Simultaneous use of Bacillus natto and Rhizopus oligosporus in the fermentation of soybean

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ABSTRACT

Japanese natto is made by fermenting intact steamed soybean with Bacillus var. natto, while Indonesian tempeh is made by fermenting dehulled steamed soybean with Rhizopus var. oligosporus. Both products have the same but distinctly respective health-care effectiveness. Although two species have different growth rates, but if both can be grown on intact steamed soybean in balanced growth, a new kind of fermented food having both health-care effects may be successfully created. Therefore, this research used intact steamed soybean as substrate, simultaneously inoculating both species, whose inocula were simultaneously or individually cultivated by different media, in the solid-state fermentation, to study the balanced growth conditions for both species, and employed Bacillus natto (Bn) cell number, glucosamine in Rhizopus oligosporus (Ro) mycelia and ammonium nitrogen content as well as the headspace oxyen percentage above fermented soybean as the criteria to evaluate whether product quality can be improved and whether two species can be grown on soybean in banlanced growth mode. No matter the tin foil covered on the fermentation plate was punctuated or not, under single Bn fermentation, final cell number could reach 109 CFU/g, and better bacterial growth yielded more ammonium nitrogen production; the punctuated group gave 0.46% of ammonium nitrogen, triple higher than that by the unpunctuated group. Based on the change of headspace oxygen percentage, BN cell number and glucosamine content, a determination of the time for both cells to actively growth could be made. For example, no matter whether punctuation is made, in single-culture fermentation, Bn cells started active growth after four hours fermetation, while Ro cells after 6-8 hours. In addition, the former growed faster than the latter. Under single RO fermentation, different inoculum preparation method resulted in different cellular growth rate and lag time; final gluccosamine content was between 9.53 and 10.63 mg/g and almost no ammonium nitrogen was produced. Steamed soybean without any inoculation during this same period yielded no ammonium nitrogen, indicating that the ammonium formed during mixed-culture fermentation came mainly from Baccillus natto. Under the operating conditions of 30-minutes autoclaving time and 37oC fermentation, mixed-culture fermentation could led to better balance growth for both species with Bn cell number over 109 CFU/g, glucosamine content over 12 mg/g, and 0.38% of ammonium nitrogen in harvested product. Among mixed-culture fermentations by both species but individually prepared by different media, simultaneous inoculation of Bn starters bought from the market and Ro cells prepared by potato dextrose broth helped best balanced growth for both species; after 24 hours fermentation, Bn cells reached 109 CFU/g and glucosamine exceeded 12 mg/g with lowest ammonium content (0.29%). And without punctuation of tin foil, the same fermentation would consume all headspace oxygen within 9 hours. If both NB and RO cells were cultivated by the same media and then inoculated together, the one covered by punctuated tin foil and fermented by the mixed inocula prepared by yeast malt broth gave better balanced growth; final Bncells reached 109 CFU/g and glucosamine exceeded 12 mg/g with lowest ammonium content (0.13%). And without punctuation of tin foil, the same fermentation would consume all headspace oxygen within 12 hours. Apparently, mixed-culture fermentation could reduce ammonium nitrogen formation and enhance Ro cellular growth. Also, punctuation of tin foil played an important role in final Bn cell number, glucosamine and nitrogen contents; punctuation could enhance growths of both species, but led to more ammonium nitrogen formation.

Keywords : mixed fermentation ; Bacillus natto ; Rhizopus oligosporus ; natto ; tempeh ; balanced growth

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