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Rapid detection model of *Bacillus subtilis* in solid-state fermentation of rapeseed meal

Zheng Xing[1,2] | Hui Jiang[1,2] | Ronghai He[1,2] | Benjamin K. Mintah[1,3] | Mokhtar Dabbour[1,4] | Chunhua Dai[1,2] | Ling Sun[1,2] | Haile Ma[1,2]

[1]School of Food and Biological Engineering, Jiangsu University, Zhenjiang, Jiangsu, China

[2]Institute of Food Physical Processing, Jiangsu University, Zhenjiang, Jiangsu, China

[3]ILSI-UG FSNTC, Department of Nutrition and Food Science, University of Ghana, Accra, Ghana

[4]Department of Agricultural and Biosystems Engineering, Faculty of Agriculture, Benha University, Qaluobia, Egypt

Correspondence

Ronghai He, School of Food and Biological Engineering, Jiangsu University, 301 Xuefu Road, Zhenjiang, Jiangsu 212013, China.
Email: heronghai1971@126.com

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Abstract

The main objective of this research was to monitor variety in the content of *Bacillus subtilis* 10160 during solid-state fermentation (SSF) of rapeseed meal by near infrared (NIR) spectroscopy and chemometrics. Observations showed the coefficient of determination (R^2) value was 0.9401 and root-mean-square deviation was 0.639 log (CFU/g) for viable cell content by synergies between four intervals 5,203.003–5,600.267, 5,604.124–6,001.388, 6,807.487–7,204.751 and 8,411.972–8,809.235 cm^{-1}. The determination coefficient of prediction (R_p^2) and root-mean-square deviation of prediction in an external validation set could reach 0.9532 and 0.628 log (CFU/g) by the viable *B. subtilis* model. These findings suggest that rapid detection of *B. subtilis* in SSF is achieved by the combination of synergy interval partial least squares and NIR spectroscopy.

1 | INTRODUCTION

Extracting protein from plant meals for use in human and livestock nutrition have been explored by some researchers (Dabbour, He, Ma, & Musa, 2018; Dabbour, He, Mintah, Tang, & Ma, 2018) due to the rich protein content of such meals. Rapeseed or canola meal, like other plant meals, is also rich in protein—approximately 360 g crude protein (CP)/kg and is a good source of protein for poultry diets (Newkirk, Classen, Scott, & Edney, 2003). However, some functional properties are relatively low in rapeseed protein (Qu, Zhang, Chen, et al., 2018), and high levels of antinutritional component and fiber have been the main bottlenecks limiting the use of rapeseed meal (RSM) in animal feed (Hansen, Skrede, Mydland, & Øverland, 2017). Presently, it has been reported that rapeseed protein isolate dextran conjugates with different grafting degrees (GD) are prepared by grafting reaction to improve protein utilization (Qu, Zhang, Han, et al., 2018). Also, some studies have reported the release of antioxidant rapeseed peptides to improve protein utilization using proteolytic enzymes (Mäkinen, Streng, Larsen, Laine, & Pihlanto, 2016), since short peptides with stronger antioxidant activity could be readily absorbed (Dai, Zhang, He, Xiong, & Ma, 2017). Jin et al. (2016) and (He et al., 2016) studied the hydrolysis of proteins into more readily absorbable peptides. M. Jiang, Yan, He, and Ma (2018) reported that using ultrasonic pretreatment to improve protein hydrolysate utilization, and some studies have also focused on converting rapeseed protein into polypeptide by SSF (Xing et al., 2018). SSF has been developed as a microbial culture with solid matrices; these systems have proven to be more effective in microbial production of useful products and metabolites since they closely mimic the habitat of microorganisms (Tang et al., 2015).

Bacillus subtilis 10160 is also presented as a high-yield protease microorganism (He et al., 2012). Dai, Ma, et al. (2017) studied the use of *B. subtilis* solid-state fermented of soy residues to upgrade nutritional value and biological activity. Viable cells are the most basic element in solid-state fermentation. Consequently, monitoring the bacteria growth is vital in determining the fermentation progress. In

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liquid fermentation, the parameters are easy to monitor due to the availability of various sensors. Therefore, several studies on liquid fermentation exist in literature (Dujardin et al., 2014; Van Kerrebroeck, Harth, Comasio, & De Vuyst, 2018). However, monitoring the bacteria growth in solid-state fermentation is still rare.

Currently, techniques including DNA for polymerase chain reaction (PCR) (Tzschoppe, Martin, & Beutin, 2012), biosensors, nucleic acid-based tests (NAT) (Umesha & Manukumar, 2018), immunomagnetic separation (Zeng et al., 2014), ELISA latex agglutination (Bayat, Tavakoli, Kousha, & Panahi, 2008) and traditional plate counting have been applied in solid-state fermentation. Among them, the nucleic acid-based techniques are broadly utilized for detection of microorganism with better sensitivity. These methods, however, are considered laborious, and have drawbacks on industrial production. Thus, exploring a rapid and more accurate method to detect microorganisms in fermentation samples is significant. NIR technology can meet this requirement. NIR spectroscopy is widely used in the fermentation, food, agricultural, petrochemical and pharmacological industries due to its rapid, noninvasive and economic advantages (H. Jiang et al., 2012; Tong, Du, Zheng, Wu, & Wang, 2015). In their study, do Nascimento, de Macedo, dos Santos, and de Oliveira (2017) reported that NIRS can be utilized for actual-time detecting of process factors in alcoholic fermentation. Andueza, Agabriel, Constant, Lucas, and Martin (2013) used NIRS to achieve the ordering of cheese samples from various regimes. Zhang et al. (2017) studied actual-time detecting and in situ of enzymatic procedure of wheat protein using miniature fiber NIR spectrometer. Xiao et al. (2018) reported the use of NIRS to monitor the developmental stage of white and red grapes. Villar et al. (2017) have successfully applied sensor system (VIS–NIR) and chemometrics to monitor the cider fermentation procedure. Nevertheless, reports on the rapid monitoring of viable cells using near infrared are limited. Hence, exploring the potential correlation between viable cells and NIR spectroscopy is imperative.

The main aim of this study was, therefore, to detect *B. subtilis* 10160 in SSF of rapeseed residue using NIR technology and to conduct a preliminary exploration of its fermentation mechanism.

2 | MATERIALS AND METHODS

2.1 | Rapeseed meal pretreatment and strain preparation

Rapeseed meal was employed as the main raw material used for the solid-state fermentation in this study. Rapeseed meal was supplied by Zheng Da Oil Company (China), and stored for about 3 weeks at 15–20°C to prevent deterioration, and then sieved (40 mesh sieve) to get uniform size particles. The contents of protein and moisture in the rapeseed meal were 40 and 9%, respectively.

B. subtilis 10160 was bought from Industrial Center of Culture Collection (China). The bacteria were transferred to the basal medium with a bacterial ring, and placed the medium in a biochemical incubator (stationary culture at 30°C for 24 hr) for strain activation.

2.2 | Fermentation and microbiological analysis

Solid-state medium was cultivated in 3-L laboratory fermentation container. A mixture of 300 ml of sterile water, 300 g sterilized rapeseed meal and 10% (6 ml) fermented seed liquid in each tank, respectively. Each medium was cultivated at 36°C. Samples were collected from eight runs of SSF of rapeseed meal trials. Sampling from each tank at 4 hr interval starting from 0 to 60 hr of a fermentation cycle. A total of 128 samples were obtained during the whole fermentation process. All samples are divided into two categories using random numbers by the Matlab software. Eighty-five were used to establish and optimize the models (64 of them are training sets and 21 are cross-validation sets). The other 43 were classified as an external validation set that is used for the feasibility evaluation of NIR technology to detect rapeseed meal fermentation.

During the fermentation, a 10 g sample was gathered and conserved at 4°C. The medium was stirred before the sample was picked to ensure the evenly mixed and necessary aeration. A mixture of 10 g conserved sample and 90 ml of distilled water was homogenized for 2 min in a homogenizer (FSH-2A, CHN) at 10,000 rpm. The mixture was diluted at a gradient of 10 times. Each mixture then transferred to a separate beef extract medium for culture. The medium (1 L) contained NaCl (5 g), tryptone (10 g), beef extract (3 g) and agar (20 g). pH value of final medium was adjusted to 7.5 by 1 M NaOH. After 24 hr of culture at 36°C, bacteria on the plate culture medium were visible to the naked eye. The dilution factor of appropriate quantity (usually 30–300) was selected by manual counting. The number of viable cells is expressed as the product of the manual count and the dilution factor (including the dilution of the previous dissolved matrix). The above method (Balestra & Misaghi, 1997) was used in counting the number of viable microorganisms.

2.3 | Spectrum collection

NIRS is the product of complex scattering and absorption features of sample components, which are the result of stretching and vibration of C—H, N—H, and O—H groups (Salzer, 2008). The diffused reflectance spectra of the samples in this study were collected using near-infrared spectroscopy (Nicolet, USA), which was in the range 4,000 to 10,000 cm^{-1} and equipped with an integrating sphere. Blank background spectra were collected as a reference prior to spectral measurements. The spectra were collected by directly illuminating the sample with near-infrared light at room temperature (25°C). The mean value was calculated from three replicates scan data for each sample to ensure adequate signal-to-noise ratio for the subsequent analysis (Wang & Peng, 2017).

2.4 | Synergy interval partial least squares and calibration models

The synergy interval partial least squares (siPLS) regression permits the assembly and combination of spectral equidistant intervals to obtain regressions that generally provide more robust prediction

models with lower errors for root-mean-square deviation of cross validation (RMSDCV) and root-mean-square estimation deviation (RMSED) (da Silva & Wiebeck, 2017). The siPLS model was evaluated based on stoichiometric indicators. This method is based on the root-mean-square deviation (RMSD):

$$\text{RMSD} = \sqrt{\frac{\sum_{i=1}^{l}(c_i - d_i)^2}{l}}, \quad (1)$$

where c_i is the reference values of the plate counting method, d_i denotes the estimated values for different samples, l is the samples in each set (Li et al., 2015). Data analysis is implemented by the Matlab software.

3 | RESULTS AND DISCUSSION

3.1 | Microorganisms analysis

In this study, the preliminary viable cell content of fermentation medium was approximately 7.08×10^4 CFU g^{-1} and the standard deviation was 1.9769. The growth curve of *B. subtilis* 10160 in rapeseed meal medium is shown in Figure 1. At 36 hr fermentation, the number of viable organism reached a maximum 2.29×10^{11} CFU g^{-1}, followed by a downward trend, possibly due to the large consumption of nutrients. This result shows similar trend as reported by Tang et al. (2015). In addition, the content of viable cells during the entire rapeseed meal SSF process ranged from 7.08×10^4 to 2.29×10^{11} CFU g^{-1}. A wide range of viable cell content facilitate in establishing a more accurate and applicable model.

3.2 | Optimal spectral preprocessing strategy

To avoid the influence of noise generated during the scanning process of the apparatus, reducing various external factors makes data processing easier, and so it is necessary to perform series of preprocessing on the obtained spectra (Blanco, Coello, Iturriaga, Maspoch, & Pagès, 2000). Scattering caused by different particle sizes can be eliminated by standard normal variate (SNV) and multivariate scatter correlation (MSC). Random noise can be effectively eliminated and improved signal-to-noise ratio using Savitzky–Golay (S-G). Normalization eliminates the effects of changes in optical path variation and sample dilution on the spectrum. Furthermore, in order to eliminate datum line offset, scattering, interference from other backgrounds, resolve overlapping peaks, and improve resolution and sensitivity, derivative correction is an effective approach. Figure 2 shows the results obtained after different pretreatment strategies.

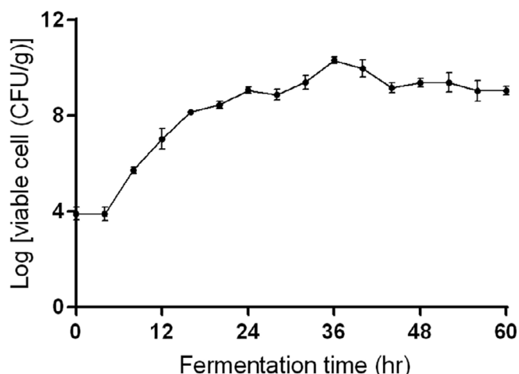

FIGURE 1 Growth curve of *Bacillus subtilis* 10160 in rapeseed meal

3.3 | siPLS regression calibration models

The best siPLS model was selected from multiple parameters research. Full spectrum is divided into intervals. A model can consist of several joint intervals. Each joint model can be established by PLS. The RMSDCV value is a measure of the accuracy of the model. Sub-interval interaction is selected based on the high precision and low RMSDCV values of the combined model. In this study, the spectral range was divided into 15 segments and combined with arbitrary four segments to build a model. Established multiple-models rely on various treatment strategies and synergy interval selection. The best pretreatment in calibration and estimation (prediction) sets usually differ. Therefore, when evaluating pretreatment it is desirable to use some automatic software routines that permit efficient testing of different validation sets and/or the execution of a large number of cross-validation passes (Fernández-Cabanás, Garrido-Varo, Pérez-Marín, & Dardenne, 2006). Table 1 lists the best results for all models. The best siPLS cross-validation models for viable cell content showed RMSDCV = 0.639 log (CFU/g) and R^2 = 0.940 with 12 latent variables (LVs). Figure 3 shows the optimal interval synergy after optimal preprocessing.

Figure 4 reveals the relationship/correlation between the experimental value of the biological content and the NIR predicted value in the rapeseed meal sample in the training group. From Table 1 and Figure 4, the best calibration model provided a consistent estimate of biomass. Therefore, we can draw a conclusion that NIR spectroscopy could be used to estimate the viable *B. subtilis* 10160 cell of rapeseed meal SSF with high precision.

3.4 | Application of models in the rapeseed meal SSF process

In order to evaluate the NIR calibration model established in SSF of rapeseed meal, the data of 43 samples was used as an external validation set. Figure 5 reveals the relation between variety in viable cell content in rapeseed meal SSF and the predictions obtained from the NIR spectral calibration model. The predicted and measured values of *B. subtilis* 10160 content revealed analogous variations during the entire fermentation process. The R^2 and root-mean-square deviation of prediction of the viable cell content between the measured and predicted values were 0.9532 and 0.628 log (CFU/g). All these results indicated that the calibration models have precise predicted for viable cell content.

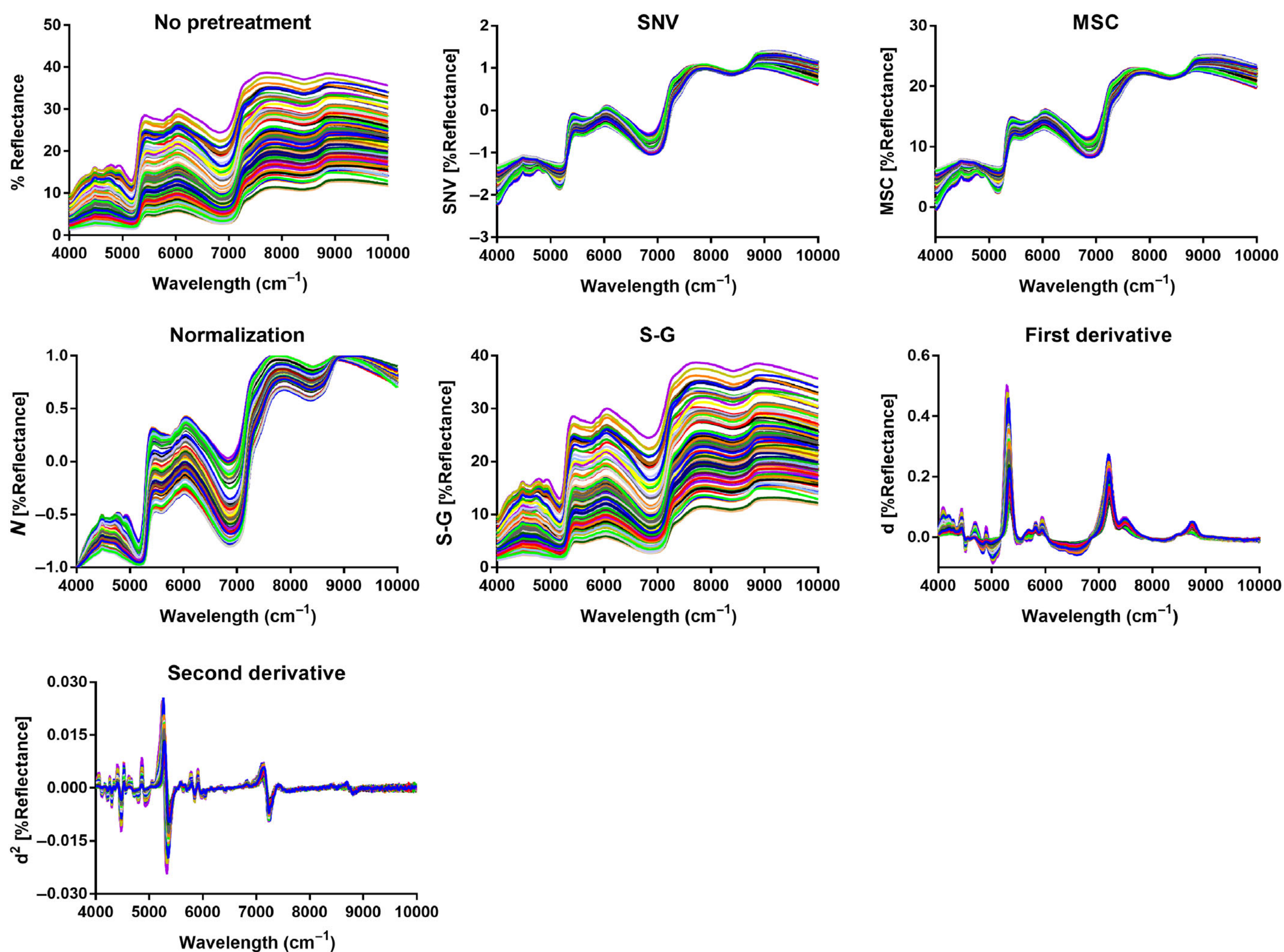

FIGURE 2 NIR spectroscopy spectra with diverse pretreatment methods

TABLE 1 Optimal near infrared spectral models of viable *Bacillus subtilis* 10160 content in SSF with diverse pretreatment conditions

Pretreatment method	LV	Synergy interval	Calibration set		Prediction set	
			R_c^2	RMSDCV	R_p^2	RMSDP
No pretreatment	12	4,5,8,12	0.9403	0.638	0.9255	0.728
SNV	12	4,5,8,12	0.9402	0.638	0.9514	0.621
MSC	12	4,5,8,12	0.9402	0.639	0.9521	0.618
Normalization	12	4,5,8,12	0.9401	0.639	0.9532	0.628
S-G	12	4,5,8,12	0.941	0.635	0.9257	0.729
First derivative	10	2,4,7,10	0.9431	0.624	0.8485	1.01
Second derivative	5	1,2,3,5	0.9445	0.616	0.7699	1.3

Abbreviations: LVs, latent variables; MSC, multivariate scatter correlation; RMSDCV, root-mean-square deviation of cross validation; RMSED, root-mean-square estimation deviation; S-G, Savitzky–Golay; SNV, standard normal variate; SSF, solid-state fermentation.

3.5 | Analysis of fermentation mechanism

The *B. subtilis* 10160 contribute to the digestion of rapeseed protein into peptides and ameliorate the amino acid composition in the rapeseed meal SSF (Xie et al., 2015). He et al. (2012) indicated that *B. subtilis* can produces a protease during SSF of rapeseed meal, which digests the rapeseed protein into rapeseed peptide. Meanwhile, Xing et al. (2018) reported that the content of rapeseed peptides began to increase rapidly on the second day, which is consistent with the results of the maximum microbial biomass at 36 hr in this study. Consequently, because certain chemical components produced by microorganisms during fermentation can establish a good relationship with the spectrum, rapid detection of microorganisms by NIRS can be achieved.

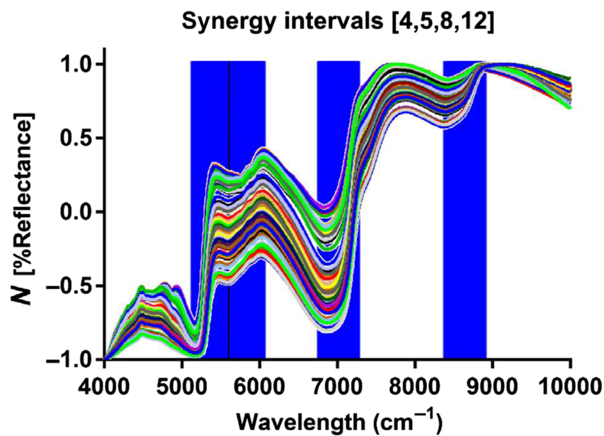

FIGURE 3 Optimal intervals after normalization pretreatment

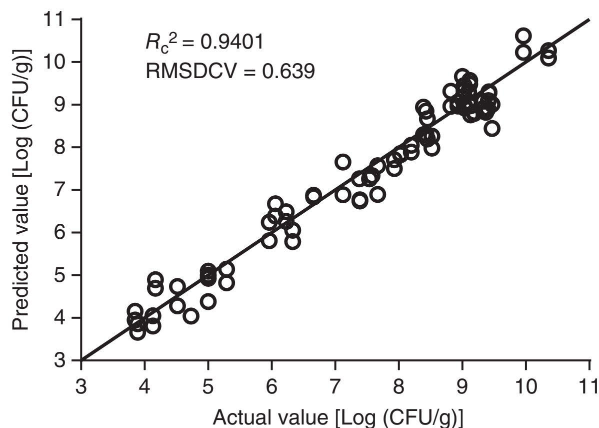

FIGURE 4 NIR spectroscopy calibration model predicted value compared with actual value after MSC pretreatment. MSC, multivariate scatter correlation; NIR, near infrared; RMSDCV, root-mean-square deviation of cross validation

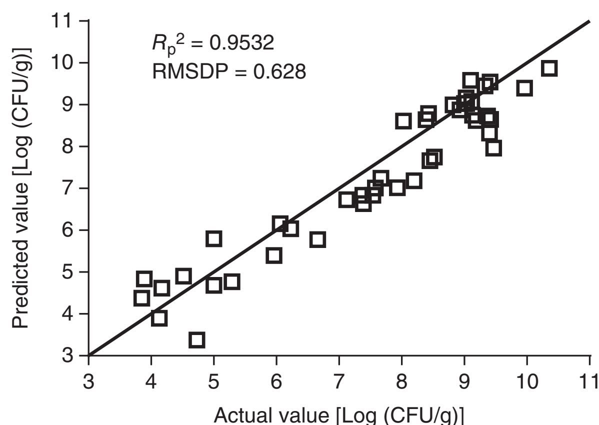

FIGURE 5 Content variety of *B. subtilis* 10160 in rapeseed meal SSF and the predicted value obtained from the NIR spectral model after MSC pretreatment. MSC, multivariate scatter correlation; NIR, near infrared; RMSDP, root-mean-square deviation of prediction; SSF, solid-state fermentation

4 | CONCLUSIONS

The results of this work showed that combining NIRS technology and siPLS regression provided a rapid and precise approach for determination of viable *B. subtilis* 10160 in SSF rapeseed residue. The predicted values obtained by the NIR spectroscopy model are similar with those observed by the traditional biological count method. Overall, the results indicated that applying fiber-optic NIR spectroscopy technology to detect the variety of viable cell content during SSF process of rapeseed meal has a desirable outcome. Therefore, this research offers a feasible approach for the determination of other microorganisms or fermentation parameters. Applying a suitable chemometric model to industrial production could contribute to real-time monitoring of fermentation parameters and furnish real-time signals on the processes of fermentation and product quality.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

ETHICS STATEMENT

This article does not contain any studies with human participants or animals performed by any of the authors.

INFORMED CONSENT

Not applicable.

ORCID

Ronghai He https://orcid.org/0000-0002-0904-0522

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