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Production of Extracellular Polysaccharide, EPS WN9, from *Paenibacillus* sp. WN9 KCTC 8951P and Its Usefulness as a Cement Mortar Admixture

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Abstract The production of extracellular polysaccharide, EPS WN9, from *Paenibacillus* sp. and its suitability as a viscosity modifying admixture for cement mortar mixing were investigated. After 48 h culture in an optimized medium, cell growth and EPS production were 1.2 g/L and 4.0 g/L, respectively. By adding EPS WN9 to mortar, it was possible to prepare a homogeneous mortar without material segregation and excess air entrapment. The optimal amount of EPS addition to mortar was found to be 0.02 to 0.05%(w/w) of the cement used. Increasing the dosage of EPS WN9 from 0 to 0.05%(w/w) resulted in a setting retardation of 0.14 h to 0.8 h and an increase in the compressive strength of mortar of 10 to 20%.

Keywords: cement mortar, microbial polysaccharide, *Paenibacillus* sp., segregational resistance

INTRODUCTION

Recently, microbial polysaccharides such as curdlan and welan have been developed as concrete viscosity modifiers to improve workability. These microbial polysaccharides have been used in cement mixture such as cement paste, mortar, and concrete. They are especially useful in the manufacture of concrete, in which both viscosity modifier and cement dispersing agent are used in combination, for example, under water concrete, high-strength concrete, lean-mix concrete, centrifugally-cast concrete, extrusion-formed cement, prepacked concrete, injection-grout, shortcrete, fiber-reinforced concrete, and self-leveling concrete [1-4].

Concrete is composed of cement, fine aggregate, coarse aggregate, and water. When water is added to the cement, it forms a paste which then hardens to a solid structure. The water loss in such a cement slurry, which is composed of cement, fine aggregate, coarse aggregate, and water, is very rapid. When this slurry is poured onto a flash board enclosing reinforcing bars, components of the concrete segregate rapidly because of differences in specific gravity and filtering. This causes the workability of the concrete to decrease and reduces its quality. Various admixtures are used in concrete cement in order to prevent material segregation and to increase workability [2,5,6].

It is possible to manufacture a concrete with excellent fluidity and segregation resistance by adding suitable additives. This is done by using a cement dispersing agent such as water reducing agent or plasticizer, and a viscosity modifier. Cellulose derivatives such as hydroxymethyl cellulose, hydroxylpropyl ethyl cellulose, and hydroxymethyl ethyl cellulose are used as viscosity modifiers. However, the use of these polysaccharide derivatives has a drawback as they show excessive air-entrapment or viscosity variation when the temperature changes and also other undesirable characteristics, such as retardation of the setting time of concrete [6].

This study deals with the production of EPS WN9 from *Paenibacillus* sp. and its evaluation as a new viscosity modifying admixture for cement mortar mixing.

MATERIALS AND METHODS

Fermentation

The fermentation medium for extracellular polysaccharide (EPS WN9) production from *Paenibacillus* sp. WN9 KCTC 8951P contained 20 g glucose, 8 g yeast extract, 1 g K_2HPO_4, 0.5 g $MgSO_4 \cdot 7H_2O$, 0.5 g KCl, 0.1 g $CaCl_2 \cdot 2H_2O$, 0.01 g $FeSO_4 \cdot H_2O$ in 1 L distilled water. The seed culture was prepared as described previously [7]. For EPS WN9 production, 40 mL of seeds were inoculated into a 3-L jar fermenter (Bioengineering AG, Switzerland) containing 2 L of the cultivation medium. Cells were cultivated at 30°C. The agitation speed and aeration rate were 700 rpm and 0.5 vvm, respectively.

Cement Mortar Mixing

Portland cement (specific gravity: 3.15, fineness: 3,200 cm^2/g, Ssangyong Co., Korea) and fine aggregate (river

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sand; specific gravity: 2.58, fineness modules: 2.56, water absorption: 1.8%, Hapchun, Korea) were used as the mortar mix, and a standard polycarbonic acid type of super plasticizer SP8N (Dongnam Co., Korea) was used as a SP source. The novel microbial extracellular polysaccharide, EPS WN9, which was prepared from the fermentation supernatant by ethanol precipitation [7] was used as thea viscosity modifying admixture.

To prepare a cement mortar, cement was dry-blended with EPS WN9 up to 0.08% on cement weight and then the blended cement and fine aggregate were mixed at a weight ratio of 1 to 1.6. At each mixing, the ratio of water/cement was adjusted to 0.45, 0.50, and 0.55, respectively. Super plasticizer was added up to 3% on cement weight. To evaluate the fluidity according to the change of surface moisture of the fine aggregates, up to 4% of water was added to the mixture.

Analyses

Concentrations of cells, EPS, and glucose were determined by methods described previously [7]. Apparent viscosities of EPS WN9 were measured with a Brookfield digital viscometer model LVDV-III fitted with a small sample adapter (spindle No. 25; Brookfield Engineering Lab., USA).

Mortar flow was measured according to the Korean standard method KS L 5105, using a small flow cone. The average diameters of the mortar pools which were created by pulling up the cone without vibration were measured and the change in flow value according to lapsed time was measured for up to 90 min at 30 min intervals. Each test mix was allowed to stand for 1 min before measurement. Air entrained in the mortar was measured using an air content measuring instrument (1 L capacity, LC-546, Sanyo Co., Japan). The setting time of the mortar was measured by the penetration resistance test at the initial and final settings specified in Korean standard method KS F 2436. The compressive strength of the cement mortar was measured according to KS L 5105 on 3, 7 and 28 days after preparation.

RESULTS AND DISCUSSION

Paenibacillus sp. was cultured in media supplied with various levels of carbon sources, nitrogen sources, and inorganic salts, and their optimal concentrations for EPS WN9 production were found to be 20 g glucose, 8 g yeast extract, 1 g K_2HPO_4, 0.5 g $MgSO_4 \cdot H_2O$, 0.5 g KCl, 0.1 g $CaCl_2 \cdot 2H_2O$, and 0.01 g $FeSO_4 \cdot 7H_2O$ in 1 L distilled water (detailed data not shown). In this optimized medium, the typical time course of cell growth and EPS WN9 production by *Paenibacillus* sp. is shown in Fig. 1. Cell growth and EPS production were paralleled. After 48 h cultivation, cell growth and EPS production were 1.21 g/L and 4 g/L, respectively. Production titre of cell growth and EPS production increased 1.3 times and 1.5 times, respectively, compared to the basal medium [7] Although half of the glucose level was re-

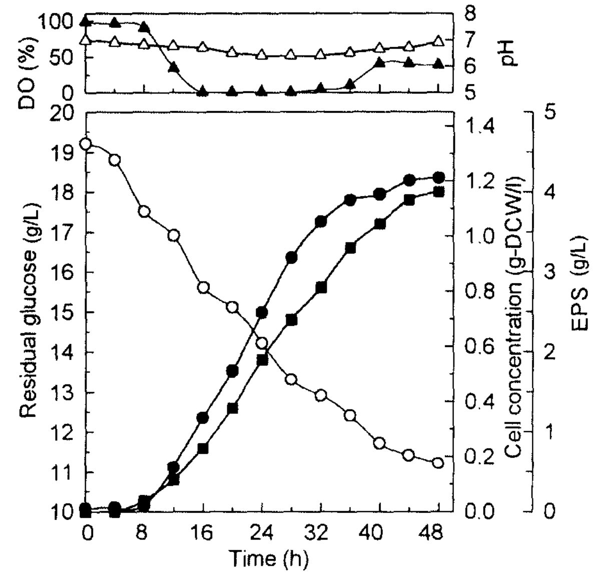

Fig. 1. A time course of cell growth and EPS WN9 production by batch fermentation with *Paenibacillus* sp. in a jar fermenter. △, DO; ▲, pH; ○, glucose; ●, cell concentration; ■, EPS production.

mained in the cultivation medium, cell growth and EPS production were not increased further. A similar profile was observed in initial glucose concentration of 10 g/L [7].

The usefulness of EPSs is determined by their ability to alter the rheological properties of water [8]. The aqueous phase of the cement mortar contains various kinds of ions such as calcium, silica, aluminium, ferrous, sulfur, potassium, and sodium and it exhibits a strong alkalinity of pH 13 [9,10]. Many EPSs including xanthan gum and A49-Pol are unstable under such condition and their viscosities generally decreased [11]. However, as shown in Table 1, viscosity of EPS WN9 in such alkaline conditions was not decreased. The effect of EPS WN9 addition to cement mortar, as a segregational resisting admixture, is shown in Fig. 2. The addition of EPS WN9 resulted in a homogeneous mortar without material segregation. The segregational resistance and viscosity change of the mortar clearly depend on the

Table 1. Viscosity of EPS WN9 solution at high alkalinity[a]

Treat condition	Viscosity (cP)
In deionized water	231
In alkaline solution[b]	208
In cement extract[c]	349
In cement extract[d]	404

[a] 0.5% solution, shear rate at 28 sec^{-1}.
[b] pH of distilled water was controlled to 13 with 2N NaOH and then EPS WN9 was dissolved.
[c] EPS WN9 was dissolved in supernatant of 50% (w/v) cement slurry.
[d] EPS WN9 solution in cement extract was applied heat at 70°C for 30 min.

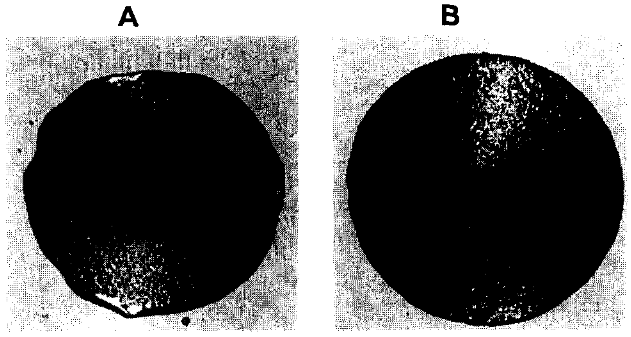

Fig. 2. Comparison of cement mortar for segregational resistance. (a) no EPS WN9 and (b) 0.02% EPS WN9 of cement content.

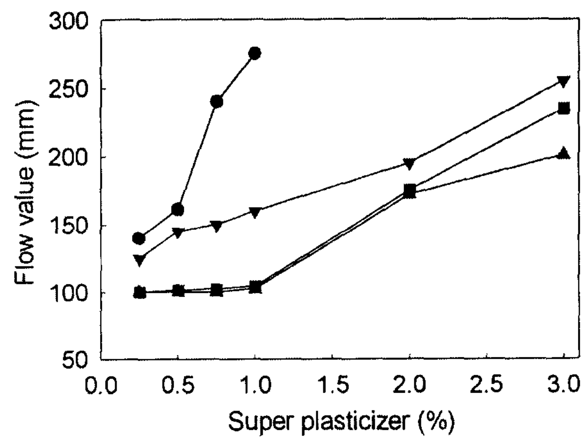

Fig. 3. Flow properties of cement mortar according to addition of EPS WN9 and super plasticizer. The ratio of w/c was 0.45. Addition of EPS WN9 to cement: ●, 0%; ▼, 0.02%; ■, 0.05%; ▲, 0.08%.

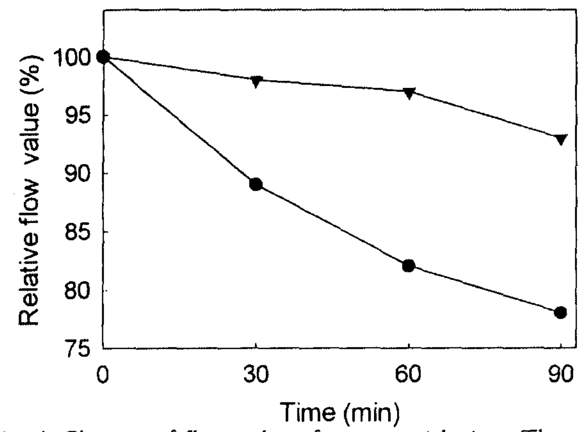

Fig. 4. Changes of flow value of mortar with time. The ratio of w/c was 0.45. Addition of EPS WN9 to cement mortar: ●, 0%; ▼, 0.02%.

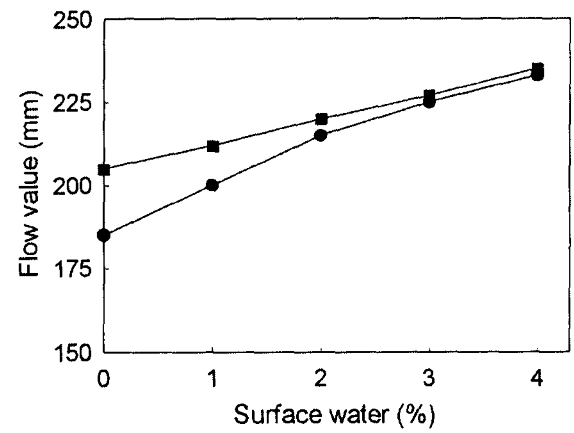

Fig. 5. Relationships between surface water content of fine aggregate and flow. The ratio of w/c was 0.45. Addition of EPS WN9 to cement: ●, 0%; ■, 0.02%.

addition of EPS WN9, but its addition amount was not a critical factor.

The flow of the mortar prepared as in Materials and Methods is shown in Fig. 3. The flow of the cement mortar without EPS WN9 addition was more than 250 mm when more than 0.75%(w/w) of super plasticizer was added and the materials segregated. To achieve uniform fluidity the amount of super plasticizer required was small. The flow decreased as the dosage of EPS WN9 was increased. Adding 0.02 to 0.08%(w/w) of EPS WN9 caused the flow value to sharply decrease and material segregation was not observed. Similar results were obtained for high ratio of water/cement mortar (detailed data not shown). There was a significant difference of flow values between the control (no addition of EPS WN9) and 0.02%(w/w) addition of EPS WN9. That is to say, the flow value with less than 0.75%(w/w) of super plasticizer added was reduced to 40% of its control value. When super plasticizer was increased to 3.0%(w/w) a slight material segregation was observed with increased flow value. The flow values at above 0.02%(w/w) of EPS WN9 showed a small change under 0.25 to 1.0%(w/w) of super plasticizer addition, but the flow value increased according to the increase of super plasticizer. It was thought that segregational resistance of material caused by the addition of EPS WN9 was due to its water absorption or thickening effect [12].

Fig. 4 shows the relative changes of flow value of the mortar according to time after mortar mixing. Adding EPS WN9 caused relatively small flow value change. After 90 min, the change in the flow value of the mortar with 0.02%(w/w) of EPS WN9 was about 93% of initial value, whereas the change of the flow value of the mortar without EPS WN9 was about 78% of its initial value.

The surface moisture of the aggregate is one of the important factors which determines the quality of the mortar and concrete. Fig. 5 shows the relationship between the flow value and the change in the surface moisture of the mortar. The flow values of mortar were increased with increased surface moisture content. The lower slope of flow value was observed in the system containing EPS WN9, and consequently the preparation

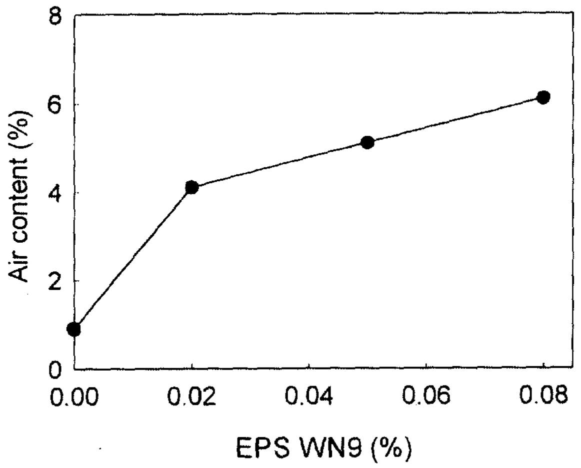

Fig. 6. Changes of air content of mortar according to addition of EPS WN9. The ratio of w/c was 0.45.

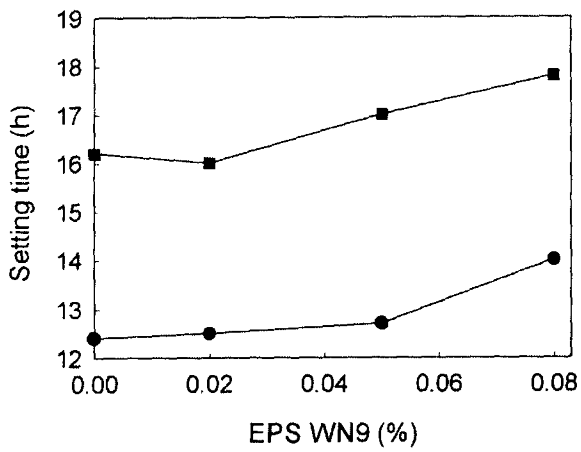

Fig. 7. Effect of EPS WN9 addition to mortar on setting time. The ratio of w/c was 0.45. ●, initial setting; ■, final setting.

of high quality mortar was possible.

Although the addition of viscosity modifier to mortar in combination with super plasticizer is effective for preventing material segregation and giving proper fluidity, some viscosity modifiers, such as cellulose derivatives show high air-entrapment and this must be controlled by adding a defoaming agent [13]. Generally, the target air content in high fluidity mortar is 4.5-5.5% (±1%) by volume. Addition of viscosity modifying admixture to the mortar results in increased air-entrapment, and the high air content in the mortar causes it to lose strength. The air content of mortar increased with the increasing amounts of EPS WN9, as shown in Fig. 6. The air content in the mortar increased from 0.9% to 6.1% with addition of EPS WN9 up to 0.08%. Similar results were obtained from experiments using polycarbonic acid super plasticizer (detailed data not shown).

Viscosity modifiers such as cellulose derivatives cause undesirable characteristics such as retardation of the time of setting. Retardation of setting time causes delays in the construction work. The time of setting of the mortar using cellulose derivatives has been shown to retard the set by three to nine hours [6,13]. It is thought that the retardation of mortar setting time is caused by the EPS, which disperses cement particles and retards hydration of the cement. In other to examine the effect of EPS WN9 addition, the mortar was prepared without super plasticizer. The effect of EPS WN9 addition on mortar setting property is shown in Fig. 7. The setting time at 0.02%(w/w) addition of EPS WN9 was similar to that of the control (no addition of EPS WN9), but the setting time with increased amounts (0.08%) resulted in a delayed setting time of 1.6 h.

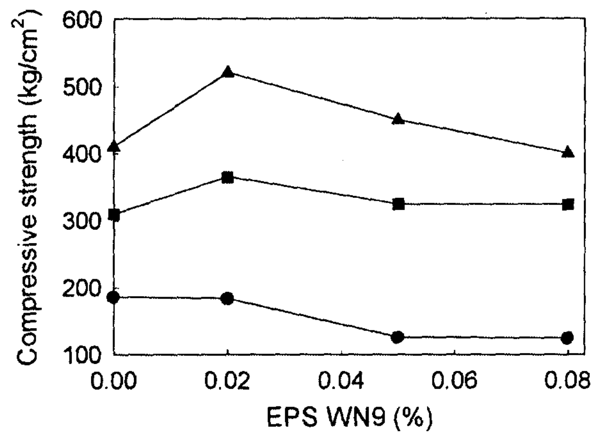

Fig. 8. Effect of EPS WN9 addition to cement on mortar compressive strength. The ratio of w/c was 0.45. Compressive strength at 3 day (●), 7 day (■), and 28 day (▲).

Fig. 8 shows the effects of EPS WN9 addition on the compressive strength of the mortar. The compressive strength showed the highest value with an addition of 0.02%(w/w) of EPS WN9. Compressive strength of mortar was increased by about 20% after the addition of 0.02%(w/w) of EPS WN9, but higher addition of EPS WN9 (0.08%, w/w) caused a slight reduction.

In conclusion, it was possible to prepare a segregational resistant and high fluidity mortar using EPS WN9 as a cement mortar admixture. It is believed that the optimum addition of EPS WN9 is between 0.02 to 0.05% of cement weight in the mortar. EPS WN9 is believed to show promise as a practical cement admixture for the preparation of high fluidity concrete, provided that further detailed study follows, especially on its combination with other types of super plasticizer and cement admixture.

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