Promotion of protein utilization from corn by-product via calcium-dependent growth stimulation of *Streptomyces* sp. SCUT-3

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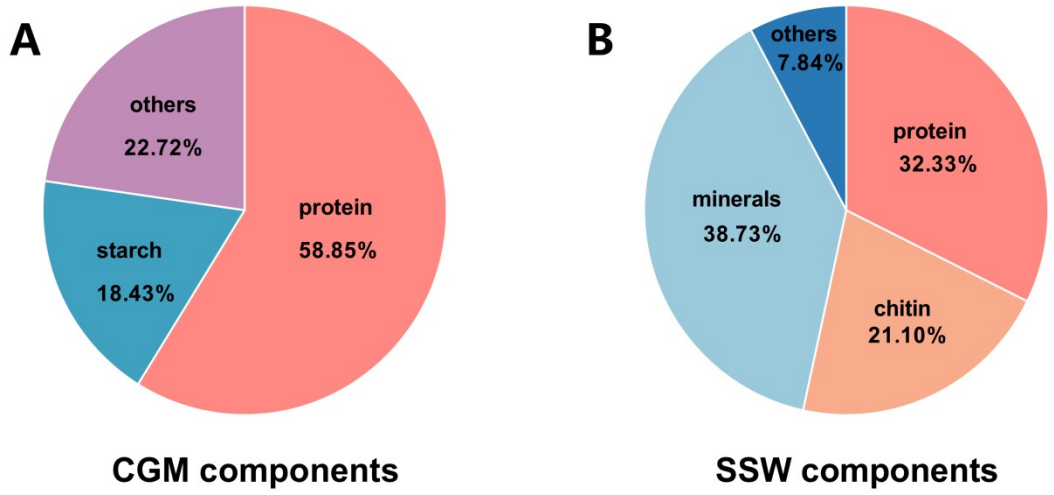

Fig. S1. (A) CGM nutritional composition (on a dry weight basis). (B) SSW nutritional composition (on a dry weight basis).

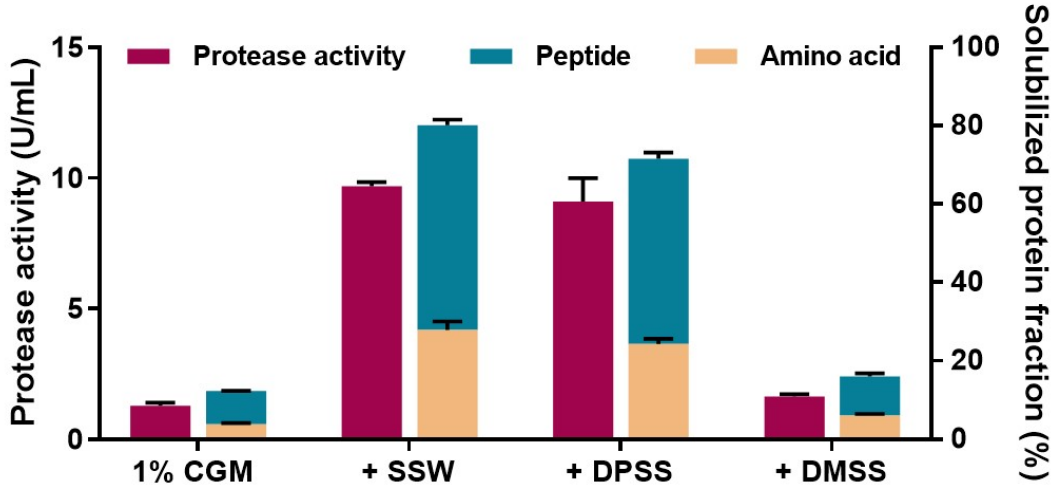

Fig. S2. Protease activity and solubilized protein fraction of SCUT-3 in 1% CGM fermentation at 24 h, supplemented with or without 0.2% SSW, DPSS, or DMSS individually. Data are presented as mean ± SD of three independent experiments (n = 3).

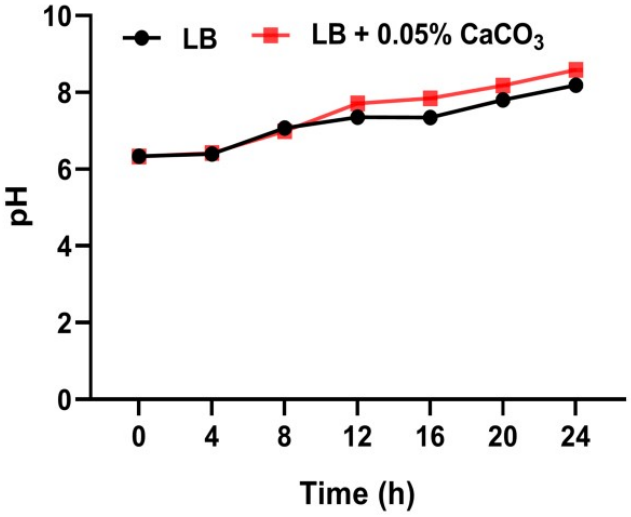

Fig. S3. pH changes during SCUT-3 cultivation in LB medium supplemented with or without 0.05% $CaCO_3$. Data are presented as mean ± SD of three independent experiments (n = 3).

Table S1. Five putative calcium-binding proteins in the genome of SCUT-3.

Subcellular location	Assembly	MW	Family
Intracellular	GQS52_00140	8.1 kDa	EFh
	GQS52_14050	21.2 kDa	
	GQS52_24710	7.6 kDa	
Membrane	GQS52_16545	4.2 kDa	Excalibur
Cytoplasm	GQS52_18945	30.4 kDa	DUF5707

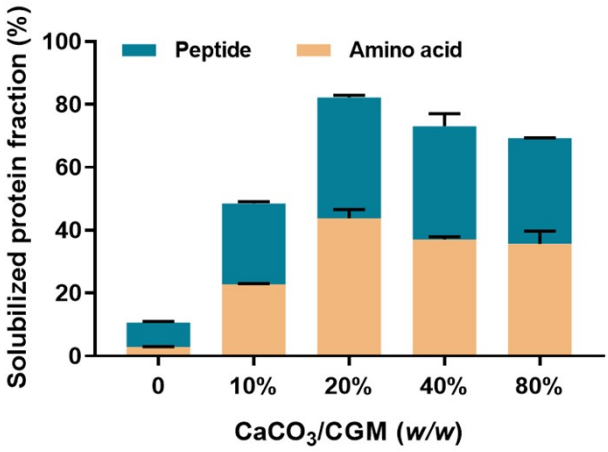

Fig. S4. Effects of different $CaCO_3$ addition ratios on CGM solid-state fermentation under the optimized SSW-CGM conditions. Data are presented as mean ± SD of three independent experiments (n = 3).

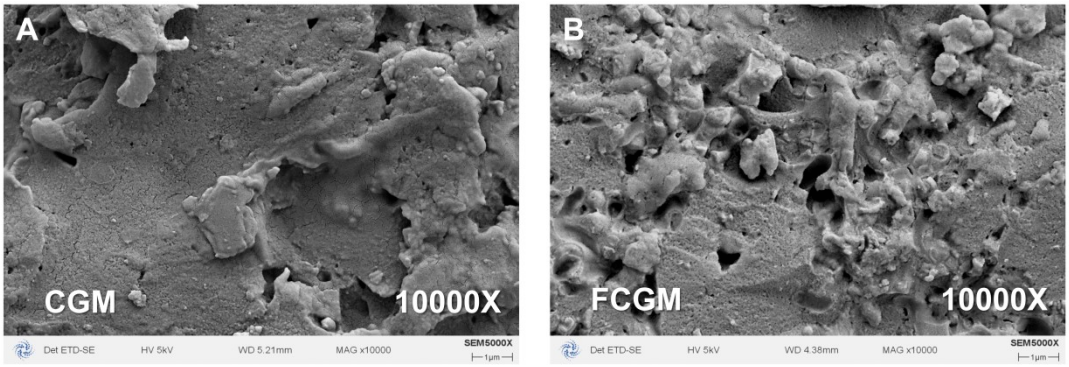

Fig. S5. The microscopic structure of CGM and FCGM. (A-B) SEM micrographs of CGM and FCGM at 10000× magnification, respectively.

Table S2. Total amino acid profiles of CGM and FCGM.

Amino acids	Abbreviation	Proportion (%)		Product (mg/g)	
		CGM	FCGM	CGM	FCGM
Aspartic acid	Asp	5.94	6.69	35.78	40.51
Threonine	Thr	3.33	4.71	20.08	28.52
Serine	Ser	5.06	4.91	30.49	29.76
Glutamic acid	Glu	21.18	22.7	127.69	137.44
Proline	Pro	8.64	8.27	52.11	50.09
Glycine	Gly	2.99	3.32	18.04	20.13
Alanine	Ala	8.65	7.53	52.13	45.58
Cysteine	Cys	0.27	0.47	1.64	2.82
Valine	Val	4.38	5.28	26.42	31.98
Methionine	Met	2.34	1.58	14.13	9.54
Isoleucine	Ile	3.65	3.50	22.00	21.17
Leucine	Leu	15.65	13.96	94.36	84.55
Tyrosine	Tyr	4.77	6.08	28.76	36.80
Phenylalanine	Phe	5.94	4.39	35.78	26.60
Lysine	Lys	1.85	1.89	11.15	11.45
Histidine	His	2.19	1.61	13.17	9.75
Arginine	Arg	3.16	3.12	19.05	18.87
Total				602.77	605.55
Essential and semi-essential amino acids (Met、Val、Lys、Ile、Phe、Leu、Thr、Arg、Tyr、His)		47.26	46.11	284.89	279.22
Umami amino acids (Glu、Asp)		27.12	29.39	163.47	177.94
Sweet amino acids (Gly、Ala、Thr、Pro、Ser)		28.67	28.75	172.84	174.08
Branched chain amino acids (Leu、Ile、Val)		23.69	22.74	142.77	137.69

Table S3. Inventory of procedures in different methods to process 10,000 tonnes CGM yearly.

Process	Agent	Pretreatment	CGM treatment
B. subtilis MTCC5480 solid-state fermentation	Procedure	Sterilization	Fermentation
	Chemicals/enzymes	-	107 tonnes glucose 50 tonnes peptone
	Reaction conditions	4600 m^3 Water; 121 °C/1 atm/20min	36 °C/1 atm/5 d
	Energy exhausted	644,000 kWh	337,000 kWh
	Waste water	-	-
	CO_2 emissions	96,342 kg	50,415 kg
S. SCUT-3 solid-state fermentation	Procedure	Sterilization	Fermentation
	Chemicals/enzymes	-	2000 tonnes SSW
	Reaction conditions	18,000 m^3 Water; 100 °C/1 atm/20min	40 °C/1 atm/3 d
	Energy exhausted	1,732,500 kWh	513,000 kWh
	Waste water	-	-
	CO_2 emissions	259,182 kg	76,745 kg

Table S4. Equivalence factors for the land use types.

Area type	Equivalence factor
Fossil land	1.26
Pasture land	0.46
Forest area	1.26
Arable land	2.51

Supplementary Methods

S1 Analysis of the basic components of CGM and SSW

S1.1 Determination of crude protein content of CGM

The protein content in corn gluten meal (CGM) was determined using the Kjeldahl method in accordance with the standard GB/T 5009.5–2016, employing a semi-automatic KDN-80C analyzer (Tuopo-Yunnong, Zhejiang, China). A precisely weighed amount of dried CGM sample was placed in a digestion tube, to which anhydrous potassium sulfate, copper sulfate, and concentrated sulfuric acid were added. The sample was digested at 450 °C for 2 h. After distillation of the digest, the released ammonia was absorbed in a 20 g/L boric acid solution, and the crude protein content was calculated by titrating the absorbed ammonia with a standard hydrochloric acid solution.

S1.2 Determination of starch content of CGM

The starch content in CGM was determined using a starch assay kit (Solarbio, Beijing, China) according to the manufacturer's instructions. Soluble sugars and starch were first extracted from the sample using 80% ethanol. Subsequently, starch was hydrolyzed into glucose by acid hydrolysis. The glucose content was then quantified using the anthrone colorimetric method, and the starch content was calculated accordingly.

S1.3 Determination of the basic components of SSW

Dried SSW powder (8 g) underwent alkaline hydrolysis with 40 mL NaOH (1 M) at 90 °C for 4 hours. The supernatant was collected for protein quantification via the Kjeldahl method using a KDN-80C analyzer (Tuopu-Yunnong, China). The residual solids were then washed to neutrality using distilled water until eluent pH reached 7.0. Subsequently, acid demineralization was performed with 40 ml HCl (1 M) at 40 °C for 6 h to determine the mineral content.The resulting precipitate was then immersed in 95% (v/v) ethanol for 4 h, After washing and drying, the remaining weight was recorded as the weight of chitin.

S2 Preparation of deproteinated and demineralized shrimp shells

The raw shrimp shells were washed thoroughly with deionized water to remove impurities and dried at 65 °C to constant weight. The dried shells were then ground into a fine powder (sieved to 0.3 mm) for subsequent treatments.

Detailed methods are provided in S1.3. Briefly, after deproteinization with NaOH, the residue was washed to neutral pH and dried at 65 °C to constant weight to obtain deproteinized shrimp shell (DPSS). DPSS was further subjected to HCl demineralization, followed by washing to neutral pH with distilled water and drying at 65 °C to constant weight to obtain deproteinized and demineralized shrimp shell (DMSS).

S3. Preparation of shrimp shell protein hydrolysate (SSPH)

SSPH was was prepared by direct enzymatic hydrolysis using alkaline protease. Briefly, shrimp shell powder was suspended in water at 10% (*w/v*), and alkaline protease was added at 1500 U/g substrate. The hydrolysis reaction was conducted at pH 8.0 and 50 °C for 6 h, followed by enzyme inactivation in a boiling water bath for 10 min. The mixture was then centrifuged at 8000 ×g for 5 min at room temperature, and the supernatant, rich in soluble peptides and free amino acids, was collected as SSPH.