.8–6.2) and rich protein (6–8.4%) [4, 5], soybean curd is perishable. The detection of spoilage microorganisms such as *Lactic acid bacteria*, *Pseudomona*, *Enterobacteriaceae*, *Bacillus* spp., *Klebsiella* spp., and *Staphylococcus* spp., in soybean curd has been reported in some research [6–8]. Different from conventional soy foods, whole soy foods including soybean flour suspension, meat analogues and WSC exhibit more nutritive value [9, 10]. However, the predominant spoilage bacteria in whole soy foods (e.g. whole soybean curd, WSC) has not been specially identified and the risk assessment of this innovative whole soy foods is not well evaluated up to now. The steps expended to assess a specified risk involve the identification of spoilage bacteria and the methodical matching to a system. Generally, the key risk factors associated with soybean curd involve the contamination levels of raw ingredients and the quality of water. Generally, soybean seeds are directly collected from soil thus easily contaminated by microorganisms such as *B. cereus* and *B. subtilis* [11]. Although the thermal treatment during curd making is able to kill vegetative cells but the spores could survive since they are heat resistant.

Spoilage microorganisms were separated from spoiled soybean curd that had been stored at 37 °C for 24 h. Colonies with different distribution ratio and morphology

Sequence details

Sequence details

Fig. 1 Reverse sequence details of 16S rDNA isolated from two spoilage strains in EzBioCloud system

on TSA medium were picked and purified. Genomic DNA of spoilage bacteria in WSC and CSC were extracted using a DNA extraction kit (DP-302, Tiangen Biotech, Beijing, China) and amplified for 16S rDNA gene and sequenced by PCR procedure with a universal primer set (27F, 5'-AGTTTGATCMTGGCTCAG-3' and 1492R, 5'-GGTTACCTTGTTACGACTT-3') [12]. The genomic 16 S rDNA were sequenced using a sequencer (Applied Biosystems, Carlsbad, CA, USA), reverse sequences were subjected to EzBioCloud system (<https://www.ezbiocloud.net>). A risk ranger software was applied to evaluate the risk profile of soybean curd [13]. The users were required to answer 11 questions in relation to probability of infective

dose, exposure degree and pathogen severity. Severity of illness (question 1), susceptibility of population (question 2) and probability of infective dose were answered based on literatures, probability of exposure (question 3-5) and question 11 relied on a questionnaire participated by 647 students and teachers, which was conducted at Sichuan Agricultural university, Sichuan province, China.

The species of spoilage bacteria in WSC and CSC were the same and the reverse sequence details of the main two strains were shown in Fig. 1, in which the length and completeness were: T-1, 1381 bp, 93.8% (47–1427 bp) and T-2, 1377 bp, 93.4% (50–1426), respectively. Taxonomic results from EzBioCloud system were exhibited in Table 1,

Table 1 Taxonomic results of spoilage organism isolated from spoiled soybean curd as identified on the basis of 16 S rDNA partial sequence

Bacteria	No.	Name	Strain	Accession no.	Pairwise similarity (%)	Completeness (%)
T-1	1	<i>Bacillus subtilis subsp. inaquosorum</i>	KCTC 13429	AMXN01000021	99.93	100
	2	<i>Bacillus tequilensis</i>	KCTC 13622	AYTO01000043	99.93	100
	3	<i>Bacillus subtilis subsp. subtilis</i>	NCIB 3610	ABQL01000001	99.86	100
T-2	1	<i>Bacillus cereus</i>	ATCC 14579	AE016877	100	100
	2	<i>Bacillus wiedmannii</i>	W8-0169	LOBC01000053	99.93	100
	3	<i>Bacillus albus</i>	N35-10-2	KJ812440	99.93	100

the spoilage microorganisms were identified as *Bacillus subtilis* subsp. *Inaquosorum* (99.93% similarity, 100% completeness) and *Bacillus cereus* (100% similarity, 100% completeness), respectively. According to the compendium of microbiological criteria for food in Australia [14], *B. cereus* was the main pathogen in soybean curd, other *Bacillus* organism connected with foodborne diseases are

originating from the *Bacillus subtilis* group (such as *B. licheniformis* and *B. pumilis*), symptoms and causative elements caused by *Bacillus subtilis* group were similar to *B. cereus*. FAO/WHO Expert Consultations (2004, 2006) categorized *Bacillus cereus* as category C: microorganisms for which causality with illness is less plausible or not yet demonstrated. As a moderate hazard, *B. cereus* usually

Table 2 Risk ranking assessment of *B. cereus* and pathogenic *B. subtilis* in soybean curd

Risk criteria	CSC	WSC
<i>Dose and severity</i>		
Q1	Mild	Mild
Q2	General	General
<i>Probability of exposure</i>		
Q3	Daily (6.68%) Weekly (50.59%) Monthly (27.15%) A few times per year (13.35%) Once every few years (0.45%)	Daily (6.68%) Weekly (50.59%) Monthly (27.15%) A few times per year (13.35%) Once every few years (0.45%)
Q4	98.22%	98.22%
Q5	647	647
<i>Probability of infective dose</i>		
Q6	Other: 41%	Other: 41%
Q7	Usually eliminates the hazards	Usually eliminates the hazard
Q8	No	No
Q9	Not relevant	Not relevant
Q10	Other: 1000	Other: 100
Q11	Usually eliminates the hazards (boiled: 38.05%, braised: 39.92%, fried: 13.28%, total 91.25%) Has no effect on the hazard (Cold mixed ^a : 8.75%)	Usually eliminates the hazards (boiled: 38.05%, braised: 39.92%, fried: 13.28%, total 91.25%) Has no effect on the hazard (Cold mixed: 8.75%)
<i>Risk estimates</i>		
Probability of illness ^b	Daily: 4.10×10^{-8} Weekly: 5.84×10^{-9} Monthly: 1.35×10^{-9} A few times per year: 3.37×10^{-10} Once every 2 years: 5.62×10^{-11}	Daily: 4.10×10^{-7} Weekly: 5.84×10^{-8} Monthly: 1.35×10^{-8} A few times per year: 3.37×10^{-9} Once every 2 years: 5.62×10^{-10}
Total predicted illnesses ^c	Daily: 9.68×10^{-3} Weekly: 1.38×10^{-3} Monthly: 3.18×10^{-4} A few times per year: 7.96×10^{-5} Once every 2 years: 1.33×10^{-5}	Daily: 9.68×10^{-2} Weekly: 1.38×10^{-2} Monthly: 3.18×10^{-3} A few times per year: 7.96×10^{-4} Once every 2 years: 1.33×10^{-4}
Risk ranking	Daily: 41 (medium risk) Weekly: 36 (medium risk) Monthly: 32 (medium risk) A few times per year: 29 (low risk) Once every 2 years: 25 (low risk)	Daily: 47 (medium risk) Weekly: 42 (medium risk) Monthly: 38 (medium risk) A few times per year: 35 (medium risk) Once every 2 years: 30 (low risk)

^aMix cold soybean curd with sauce

^bProbability of illness per day per consumer of interest

^cPer annum in population of interest

causes illness of short duration and have no sequelae, and all population might be susceptible to *B. cereus* poisoning, existing research indicated that for the production of emetic toxin in foods, the number of *B. cereus* population was required to reach 10^6 to 10^8 CFU/g [15], Food Standards Australia New Zealand (FSANZ) categorized satisfactory evaluation for foods if *B. cereus* level was below 10^2 CFU/g, however, it was deemed that potentially hazardous evaluation was considered if *B. cereus* level were over 10^5 CFU/g. From this point of view, there was an observed increase (10^3) from the initial contamination level to infection dose. As reported by Ross and Sumner (2002), low risk evaluation was associated to risk ranking value below 32 (< 32), medium risk was for value within 32–48, high risk was for value over 48 (> 48). As shown in Table 2, *B. cereus* gave medium risk scores for CSC with two exception (a few times per year, risk rating of 29, once every 2 years, risk rating of 25), whereas risk scores of WSC were higher compared with CSC but most groups still displayed medium risk scores, except for the group “once every 2 years”. Overall, risk ranking of whole-soybean curd was within an acceptable range even if people of interest consume whole-soybean curd everyday using cold-mix pre-preparation methods.

In summary, this is the first report to identify spoilage bacteria and evaluate the food safety risk of WSC. Two main spoilage strains were isolated from spoiled WSC and then identified as *B. subtilis* and *B. cereus*. Risk ranger software suggested that, compared with CSC, risk ranking of WSC became higher but was still within medium risk even if people of interest consume WSC everyday using cold-mix pre-preparation methods. Thus, introduction of okara into soybean curd reinforced the initial level of contamination but didn't significantly increase risk profile of WSC.

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