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Sourcing Starter Cultures for *Parkia biglobosa* Fermentation III: Preparation of Starter Cultures for Industrial Scale Fermentation

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Authors' contributions

This work was carried out in collaboration between all authors. Author WV designed the study. Author EYA wrote the protocol, performed the laboratory analyses and statistical analysis, wrote the first draft of the manuscript and managed literature searches. Author DJ designed the Questionnaire, coordinated the conduct of the Sensory Evaluation and analyzed the data. All authors EYA, WV and DJ managed the analyses of the study.

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ABSTRACT

Aim: To prepare starter cultures for industrial-scale fermentation of *Parkia biglobosa*, using two strains of *Bacillus* species, 2B and BC4333.
Study Design: Three substrates, African locust bean (ALB), soybean meal (SBM) and de-hulled soybean (SB) were tested for propagation of the bacteria. Four powdered materials, SBM, SB, ALB and Starch (STA) were tested as diluents/carriers for the starter cultures.
Place and Duration of Study: Food Biotechnology Research Unit, National Center for Genetic Engineering and Biotechnology (BIOTEC), Pathumthani, Thailand, between June and August, 2011.
Methodology: Microbial load, with respect to standard plate count and spore count of milled, dried

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starter cultures was determined. The potency of the starters to effect fermentation of beans was screened by starter-culture fermentation. Products were analyzed for sensory evaluation, total microbial load and pH.

Results: The three substrates, ALB, SBM and SB were found suitable for the propagation of the 2 strains of *Bacillus,* 2B and BC4333 as starter cultures. The starter cultures can be dried at 60°C for 24h and milled using sterile dry-mill blender. Powdered ALB, SBM, SB and starch were also suitable diluents/ carriers for the stock starter cultures. However, on the basis of physical appearance of products, starch is a preferred carrier.

Conclusion: The dried starter culture preparations can be used as inocula for large scale fermentation at ratio ranging: 1 g starter: 33 kg to 1 g starter: 1000 kg of African locust bean substrate.

Keywords: Bacillus; starter cultures; fermentation; Parkia biglobosa.

1. INTRODUCTION

African locust bean (*Parkia biglobosa*) cotyledon is fermented in most West African belt countries to produce a soup condiment, known as 'iru', 'dawadawa', 'kinda', 'netetou' 'ogiri' 'afintin', or 'soumbala', depending on the ethnic group [1-3]. Till date, the production process is a traditional art; and the fermentation is carried out by indigenous microflora derived from the immediate environment. Thus the product is characterized by inconsistent quality, short shelf-life; and development of strong ammonia odor, brownish coloration and stringy film of bacterial spoilage organisms. Apart from the nutritional benefit, 'iru' is also used as a local remedy in the treatment of eye infections [4]. Twenty five isolates of *Bacillus* species were obtained from ten commercial samples of 'iru' bought from local vendors in Southwest Nigeria. The 25 isolates were grouped to 13 on the basis of fingerprinting of their DNA band patterns. On the basis of 16S rRNA sequencing, the isolates were found to be strains of *Bacillus subtili s*and *B. licheniformis* [3]. Starter culture experiments showed that five of the 15 strains screened performed better than the others, on the basis of sensory evaluation. None of the 5 strains being chosen (BC4333, 8B, 2B, 7A & 5A), was haemolytic on blood agar; and all have potentials of being developed as starter cultures for use in industrial production of 'iru'. Of particular interest was the strain BC4333, which produced very soft-textured type, locally called 'iru-pete' and had low level of ammonia, which are some of the desirable qualities in 'iru' [5].

1.1 Rationale and Objective

The use of starter cultures in indigenous food fermentations enhances the quality of the product, with respect to microbiological quality, shelf life and sensory properties [6]. It was reported that the use of starter culture resulted in higher rate of fermentation than the natural fermentation of soybean [7]. Two strains of *Bacillus subtilis*, BC4333 and 2B, which can produce soft-type ('iru-pete') and hard-type ('iru-woro') of 'iru', respectively, were selected for further studies. The objective of this work is to develop starter cultures from the two strains of *B. subtilis* group, for application in industrial scale production of 'iru'.

2. MATERIALS AND METHODS

2.1 Determination of Substrate to be Used for Propagation of Starter Cultures

Two strains of *Bacillus* species, BC4333 and 2B were chosen from the best 5 strains, which can produce soft-type and hard-type, respectively [5]. The substrates tested were: African locust bean (ALB), soybean meal (SBM) and de-hulled soybean (SB). Sterile substrates (300 g each) were inoculated with broth culture of test organisms to give 10^4 $cfug^{-1}$. Fermentation was done at 35°C for 36 h.

Starter cultures were dried (Oven-Drier: Memmert loading Model 100-800) at 60°C for 24 h and ground, using sterile blender (OSTER 12-speed Blender model 6684) mill. The dried starter cultures were analyzed for total microbial load ($cfug^{-1}$) and spore count per gram.

2.2 Starter-culture Fermentation (i.e. Confirmation of the Potency of Starters to Effect Fermentation of Beans)

Starter-culture fermentation was performed using the ground starter cultures (from 2.1 above) to produce 'iru'. Four powdered materials (soybean

meal, SBM; de-hulled soybean, SB; African locust bean, ALB and Starch, STA) were tested as diluents/carriers for the dried, ground starter cultures. Sixty grams (60 g) of sterile substrate (ALB) was inoculated at ratio 0.5 % (w/w) and 10^4 cfug^{-1}. Fermentation was done at 35°C, and samples were taken at 24 h, 36 h and 48 h for analyses. Products were analyzed for:

- Sensory Evaluation (bacterial film, color, odor, texture and overall acceptability);
- Total Plate Count;
- Spore Count;
- pH;
- Moisture content

2.2.1 Sensory evaluation (growth of bacterial film, color, odor, texture and overall acceptability)

Sensory evaluation was performed on the starter-culture fermented samples, using a 5-point Hedonic scale. Fifteen trained Panelists were involved in assessing the products. The parameters assessed were profuseness of bacterial film (growth) on the surface of the cotyledons, texture, color, odor and overall acceptability. Scores were analyzed, using ANOVA in SPSS 17.0.

2.2.2 Total plate count

Ten-fold serial dilutions were made in sterile 0.1% peptone water. Spread-plate technique was adopted, using nutrient agar (NA). Plates were incubated at 35°C for 24 hours. Colonies were counted and total plate count was calculated and expressed as cfug^{-1}. Plating was in triplicates.

2.2.3 Spore count

One gram of sample was weighed into 9 ml of sterile 0.1 % peptone water, mixed by vortex and heated in water bath at 80°C for 30 min. The 10^{-1} dilution tube was diluted serially in sterile 10-fold dilution tubes to 10^{-7}. One ml of the 10^{-7} dilution was plated (NA, pour-plate) in duplicates; and plates were incubated at 35°C for 24 h.

2.2.4 pH

One gram of sample was ground, using a mortar and pestle. Five milliliters of distilled water was added to form slurry. The pH was determined in triplicates, using a pH Meter (Metler Toledo), after standardization with buffers at pH 7.0 and 9.0.

2.2.5 Moisture content

The moisture content of samples was determined by weighing two grams into the flat aluminium plate (10 cm-diameters). The plate and content was placed in the Moisture Balance (Sartorius MA 30 S), till dryness was achieved. The difference in weights was used to calculate the moisture content, which was expressed in percentage of the initial weight of the wet sample.

3. RESULTS AND DISCUSSION

3.1 Choice of Substrate for Propagation of Starter Cultures

The pH and moisture content of the substrates before incubation and are shown in Table 1. The moisture content of the substrates ranged between 65.16% and 69.44%; while the pH ranged between 6.00 and 6.92. Plates 1-3 and 4-6 show the fresh and dried starter cultures of the two strains of *Bacillus subtilis* group, 2B and BC4333, respectively. There was evidence of good growth of the 2 strains on the three substrates (African locust bean, soybean and soybean meal) tested, as the organisms colonized the surfaces of the substrates profusely.

Table 1. Moisture content and pH of substrates (used for production of starter-cultures) before incubation

Sample code	Moisture content (%)		pH	
	Average	St dev	Average	St dev
SB/2B	66.93	0.141	6.87	0.025
SB/BC4333	66.99	0.085	6.92	0.015
SBM/2B	68.16	0.064	6.27	0.015
SBM/BC4333	69.44	0.021	6.29	0.015
ALB	65.16	0.339	6.00	0.017

Legend: SB: Soybean, SBM: Soybean meal, ALB: African locust bean; 2B: Bacillus strain 2B; BC4333: Bacillus strain BC4333;STDEV: Standard Deviation

The microbial loads of starter cultures are shown in Figs 1 and 2. The microbial load of the fresh starter cultures ranged between 3.25×10^7 and 3.5×10^9 cfug^{-1} (total plate count); and 6.7×10^7 and 3.3×10^9 cfug^{-1} (spore count), respectively.

Bacillus 2B ***Bacillus BC4333***

Plate 1. Starter cultures of *Bacillus* strains 2B and BC4333 grown for 36h on dehulled soybean (SB)

Bacillus 2B ***Bacillus BC4333***

Plate 2. Starter cultures of *Bacillus* strains 2B and BC4333 grown for 36h on soybean meal (SBM)

Bacillus 2B ***Bacillus BC4333***

Plate 3. Starter cultures of *Bacillus* strains 2B and BC4333 grown for 36h on dehulled African locust bean (ALB)

Bacillus 2B *Bacillus BC4333*

Plate 4. Dried starter cultures of *Bacillus* strains 2B and BC4333 grown on dehulled soybean (SB)

Bacillus 2B *Bacillus BC4333*

Plate 5. Dried starter cultures of *Bacillus* strains 2B and BC4333 grown on soybean meal (SBM)

Bacillus 2B *Bacillus BC4333*

Plate 6. Dried starter cultures of *Bacillus* strains 2B and BC4333 grown on dehulled African locust bean (ALB)

3.2 Starter-Culture Fermentation

The microbial load and pH of the unfermented substrate and the fresh starter culture-fermented 'iru' products are shown in Tables 2-4. Within 24 h of fermentation, there was significant increase in pH of all the samples, from pH 5.8 to pH > 8.0. The cultures had achieved optimum growth within 24-hour of incubation; indicating that fermentation process could be completed within

that period. Proteolysis would have been a major biochemical activity, which has been reported to characterize *Bacillus* species-mediated fermentation processes [8]. Rapid increase in pH during fermentation of soybean to produce 'thua-nao', was attributed to release of ammonia. This phenomenon was regarded as an advantage, as the alkaline fermentation not only provides a selective condition for *Bacillus* species, it also hinders the growth of other organisms [9].

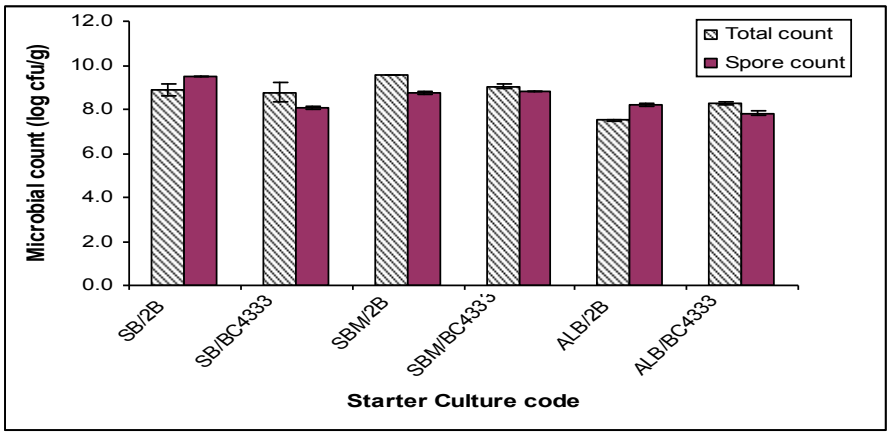

Fig. 1. Total count and spore count (log cfu/g) of fresh starter cultures
Key to codes: SB, Soybean; SBM, Soybean meal; ALB, African locust bean; 2B, *Bacillus subtilis* strain 2B; BC4333, *Bacillus subtilis* strain BC4333

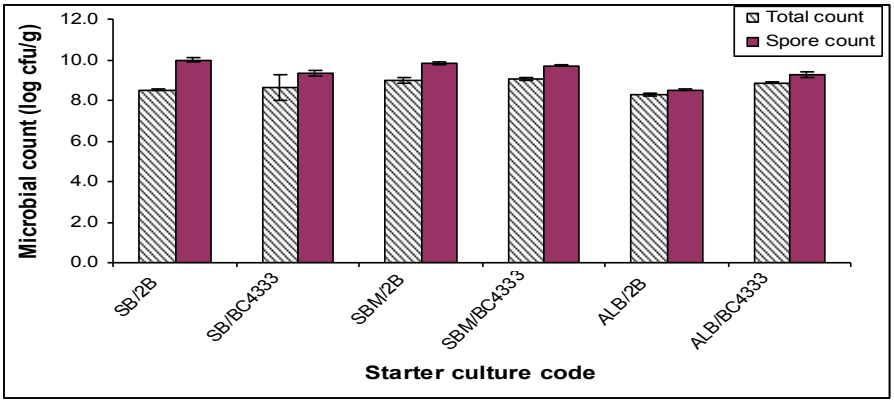

Fig. 2. Total count and spore count (log cfu/g) of dried starter cultures
Key to codes: SB, Soybean; SBM, Soybean meal; ALB, African locust bean; 2B, *Bacillus subtilis* strain 2B; BC4333, *Bacillus subtilis* strain BC4333

Table 2. Total plate count (log cfug^{-1}) and PH of 24-hour starter-culture fermented samples

Sample code	Total plate count (log cfug^{-1})		pH	
24-hour	Mean	± Std. Dev.	Mean	± Std. dev.
SB/2B	7.962	0.219	8.16	0.03
SBM/2B	8.633	0.072	8.15	0.02
ALB/2B	8.746	0.009	8.19	0.03
Starch/2B	8.537	0.057	8.11	0.06
SB/BC4333	7.826	0.220	8.00	0.02
SBM/BC4333	8.501	0.194	8.06	0.02
ALB/BC4333	8.405	0.164	8.11	0.04
Starch/BC4333	8.581	0.174	7.98	0.02
Unfermented control			5.80	0.03

The result of sensory evaluation of the starter-culture fermented products is shown in Figs 3-7. Significant differences were observed only in the profuseness of the bacterial film (growth) on the surface of the cotyledons (24 h, 36 h & 48 h); and texture (24 h only).

Table 3. Total plate count (log cfu/g) and PH OF 36-hour starter-culture fermented samples

Sample code	Total plate count (log cfug^{-1})		pH	
36-hour	**Mean**	**± Std. Dev.**	**Mean**	**± Std. dev.**
SB/2B	7.943	0.461	8.17	0.02
SBM/2B	8.011	0.145	8.14	0.02
ALB/2B	7.301	0.000	8.18	0.06
Starch/2B	7.842	0.063	8.13	0.02
SB/BC4333	7.699	0.000	8.07	0.05
SBM/BC4333	7.971	0.135	8.02	0.01
ALB/BC4333	7.981	0.071	8.02	0.15
Starch/BC4333	7.837	0.102	806	0.02
Unfermented control			5.80	0.03

Table 4. Total plate count (log cfu/g) and PH of 48-hour starter-culture fermented samples

Sample code	Total plate count (log cfug-1)		pH	
48-hour	**Mean**	**± Std. Dev.**	**Mean**	**± Std. dev.**
SB/2B	7.810	0.131	8.04	0.02
SBM/2B	7.796	0.084	8.05	0.02
ALB/2B	7.793	0.199	7.89	0.07
Starch/2B	8.155	0.046	7.98	0.01
SB/BC4333	7.913	0.187	8.12	0.01
SBM/BC4333	7.933	0.080	7.86	0.02
ALB/BC4333	7.874	0.192	8.10	0.05
Starch/BC4333	8.001	0.330	7.95	0.09
Unfermented control			5.80	0.03

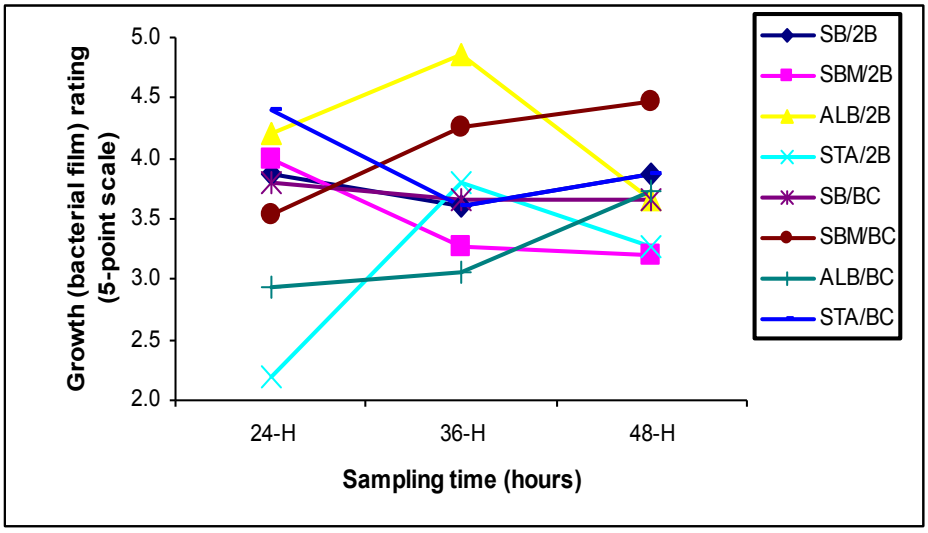

Fig. 3. Growth (bacterial film) rating of starter-culture fermented 'iru' samples

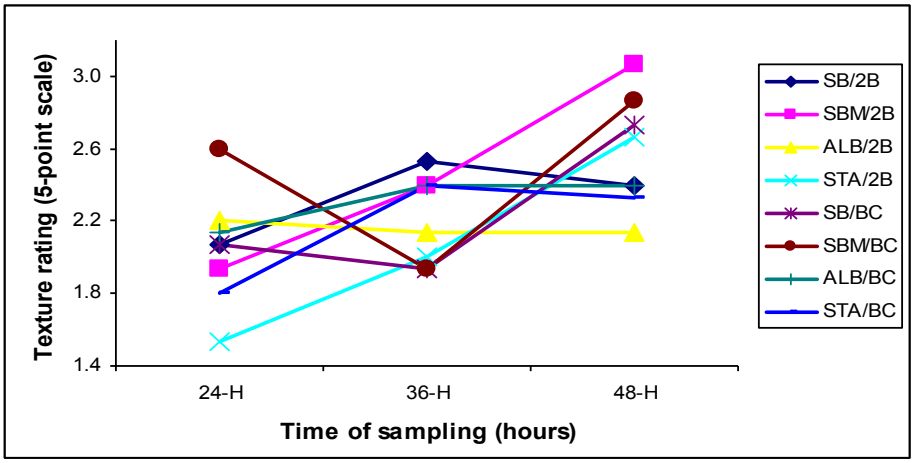

Fig. 4. Texture rating of starter-culture fermented 'iru' samples

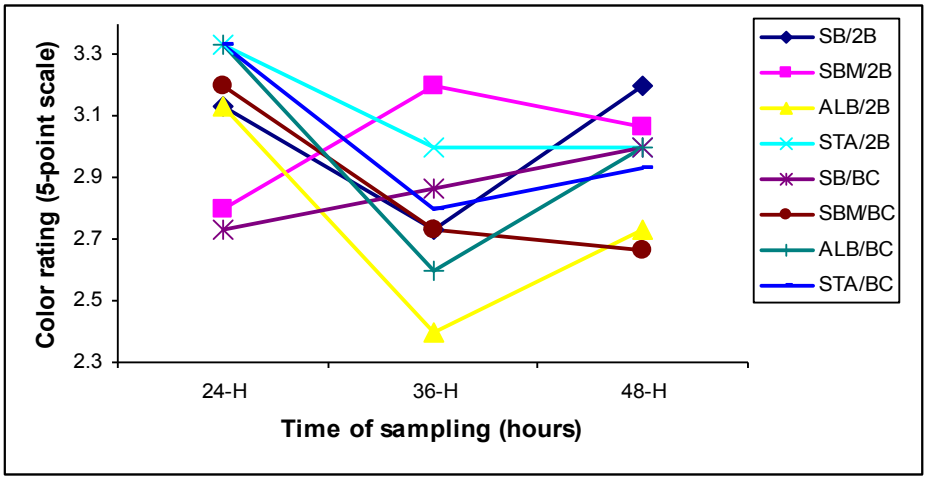

Fig. 5. Color rating of starter-culture fermented 'iru' samples

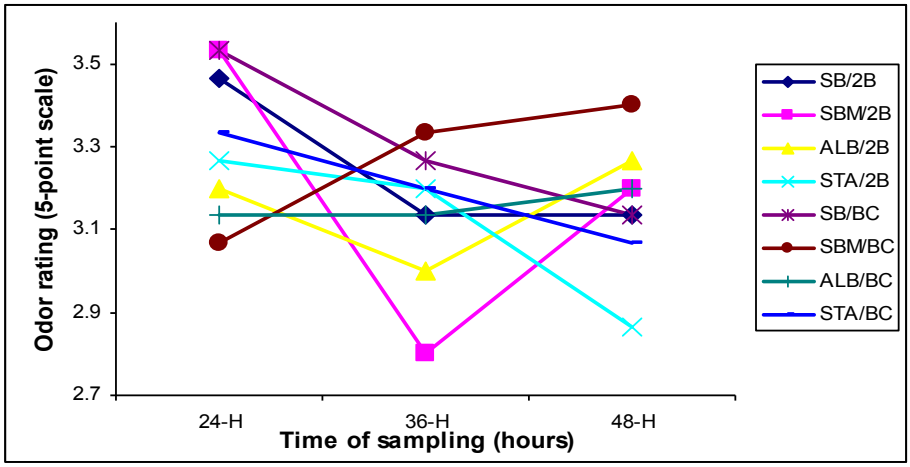

Fig. 6. Odor rating of starter-culture fermented 'iru' samples

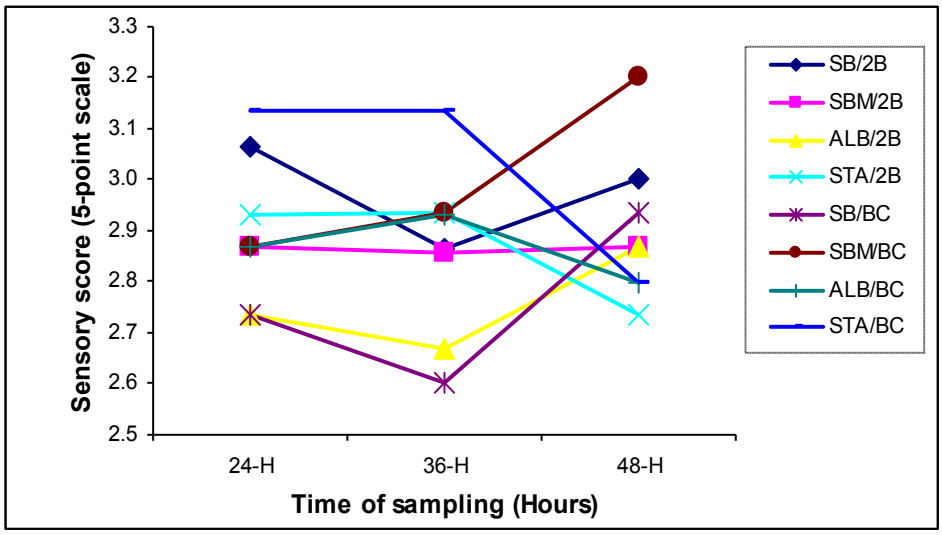

Fig. 7. Overall acceptability of starter-culture fermented 'iru' samples

4. CONCLUSION

African locust bean, soybean and soybean meal are suitable substrates for the propagation of the 2 strains of *Bacillus,* 2B and BC4333 as starter cultures. The starter cultures can be dried at 60°C for 24 hours and milled using sterile dry-mill blender. The starter cultures can be used as inocula for large scale fermentation at ratio ranging from 1 g starter: 33 kg to 1 g: 1000 kg of African locust bean substrate. Powdered African locust bean, soybean meal, soybean and starch are suitable diluents/carriers for stock starter cultures. However, on the basis of physical appearance of products, starch is a preferred carrier.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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