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Effects of Starter Candidates and NaCl on the Production of Volatile Compounds during Soybean Fermentation

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Running title: Starters and Volatiles Production

Abstract

We inoculated different combinations of three starter candidates of *Bacillus licheniformis*, *Staphylococcus succinus*, and *Tetragenococcus halophilus* into sterilized soybeans to predict their contributions to volatile compounds production through soybean fermentation. Simultaneously, we added NaCl to soybean cultures to evaluate its effect on the volatile compounds profile. Cells in soybean cultures (1.5% NaCl) reached almost their maximum growth in a day of incubation, while cell growth was delayed by increasing NaCl concentration in soybean cultures. The dominance of *B. licheniformis* and *S. succinus* in the mixed culture of three starter candidates switched to *T. halophilus* as the NaCl concentration increased from 1.5% to 14% (w/w). Seventeen volatile compounds were detected from the control and starter candidate-inoculated soybean cultures with and without the addition of NaCl. Principal component analysis of these volatile compounds concluded that *B. licheniformis* and *S. succinus* made major contributions to producing a specific volatile compound profile from soybean cultures where both species exhibited good growth. 3-Hydroxybutan-2-one, butane-2,3-diol, and 2,3,5,6-tetramethylpyrazine are specific odor notes for *B. licheniformis*, and 3-methylbutyl acetate and 2-phenylethanol are specific for *S. succinus*. Octan-3-one and 3-methylbutan-1-ol were shown to be decisive volatile compounds for determining the involvement of *S. succinus* in the soybean culture containing 7% NaCl. 3-Methylbutyl acetate and 3-methylbutan-1-ol were also produced by *T. halophilus* during soybean fermentation at an appropriate level of NaCl. Although *S. succinus* and *T. halophilus* exhibited growth on the soybean cultures containing 14% NaCl, species-specific volatile compounds determining the directionality of the volatile compounds profile were not produced.

Keywords: Doenjang, *Bacillus licheniformis*, *Staphylococcus succinus*, *Tetragenococcus halophilus*, 3-methylbutyl acetate, 3-methylbutan-1-ol

Introduction

Doenjang, a traditional Korean soybean paste, is fermented by a two-stage process. First, boiled soybeans are spontaneously fermented and dried for 1–2 months, which results in the growth of microorganisms as well as the degradation of macromolecules in the soybeans. The second stage starts by mixing the fermented soybeans, called meju, with a high-salt brine (approximately 18%, w/v). After approximately 2 months, the liquid portion of the mixture is separated, resulting in a traditional type of soy sauce, ganjang. The remaining solid portion, doenjang, is subsequently mashed and fermented for >6 months.

To industrialize the traditional doenjang manufacturing process, a number of studies including microbial community analysis and starter development have been performed. Naturally occurring fungi, yeast, and bacteria are involved in the ripening of doenjang [1]. Species of *Aspergillus* and *Bacillus* have been considered to play major roles in the doenjang manufacturing process based on their high rate of detection in meju and doenjang [2-4]. These results prompted the use of *Aspergillus* and *Bacillus* species in the production of doenjang and they were reported to be effective starting materials for this purpose [5-9]. However, recent culture-independent microbial studies have suggested the presence of a wider variety of microorganisms in meju and doenjang [10-14].

In our previous bacterial community analysis using culture-dependent methods, we identified enterococci, coagulase-negative staphylococci (CNS), and *Tetragenococcus halophilus* along with bacilli as the predominant groups of bacteria during the doenjang fermentation process [1]. We performed assessments of the safety, proteolytic and lipolytic enzyme activities, and salt tolerance of the predominant isolates to select novel starting materials for industrializing the process of doenjang manufacture, and then selected safe and

functional starter candidates [15-18]. The five selected starter candidates of *Enterococcus faecium*, *T. halophilus*, *Bacillus licheniformis*, *Staphylococcus saprophyticus*, and *Staphylococcus succinus* were inoculated into sterilized soybeans and the volatile compounds produced from each starter candidate during soybean culture were analyzed [19]. *E. faecium* and *T. halophilus* produced similar profiles of volatile compounds to soybeans. *B. licheniformis* and *S. succinus* produced the crucial volatile compounds that distinguish the volatile compounds profile of soybean. Meanwhile, *S. saprophyticus* was not as effective a volatile compound producer as *S. succinus*.

We finally selected three starter candidates of *B. licheniformis*, *S, succinus*, and *T. halophilus* based on their salt tolerance and volatile compound profile from soybean cultures. A better understanding of the growth kinetics of these starter candidates and their associations with flavor production is required for further optimization of their use in starter cultures for food fermentation. In the present study, we analyzed the volatile compounds produced by starter candidate combinations with *B. licheniformis*, an abundant species in doenjang, during soybean culture to predict the flavor profiles produced by future starter applications in soybean-based fermented food. We also analyzed the effects of NaCl on the volatile compound profiles produced from soybean cultures inoculated by the starter combinations to predict the flavor profiles in high-salt soybean fermentation.

Materials and methods

Bacterial strains and culture conditions

Three starter candidates applied in the current study, *B. licheniformis* 14BML13, *S. succinus* 14BME20, and *T. halophilus* 7BDE23, were selected through assessments of their safety, proteolytic and lipolytic enzyme activities, and salt tolerance followed by analyses of volatile compounds produced from their soybean cultures [16-18]. Strains were cultured in

tryptic soy broth (TSB; Difco, Detroit, MI, USA) and tryptic soy agar (TSA; Difco) at 30°C for 24 h. In the culture of *T. halophilus* 7BDE23, 3% (w/v) NaCl was supplemented into the same medium.

Inoculation of starter candidate combinations into sterilized soybeans

Soybeans (50 g, Korean Bactae, *Glycine max* L.) were washed and then soaked in 50 mL of water for 24 h at room temperature. After absorbing water, the soybeans were placed in 250-mL reagent bottles and then autoclaved for 30 min at 121°C. Samples were prepared in duplicate and each logarithmic-phase cell cultured in TSB was inoculated into the sterilized soybeans at an equal ratio at a level of 5×10^5 colony forming units (CFU)/g and then mixed thoroughly. The effects of NaCl on soybean cultures were determined in the samples additionally supplemented with 6 g and 12 g of NaCl. The inoculated soybean samples were incubated aseptically at 25°C for 28 days along with two sterilized soybean samples as controls. Samples in two bottles were collected every 7 days and stored at −80°C until chemical and microbiological analyses were undertaken.

Analysis of pH and NaCl content in soybean cultures

Ten grams of each sample was mixed thoroughly with 40 mL of deionized water for 5 min, filtered through Whatman filter papers (No. 2; GE Healthcare Life Sciences, Chicago, IL, USA), and then the pH of the filtrates was measured using a pH meter. The NaCl content of samples was quantified by titration with silver nitrate, in accordance with the Mohr method [20] after crushing the macerated soybeans with a mortar and pestle. All experiments were conducted in duplicate on two independent samples prepared in the same way.

Growth monitoring of the inoculated starter candidates during soybean culture

The filtrates prepared for pH analysis were spread onto plate count agar plates (Oxoid, Basingstoke, UK) after tenfold dilutions using saline and then incubated at 30°C for 24 h to determine the number of viable cells. After the colonies grown on agar plates had been counted, 100 distinguishable colonies were randomly picked and transferred onto selective media that can differentiate bacilli, staphylococci, and tetragenococci to estimate the proportion of each inoculated species in soybean cultures. The number of *B. licheniformis* was identified on nutrient agar (Difco) containing 50 mg/L penicillin to inhibit the growth of staphylococci and tetragenococci. The number of *T. halophilus*, which grows under facultatively aerobic to microaerophilic conditions, was determined on SF agar (Difco) containing 0.1% (w/v) cysteine-HCl and that of *S. succinus* was determined on mannitol salt agar (Difco). All agar media were incubated for 3 days at 30°C. All analyses were performed in duplicate on two independent samples prepared in the same way.

Analysis of volatile compounds by GC-MS

Soybean cultures (1 g) were mixed with 3.25 mL of water and 0.15 g of NaCl in a 20-mL vial with a silicon/Teflon septum (Supelco, Bellefonte, PA, USA), followed by maintenance at 60°C for 30 min to reach equilibrium. The headspace volatile compounds were extracted using an SPME device (Supelco) with a 50/30-μm divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) fiber for 15 min and then eluted at 220°C for 10 min. The volatiles were automatically injected into GC-MS through a transfer line set at 230°C (ISQ™ Series Single Quadrupole GC-MS systems; Thermo Scientific, West Palm Beach, FL, USA). The separation was performed using a DB-WAXetr capillary GC column (50-m length × 0.32-mm i.d. × 1-μm film thickness) (Agilent Technologies, Santa Clara, CA, USA). The conditions were as follows: oven temperature program, 40°C for 6 min, raised at 4°C/min until 90°C, 90°C for 5 min, 9°C/min until 220°C,

and then held for 17 min at 220°C; carrier gas (He) flow rate 1.0 mL/min (constant flow); ionization energy 70 eV; and scan range 50–500 m/z. The retention indices and mass spectral data were used to identify each compound. The retention indices of the volatile compounds were determined using a C_8–C_{20} alkane standard (Sigma, St. Louis, MO, USA) under the same chromatographic conditions and calculated according to the Kratz formula [21, 22]. The mass spectral data were also compared with mass spectral libraries provided by the National Institute of Standards and Technology (including Wiley and Mainlib). Only compounds whose similarity was more than 750 (maximum similarity, 1000) are reported here. All analyses were performed in duplicate on two independent samples prepared in the same way and quantitative analysis was based on the peak area of a particular component.

Statistical analysis

One-way ANOVA followed by Duncan's multiple range test was used to evaluate significant differences between the average values obtained in the volatile compound analyses. Values of $p<0.05$ were considered to be statistically significant. To visualize the differences between the volatile compounds produced from the sterilized soybeans by the inoculated bacteria, principal component analysis (PCA) was applied with maximum variation rotation. All statistical analyses were performed using the SPSS software package (version 22.0; SPSS, IBM, Armonk, NY, USA).

Results

Effect of NaCl on the growth of starter candidates and pH changes in soybean cultures

The NaCl concentration in sterilized soybean samples was quantified and found to be 1.5% (w/w). Those in samples supplemented with 6 g and 12 g of NaCl were determined to be 7% and 14%, respectively. No bacterial cells were detected in the control soybean samples

and the pH remained almost constant during monitoring, which means that our sterilization conditions were adequate to eliminate resident bacteria on soybean (Fig. 1).

Cells in soybean cultures reached almost their maximum growth in a day of incubation (approximately 10^8 CFU/g), while cell growth was delayed by the increase of NaCl concentration in soybean cultures. The cell numbers in *B. licheniformis*- and *S. succinus*-inoculated samples (BS samples) were the highest among the samples of mixed cultures regardless of the NaCl concentration at day 1, which might have been caused by the high growth rate of *S. succinus*. *S. succinus* exhibited relatively good growth on soybean cultures regardless of the NaCl concentration, which was shown by its high proportion within the total population in BS samples and the mixed cultures of *B. licheniformis*, *S. succinus*, and *T. halophilus* (BTS samples). After day 1, the cell numbers in BTS samples surpassed those of other mixed culture samples regardless of the NaCl concentration. The slow growth rate of *T. halophilus* on soybean cultures might have retarded the increase of cell numbers in *T. halophilus*-inoculated samples. The slow growth rate of this species was clearly demonstrated on the NaCl-added cultures of *B. licheniformis* and *T. halophilus* (BT samples), on which *B. licheniformis* failed to exhibit good growth and the maximum cell numbers in samples were reached at day 28. Although *B. licheniformis* strain 14BML13 was reported to grow on TSA supplemented with 15% NaCl [19], its growth on NaCl-added soybean cultures was not as active as that of *S. succinus* 14BME20 and *T. halophilus* 7BDE23. In the mixed cultures of three species (BTS samples), the dominance of *B. licheniformis* and *S. succinus* on soybean culture was shifted to that of *T. halophilus* by the addition of 14% NaCl. The highest cell numbers in BTS samples among mixed culture samples between days 7 and 21 might have been accomplished by the cumulative growth of *S. succinus*, *B. licheniformis*, and *T. halophilus* due to their different growth rates.

At each NaCl concentration, BS samples scored the lowest pH among mixed culture

196 samples at day 1, which corresponded well with the high growth rate of *S. succinus*, while the
197 change of pH in BS samples after day 1 was not as dramatic as that in BT and BTS samples.
198 The pH levels of BT samples were the highest among the mixed culture samples at day 1 and
199 then dropped to below those of BS samples regardless of the NaCl concentration after day 21.
200 This indicated that *T. halophilus* exhibited higher acid production than *S. succinus* during
201 fermentation, despite its slow growth in soybean culture. The decreases of pH in BS samples
202 with the three NaCl concentrations displayed similar patterns, which suggests that *B.*
203 *licheniformis* makes a small contribution to acid production when cultured with *S. succinus*.
204 The contributions of the three species to acid production during soybean fermentation may be
205 in the following order: *T. halophilus* > *S. succinus* > *B. licheniformis*. BTS samples
206 maintained the lowest pH level during fermentation, which matched well with their highest
207 cell numbers during fermentation.
208
209 **Effects of starter candidates and NaCl on the production of volatile compounds in**
210 **soybean cultures**
211 Three combinations of starter candidates were inoculated into sterilized soybeans and
212 the produced volatile compounds were monitored after 1 and 28 days of incubation. The
213 effect of NaCl on the volatile compound production was also considered by adding NaCl to
214 soybean cultures. Seventeen volatile compounds, such as acids, alcohols, carbonyls, esters,
215 furans, and pyrazines, were monitored in the soybean cultures as well as the NaCl-added
216 soybean cultures (Table 1).
217 Butane-2,3-diol and 3-hydroxybutan-2-one were dramatically increased only on
218 soybean cultures (1.5% NaCl) and their highest production was identified from the BT
219 sample. Considering the slow growth of *T. halophilus* on soybean cultures, *B. licheniformis* is
220 the major producer of both compounds. 2,3,5-Trimethylpyrazine and 2,3,5,6-

tetramethypyrazine were detected from all of the soybean culture samples of day 28. Commonly inoculated *B. licheniformis* makes the main contribution to the production of both of these compounds. 2,3,5-Trimethylpyrazine was also detected from all of the samples containing 7% NaCl and a significant increase was identified only from the BS sample containing 7% NaCl. *S. succinus* might also contribute to increasing the level of the compound during fermentation because this species grows well at 7% NaCl. A small amount of octan-3-one was detected from all samples, but significant production was identified from the BS sample containing 7% NaCl. 2-Phenylethanol was significantly increased from all of the BS samples regardless of the NaCl concentration. Salt-tolerant *S. succinus* may be the major producer of both compounds. The maximum production of acetic acid occurred in *T. halophilus*-inoculated samples with a 7% NaCl concentration and the role of *T. halophilus* in acetic acid production was clearly exhibited at a NaCl concentration of 14%. 3-Methylbutyl acetate and 3-methylbutan-1-ol were produced from all of the BS and BTS samples regardless of the NaCl concentration. *T. halophilus* as well as *S. succinus* may produce both compounds on soybean cultures when an appropriate concentration of NaCl is supplied.

PCA for volatile compounds produced by mixed cultures and the effect of NaCl on the profile

In the present study, statistics on the 17 volatile compounds identified from sterilized soybeans by the growth of starter candidate combinations were subjected to PCA. Simultaneously, the results obtained by the addition of NaCl to soybean cultures were added to the analysis (Fig. 2). The PCA score plot of the soybean cultures at three NaCl concentrations after 1 and 28 days of incubation is shown in Fig. 2B. The factor scores of the samples containing 1.5% and 7% NaCl were clearly distinguished according to fermentation times, while those of the samples containing 14% NaCl were not clearly separated. Most of

246 the factor scores at day 1 clustered near the original point, implying that the volatile
247 compounds produced by starter candidates in the early stages did not exhibit marked
248 differences. Meanwhile, the day 1 factor scores of soybean culture samples containing 7%
249 NaCl were located in the positive part of the PC1 dimension.

250 After 28 days of incubation, the factor scores for the samples containing 1.5% and
251 7% NaCl congregated in the positive parts of the PC2 and PC1 dimensions, respectively.
252 Meanwhile, the production of volatile compounds at a NaCl concentration of 14% did not
253 have directionality. The major volatile compounds contributing to the difference on the PC2
254 dimension were 3-hydroxybutan-2-one, butane-2,3-diol, 2,3,5,6-tetramethylpyrazine, 3-
255 methylbutyl acetate, and 2-phenylethanol. The levels of all five of these compounds detected
256 in the samples containing 1.5% NaCl increased as fermentation proceeded. Among these five
257 compounds, 3-methylbutyl acetate and 2-phenylethanol also increased in the samples
258 containing 7% and 14% NaCl, while 3-hydroxybutan-2-one, butane-2,3-diol, and 2,3,5,6-
259 tetramethylpyrazine were not produced from the samples supplemented with these levels of
260 NaCl. The results of PCA correspond well with our conclusions drawn from the comparison
261 of volatile compounds produced at the three NaCl concentrations (Table 1) that 3-
262 hydroxybutan-2-one, butane-2,3-diol, and 2,3,5,6-tetramethylpyrazine are specific for *B.*
263 *licheniformis*, and 3-methylbutyl acetate and 2-phenylethanol are mainly produced by *S.*
264 *succinus*.

265 The main volatile compounds contributing to differences in the PCA factor scores of
266 samples containing 7% NaCl were octan-3-one, 3-methylbutan-1-ol, oct-1-en-3-ol, hexan-1-
267 ol, oct-3-en-1-ol, pentan-1-ol, phenylmethanol, and benzaldehyde. All of these compounds
268 were produced from control as well as starter-inoculated samples and dramatic changes of
269 their production levels were identified from the samples containing 7% NaCl. Bacterial
270 metabolism as well as oxidation might have been involved in their production and an

appropriate concentration of NaCl might have accelerated their production. According to the fermentation times of samples containing 7% NaCl, the factor scores of BT and BTS samples developed in similar directions, while the direction of factor score development in the BS sample differed from that of the other samples. Octan-3-one and 3-methylbutan-1-ol contributed volatile compounds to determine the volatile compounds profile of the BS sample containing 7% NaCl. The production of both compounds was highest in the BS sample containing 7% NaCl among all samples. Therefore, halophilic *T. halophilus* commonly inoculated into BT and BTS samples might make the major contribution to producing the specific volatile compounds profile for soybean cultures containing 7% NaCl and *S. succinus* may add an authentic volatile compounds profile under the same conditions.

Even *S. succinus* and *T. halophilus* exhibited growth on the soybean cultures containing 14% NaCl, but species-specific volatile compounds determining the directionality of the volatile compounds profile were not produced. The NaCl concentration of 14% in soybean samples may be more than enough to produce a species-specific volatile compounds profile, even for halophilic *T. halophilus*.

Discussion

This study indicated that *B. licheniformis*-specific volatile compounds produced from soybean fermentation are 3-hydroxybutan-2-one, butane-2,3-diol, and 2,3,5,6-tetramethylpyrazine. *S. succinus*-specific volatile compounds were octan-3-one, 2-phenylethanol, 3-methylbutyl acetate, and 3-methylbutan-1-ol. The involvement of *T. halophilus* in the production of 3-methylbutyl acetate and 3-methylbutan-1-ol in $\geq$7% NaCl was also revealed.

3-Hydroxybutan-2-one, butane-2,3-diol, and 2,3,5,6-tetramethylpyrazine have been reported to be key soy sauce flavor compounds in maotai liquor, the most well-known

296 Chinese liquor made by the distillation of fermented sorghum [23]. Wu and Xu [24] isolated
297 a *B. licheniformis* strain producing a soy sauce flavor during the process of making maotai
298 liquor and identified the increase of three compounds in the culture of the strain in a wheat
299 bran medium. These studies strongly suggest that *B. licheniformis*-specific production of 3-
300 hydroxybutan-2-one, butane-2,3-diol, and 2,3,5,6-tetramethylpyrazine occurs in food
301 fermentation.

302 In our previous single-starter candidate application experiment, significant amounts
303 of 3-methylbutyl acetate and 2-phenylethanol were only identified from *S. succinus* soybean
304 culture [19]. A lipase preparation from a CNS species has been used to synthesize 3-
305 methylbutyl acetate from acetic acid and isoamyl alcohol [25]. 3-Methylbutan-1-ol has been
306 considered as a biomarker for the contribution of CNS to the flavor of fermented dry sausage
307 [26] and its production by *S. succinus* in the Southern European type of fermented dry
308 sausage was reported [27]. We also found earlier reports supporting the production of 3-
309 methylbutyl acetate, 2-phenylethanol, and 3-methylbutan-1-ol by *S. succinus*; however, we
310 were not successful in finding persuasive clues for the involvement of the species in the
311 production of octan-3-one. The amount of octan-3-one identified in this research was very
312 small compared with that of 3-methylbutan-1-ol, which means that this compound can hardly
313 be detected in fermented food depending on spontaneous fermentation. The amount of 3-
314 methylbutan-1-ol identified from Japanese fermented soybean paste was >100 times more
315 than that of octan-3-one [28] and moreover a very small amount of octan-3-one was detected
316 from a traditional type of fermented soybean paste from Korea [21]. Further studies are
317 required to prove the involvement of *S. succinus* in the production of octan-3-one, while the
318 production of 3-methylbutyl acetate, 2-phenylethanol, and 3-methylbutan-1-ol implies that *S.*
319 *succinus* is involved in food fermentation. To the best of our knowledge, the present study is
320 the first to identify 3-methylbutyl acetate and 3-methylbutan-1-ol produced by *T. halophilus*

during soybean fermentation in the presence of an appropriate concentration of NaCl.

The application of three starter candidate combinations to soybean cultures in the presence of NaCl stress confirmed that microbial dominance and flavor profile can be changed by the addition of NaCl. The dominance of *B. licheniformis* and *S. succinus* in the mixed culture of three starter candidates shifted to *T. halophilus* as the NaCl concentration was increased from 1.5% to 14%. We identified species-specific volatile compounds from soybean cultures, even when the three starter candidates coexisted. Previous studies support that the identified species-specific volatile compounds can be produced from several types of food fermentation regardless of the raw materials. Therefore, it is suggested that the identified species-specific volatile compounds are potential biomarkers to consider the involvement of each species in food fermentation. This study indicates the possibility that bacterial starter cultures as well as NaCl can be used to produce fermented food with a customized flavor profile.

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427

Figure legends

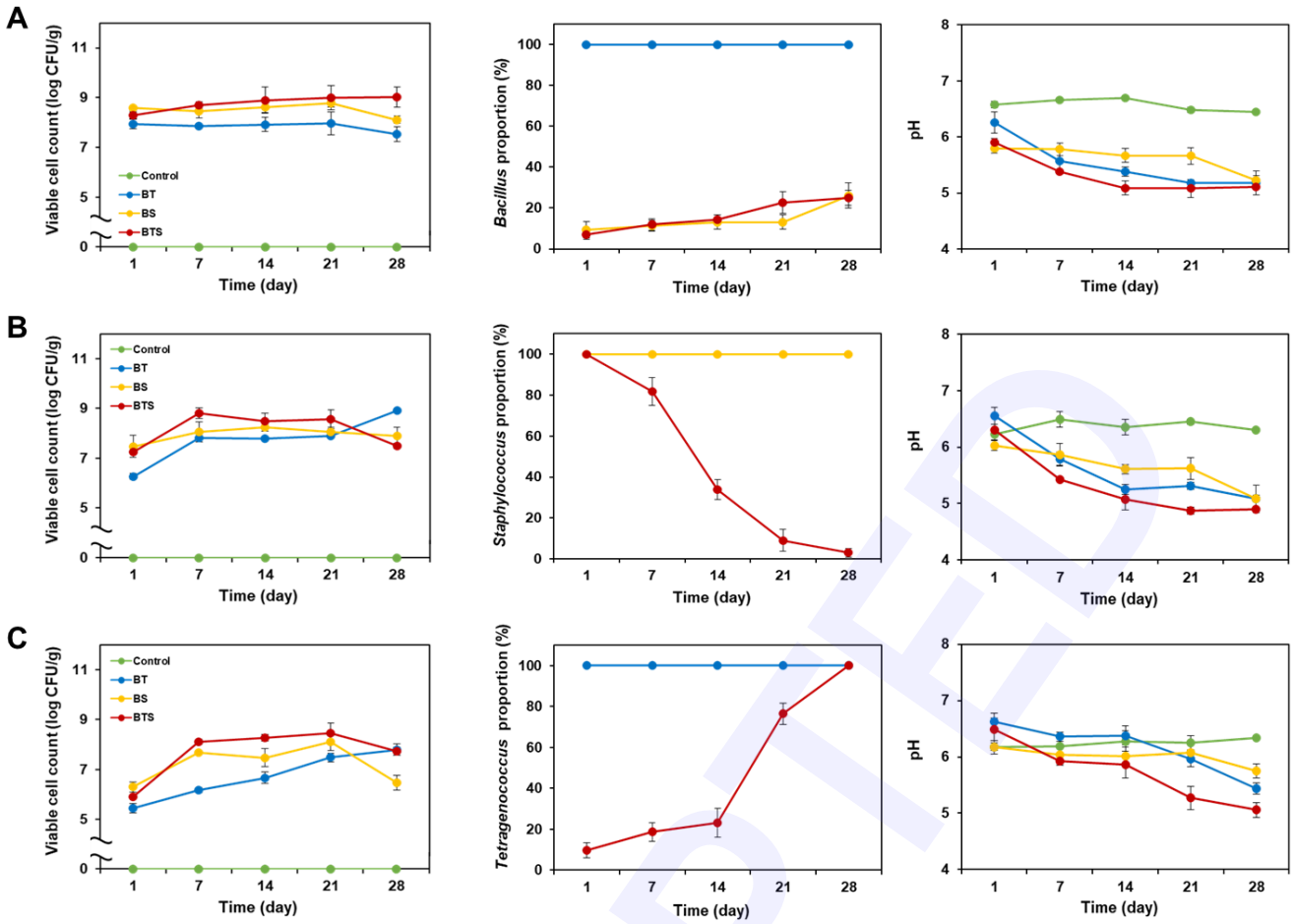
Fig. 1. Effects of NaCl on the growth of starter candidates, the proportions of each inoculated species among 100 colonies, and the pH changes during soybean fermentation. The quantified concentrations of NaCl (w/w) in sterilized soybean samples are 1.5% (A), 7% (B), and 14% (C). Inoculation: control, not inoculated; BT, *B. licheniformis* and *T. halophilus*; BS, *B. licheniformis* and *S. succinus*; and BTS, *B. licheniformis*, *S. succinus*, and *T. halophilus*. The analyses were performed in duplicate on two independent samples prepared in the same way.

Fig. 2. Principal component analysis loadings from starter candidate-inoculated soybean cultures over 28 days of incubation for (A) 17 volatile compounds identified (represented according to their chemical class) and (B) factor scores (numbers indicate incubation time of samples in days). Volatile compounds identified at the NaCl concentrations of 1.5%, 7%, and 14% were subjected to the analysis simultaneously. Inoculation: control, not inoculated; BT, *B. licheniformis* and *T. halophilus*; BS, *B. licheniformis* and *S. succinus*; and BTS, *B. licheniformis*, *S. succinus*, and *T. halophilus*.

Table 1. Effects of starter candidates and NaCl on the production of volatile compounds from the soybean cultures at days 1 and 28.

Volatile compound	RI	NaCl 1.5 % (w/w)								NaCl 7 % (w/w)								NaCl 14 % (w/w)							
		Control		BT		BS		BTS		Control		BT		BS		BTS		Control		BT		BS		BTS	
		Day 1	Day 28	Day 1	Day 28	Day 1	Day 28	Day 1	Day 28	Day 1	Day 28	Day 1	Day 28	Day 1	Day 28	Day 1	Day 28	Day 1	Day 28	Day 1	Day 28	Day 1	Day 28	Day 1	Day 28
Acids																									
Acetic acid	1482	nd^{a}	20.43^{ab}	82.56^{c}	137.99^{d}	61.92^{bc}	69.13^{bc}	29.56^{ab}	32.12^{abc}	2.72^{a}	1.52^{a}	6.58^{ab}	606.15^{d}	8.96^{ab}	30.56^{b}	8.77^{ab}	515.94^{c}	nd^{a}	nd^{a}	13.95^{bc}	14.51^{c}	nd^{a}	2.93^{ab}	177.89^{d}	232.41^{c}
Alcohols																									
3-Methylbutan-1-ol	1229	2.95^{a}	3.51^{a}	6.12^{a}	18.61^{ab}	37.28^{bc}	71.20^{c}	39.29^{b}	33.16^{b}	47.46^{a}	48.50^{a}	32.79^{a}	60.44^{a}	524.34^{d}	712.30^{e}	227.81^{b}	359.71^{c}	3.00^{a}	3.72^{ab}	6.90^{ab}	9.92^{bc}	13.70^{c}	13.93^{c}	82.64^{d}	92.95^{e}
Pentan-1-ol	1274	2.86^{abc}	2.43^{abc}	3.23^{bc}	2.25^{abc}	3.64^{c}	1.51^{a}	2.63^{abc}	2.15^{ab}	12.57^{a}	12.27^{a}	8.09^{a}	13.23^{a}	4.24^{a}	12.98^{a}	8.55^{a}	8.29^{a}	0.75^{a}	0.57^{a}	2.31^{b}	2.67^{b}	2.35^{b}	2.75^{b}	1.61^{ab}	2.34^{b}
Hexan-1-ol	1372	10.78^{a}	13.93^{a}	95.51^{c}	102.53^{cd}	95.87^{c}	42.94^{b}	116.75^{c}	86.92^{d}	80.52^{a}	71.89^{a}	263.62^{d}	385.34^{e}	325.27^{cd}	486.82^{e}	189.60^{b}	287.29^{c}	7.49^{a}	17.60^{a}	29.80^{a}	25.35^{a}	68.19^{b}	75.07^{b}	84.98^{b}	136.03^{c}
Oct-3-en-1-ol	1402	1.50^{a}	1.53^{a}	17.81^{bc}	17.91^{bc}	18.23^{bc}	16.38^{bc}	19.03^{c}	15.50^{b}	10.27^{a}	13.31^{a}	33.39^{b}	51.22^{c}	50.97^{c}	56.46^{c}	25.74^{b}	47.80^{c}	1.17^{a}	3.60^{a}	5.20^{a}	5.41^{a}	7.72^{ab}	12.84^{bc}	27.34^{d}	19.13^{c}
Oct-1-en-3-ol	1467	93.41^{a}	90.94^{a}	174.08^{b}	173.79^{b}	195.47^{b}	181.41^{b}	194.38^{b}	165.89^{b}	151.03^{a}	170.81^{a}	230.58^{b}	266.22^{cd}	295.47^{de}	323.49^{e}	157.35^{a}	235.46^{bc}	16.93^{a}	46.35^{ab}	66.09^{ab}	56.09^{ab}	96.05^{d}	241.97^{bc}	151.37^{c}	103.60^{bc}
Butane-2,3-diol	1604	nd^{a}	nd^{a}	14.89^{ab}	154.97^{c}	nd^{a}	4.80^{a}	nd^{a}	28.84^{b}	nd^{a}	nd^{a}	nd^{a}	nd^{a}	nd^{a}	nd^{a}	nd^{a}	nd^{a}	nd^{a}	nd^{a}	nd^{a}	nd^{a}	nd^{a}	nd^{a}	nd^{a}	nd^{a}
Phenylmethanol	1938	nd^{a}	nd^{a}	nd^{a}	nd^{a}	nd^{a}	nd^{a}	nd^{a}	nd^{a}	nd^{a}	1.08^{a}	nd^{a}	5.41^{b}	nd^{a}	31.61^{c}	nd^{a}	8.13^{b}	nd^{a}	nd^{a}	nd^{a}	nd^{a}	nd^{a}	nd^{a}	nd^{a}	nd^{a}
2-Phenylethanol	1983	nd^{a}	nd^{a}	nd^{a}	8.86^{a}	21.63^{a}	348.81^{b}	nd^{a}	28.85^{a}	4.27^{ab}	4.57^{ab}	3.81^{a}	9.81^{c}	13.27^{c}	42.61^{e}	9.08^{bc}	18.63^{d}	nd^{a}	nd^{a}	1.53^{a}	2.06^{a}	9.25^{b}	24.71^{c}	5.08^{ab}	22.10^{c}
Carbonyls																									
Octan-3-one	1281	0.52^{a}	0.26^{a}	3.72^{ab}	7.98^{a}	3.77^{ab}	5.08^{ab}	4.25^{ab}	3.55^{ab}	6.94^{ab}	4.62^{a}	5.41^{ab}	13.35^{b}	25.50^{c}	31.61^{c}	8.68^{ab}	8.13^{ab}	0.46^{a}	1.75^{ab}	1.90^{ab}	3.30^{bc}	3.22^{abc}	3.47^{bc}	5.67^{c}	11.87^{d}
3-Hydroxybutan-2-one	1329	10.94^{a}	15.00^{a}	163.39^{c}	327.49^{d}	23.66^{a}	107.01^{b}	24.51^{a}	95.77^{b}	nd^{a}	nd^{a}	nd^{a}	nd^{a}	nd^{a}	nd^{a}	nd^{a}	nd^{a}	nd^{a}	nd^{a}	nd^{a}	nd^{a}	nd^{a}	nd^{a}	nd^{a}	nd^{a}
Benzaldehyde	1594	7.52^{c}	16.71^{d}	5.78^{bc}	2.53^{ab}	2.39^{ab}	2.69^{ab}	1.55^{a}	4.79^{abc}	25.33^{b}	38.93^{c}	23.27^{b}	25.01^{b}	11.98^{a}	10.89^{a}	9.25^{a}	24.71^{b}	2.55^{a}	3.79^{a}	7.35^{a}	7.91^{a}	14.74^{b}	14.39^{b}	6.43^{a}	8.72^{ab}
Esters																									
Ethyl acetate	908	10.52^{a}	10.09^{a}	10.45^{a}	18.27^{bc}	12.47^{ab}	23.05^{c}	12.02^{ab}	13.51^{ab}	45.74^{c}	61.27^{d}	20.28^{a}	37.06^{bc}	22.40^{a}	35.59^{bc}	18.67^{a}	34.49^{b}	2.14^{a}	2.90^{a}	5.89^{a}	5.91^{a}	10.46^{a}	13.65^{a}	14.76^{a}	36.82^{b}
3-Methylbutyl acetate	1151	nd^{a}	nd^{a}	nd^{a}	13.61^{a}	8.26^{a}	276.17^{b}	20.25^{a}	22.80^{a}	nd^{a}	nd^{a}	nd^{a}	7.63^{a}	17.80^{a}	98.88^{b}	nd^{a}	139.77^{c}	nd^{a}	nd^{a}	0.41^{a}	2.29^{a}	15.09^{a}	18.43^{a}	17.95^{a}	141.23^{b}
Furans																									
2-Pentylfuran	1258	1.22^{a}	1.11^{a}	2.56^{abc}	1.83^{a}	3.69^{bc}	2.17^{ab}	4.26^{c}	1.42^{a}	7.85^{a}	7.70^{a}	11.95^{ab}	8.15^{a}	27.37^{c}	15.71^{b}	12.30^{ab}	5.48^{a}	3.27^{a}	2.02^{a}	7.89^{ab}	16.51^{c}	12.47^{bc}	4.91^{ab}	6.56^{ab}	2.86^{a}
Pyrazines																									
2,3,5-Trimethylpyrazine	1444	nd^{a}	0.79^{a}	nd^{a}	5.18^{a}	nd^{a}	6.01^{a}	nd^{a}	2.24^{a}	nd^{a}	0.53^{a}	2.01^{b}	1.70^{b}	1.72^{b}	2.85^{c}	1.59^{b}	1.42^{b}	nd^{a}	nd^{a}	nd^{a}	nd^{a}	nd^{a}	nd^{a}	nd^{a}	nd^{a}
2,3,5,6-Tetramethylpyrazine	1518	nd^{a}	1.41^{a}	0.78^{a}	2.62^{a}	nd^{a}	1.49^{a}	nd^{a}	1.93^{a}	nd^{a}	nd^{a}	nd^{a}	nd^{a}	nd^{a}	nd^{a}	nd^{a}	nd^{a}	nd^{a}	nd^{a}	nd^{a}	nd^{a}	nd^{a}	nd^{a}	nd^{a}	nd^{a}

Starter candidates inoculated onto samples were: control, not inoculated; BT, *B. licheniformis* and *T. halophilus*; BS, *B. licheniformis* and *S. succinus*; and BTS, *B. licheniformis*, *S. succinus*, and *T. halophilus*. Different superscripts within a row denote a significant difference between mean values (p<0.05) according to Duncan's multiple range test. Quantitative analysis was based on the peak area of a particular component.



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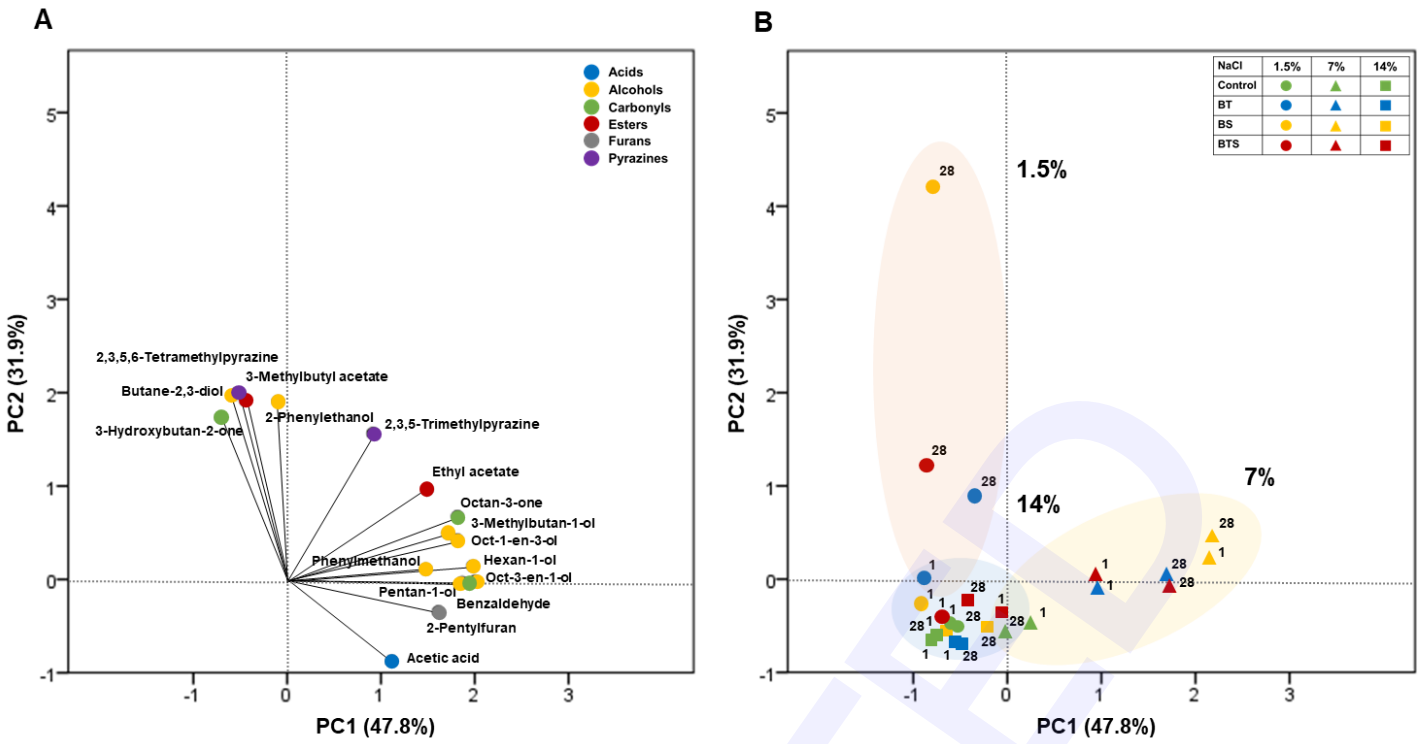

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