

Effect of volcanic ash on cell growth and production of exopolymers

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Abstract

Effect of volcanic ash on cell growth of *Aspergillus* sp. and production of exopolymers by *Agrobacterium* sp. and *Aureobasidium pullulans* was investigated. The volcanic ash contained various mineral salts such as SiO₂, Al₂O₃, CaO, K₂O. Maximal cell growth of *Aspergillus* sp. was obtained when 0.3% volcanic ash was added to medium. Cell growth of *Aspergillus* sp. increased with higher concentration of volcanic ash in medium. Amount of cell growth with 0.3% volcanic ash was 6.7 times higher than that without volcanic ash. Volcanic ash also stimulated production of exopolymer as well as cell growth. Production of curdlan with 0.1% volcanic ash was 12.40 g/l whereas that without volcanic ash was 9.15 g/l. Production of pullulan with volcanic ash was also higher than that without volcanic ash.

Introduction

Aspergillus sp. is one of major microorganism in a commercial product which is used for increase of agricultural product. *Agrobacterium* sp. ATCC 31749 produced curdlan, which is extracellular, unbranched homo- β -(1-3)-glucan. Curdlan is one of gelling agent used in food industry.¹⁾ Pullulan produced by *Aureobasidium pullulans* is an extracellular, unbranched homo-polysaccharide which consists of maltotriose and maltotetraose units with both α -(1 \rightarrow 6) and α -(1 \rightarrow 4) linkages. Pullulan produces high-viscosity solutions at relatively low concentrations and can be utilized to form oxygen-impermeable films, thickening or extending agents, or adhesives.

Materials and methods

Volcanic ash volcanic ash was kindly donated from San Rok Plastics Co. Ltd. Its composition was shown in Table 1.

Microorganisms and media *Agrobacterium* sp. ATCC 31749 was grown and

maintained on plates containing 2% glucose, 0.5% yeast extract, 1.0% calcium carbonate and 1.5% agar powder.²⁾ *Aureobasidium pullulans* ATCC 42023 and *Aspergillus sp.* maintained on plates containing 2% glucose, 0.5% K₂HPO₄, 0.1% NaCl, 0.02% MgSO₄ · 7H₂O, 0.06% (NH₄)₂SO₄, 0.25% yeast extract and 2% agar powder.^{3),4)}

Cell culture and production of exopolymer The mineral salt medium (MSM) was used for the production of curdlan. The MSM contained the following components (g/l): KH₂PO₄, 1.74; K₂HPO₄, 0.49; Na₂SO₄·10H₂O, 3.7; MgCl₂·6H₂O, 0.25; FeCl₃·6H₂O, 0.024; CaCl₂·2H₂O, 0.015; MnCl₂·4H₂O, 0.01; citrate, 0.21; NH₄Cl, 2.1; and 2% (w/v) of carbon source⁵⁾. Culture medium for *Aspergillus sp.* was same as that for *A. pullulans* ATCC 42023. The medium used for *A. pullulans* ATCC 42023 contained the following composition (g/l): K₂HPO₄, 5.0; NaCl 1.0; MgSO₄ · 7H₂O, 0.2; (NH₄)₂SO₄, 0.6; yeast extract, 2.5; and glucose, 20 (Ueda et al., 1963). the pH of medium was adjusted to 6.5 - 6.7 before sterilization. The carbon source was autoclaved separately for 20 min at 120°C and added to the medium under aseptic conditions. Cultures of *Aspergillus sp.*, *Agrobacterium sp.* ATCC 31749, and *Aureobasidium pullulans* ATCC 42023 were incubated at 30°C for 2 to 5 days. Purification of curdlan from the culture broth of *Agrobacterium sp.* ATCC 31749 was followed by Nakanishi's method.⁷⁾ Purification of pullulan was carried out by Ueda's method^{3),4)}

Analysis Cell growth of *Aspergillus sp.* was measured with dilution method⁶⁾ and visible colony was counted in agar plates. Dry cells weight (DCW) was determined by direct weighting of the cell fraction after drying to constant weight at 100 - 105°C. The yield of exopolymers were determined by the same procedure.

Results and discussions

Analysis of volcanic ash consist of volcanic ash was obtained that contained various mineral salt and major component was SiO₂ (Table 1)

Table 1. Composition of volcanic ash

Components	Mean	Absolute error	A relative concept error
SiO ₂	74.99	0.03	0.04
Al ₂ O ₃	12.26	0.02	0.17
Fe ₂ O ₃ ¹⁾	1.14	0.00	0.00
TiO ₂	0.13	0.00	0.00
MnO	0.07	0.00	0.00
CaO	2.61	0.00	0.00
MgO	0.12	0.00	0.00
K ₂ O	4.41	0.00	0.00
Na ₂ O	2.70	0.00	0.00
P ₂ O ₅	0.01	0.00	0.00
L.O.I	1.62	-	-
Total	100.5	-	-

1) The result of Fe_2O_3 was obtained amount of total Fe

Effect of volcanic ash on cell growth by *Aspergillus sp.* The effect of volcanic ash on cell growth of *Aspergillus sp.* was shown in Table 2. The cell growth of *Aspergillus sp.* increased with higher concentration of volcanic ash. Amount of cell growth with 0.3% volcanic ash was 6.7 times higher than that without volcanic ash.

Table 2. Effect of volcanic ash on cell growth by *Aspergillus sp.*

Volcanic ash (%;w/v)	pH	Final status ¹⁾	
		CFU/ml	Increase Rate ²⁾ (%)
0.00	3.97	7.50×10^7	1765
0.05	4.06	2.20×10^8	5176
0.10	4.14	5.00×10^7	1176
0.15	3.97	2.23×10^8	5247
0.20	4.22	3.93×10^8	9247
0.25	4.45	4.80×10^8	11294
0.30	4.07	5.01×10^8	11788

1) The result was obtained after 2 days

2) Initial inoculum was 100 percent, equal to 4.25×10^6 CFU/ml

Effect of volcanic ash on production of curdlan The effect of volcanic ash on production of curdlan by *Agrobacterium sp.* ATCC 31749 was shown in Table 3. Cell growth with 0.5% volcanic ash was more than 2 times higher than that without volcanic ash. Maximal production of curdlan was obtained with 0.1% volcanic ash in medium.

Table 3. Effect of volcanic ash on production of curdlan by *Agrobacterium sp.*

ATCC 31749

Volcanic ash (%;w/v)	pH	Final status ¹⁾	
		DCW (g/l)	Curdlan (g/l)
0.00	6.39	2.55	9.15
0.10	6.48	2.05	12.40
0.20	6.59	4.05	11.10
0.30	6.48	5.25	10.50
0.40	6.55	8.25	9.25
0.50	6.55	6.80	8.70

1) The result was obtained after 5 days

Effect of volcanic ash on production of pullulan The effect of volcanic ash on production of pullulan by *Aureobasidium pullulans* ATCC 42023 was shown in Table 4. Cell growth increased with volcanic ash. Cell growth with 0.2% volcanic ash was about 2 times higher than that without volcanic ash. Production of pullulan with over 0.02% volcanic ash was higher than that without volcanic ash.

Table 4. Effect of volcanic ash on production of pullulan by *Aureobasidium pullulans* ATCC 42023

Volcanic ash (%;w/v)	Final status ¹⁾		
	pH	DCW (g/l)	Pullulan (g/l)
0.00	5.90	5.83	3.85
0.02	6.24	7.23	4.73
0.04	6.35	7.38	4.75
0.06	6.34	7.75	4.70
0.08	6.35	8.23	4.23
0.10	6.34	8.58	4.30
0.15	6.33	8.65	4.70
0.20	6.37	9.50	4.78

1)The result was obtained after 4 days

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