

Supplementary Material

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Optimizing Lipase production by *Bacillus subtilis* on cheese whey and evaluating its Antimicrobial, Antibiofilm, Anti virulence and biosafety properties

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Table S1: Levels of different factors tested in Plackett–Burman design for lipase production by *B. subtilis*

Factor	Unit	Symbol	Coded levels	
			-1 (Low)	+1 (High)
Frying oil waste	mL/L	(A)	15	30
Whey	mL/L	(B)	20	40
Tryptone	g/L	(C)	15	30
pH		(D)	6	8
MgSO ₄	g/L	(E)	0.2	2
Peptone	g/L	(F)	15	30
Mannitol	g/L	(G)	10	20
Agitation speed	rpm	(H)	100	250

Table S2: Levels of significant factors tested in the CCD design for lipase production by *B. subtilis*

Factor	Symbol	Levels of the variables tested in CCD	
		-1 (Low)	+1 (High)
Whey	(A)	20	40
Peptone	(B)	15	30
Agitation speed	(C)	100	250

Table S3: Antibiotic susceptibility test against *S. aureus*

Antibiotic	Conc. (mg/mL)	IZD (mm)	Susceptibility mode
Amoxicillin (AX)	10.0	18.5 ± 7.5 ^f	I
Ampicillin (AMP)	10.0	0.000 ± 0 ^g	R
Azithromycin (AZM)	15.0	0.000 ± 0 ^g	R
Ciprofloxacin (CIP)	5.00	35.4 ± 6.2 ^c	S
Doxycycline (DOX)	30.0	40.0 ± 1.5 ^b	S
Norfloxacin (NX)	10.0	29.5 ± 9.2 ^c	I
Gentamicin (GEN)	10.0	29.7 ± 1.5 ^d	I
Kanamycin (K)	30.0	22.9 ± 1.6 ^e	I
Rifampin (RIF)	5.00	28.9 ± 3.1 ^d	I
Tetracycline (TE)	10.0	35.9 ± 4.2 ^b	I
Crude lipase	100%	54.0 ± 4.2 ^a	S

R: Resistant; I, intermediate; S, sensitive; CLSI, Clinical Laboratory Standards Institute; EUCAST, European Committee on Antimicrobial Susceptibility Testing; BSAC, British Society for Antimicrobial Chemotherapy; NI, No Inhibition. All values are the mean of three replicates ± standard deviation (SD).