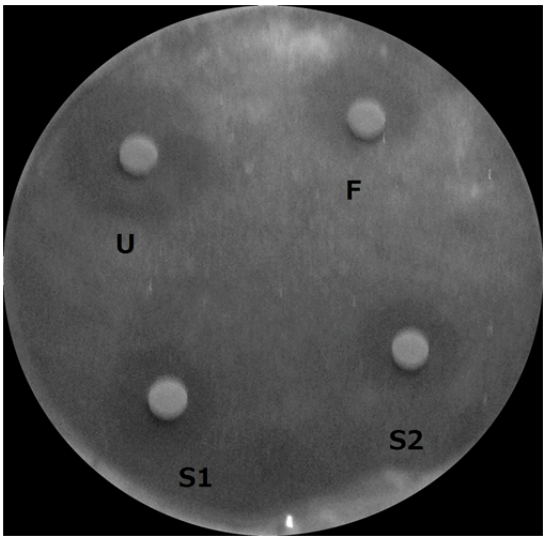

Figure 5. A fibrin plate showing: Ur, urokinase standard; S1 and S2, *Natto-1.1 kb* and *Natto-1.3 kb*, respectively; F, fermentation product.

Table 1. Comparison between the fibrinolytic activities

Enzyme	Specific activity (U/mg)[a]
Natto-1.1 kb	1187.5 ± 134
Natto-1.3 kb	1000 ± 101
Fermentation product (F)	400±97

[a]The data shown are expressed as a mean ± SD, based on three independent experiments.

Comparative fibrinolytic activity

The fibrinolytic activities of the cloned *Natto-1.1 kb* and *Natto-1.3 kb* were compared to that of the fermentation product using urokinase as a reference standard. Serial dilutions of urokinase (100 to 0.001 U) were added to the fibrin plate and the clear zones resulting from hydrolysis of the fibrin clot were recorded (Fig. 4). Ten µl of each of the expressed NK samples (*Natto-1.1 kb* and *Natto-1.3 kb*) and the fermentation product (F) containing 8, 10 and 75 mg/mL protein, respectively, were added to the fibrin clot. All samples revealed fibrinolytic effect as indicated by the clear zone of 1.6 and 1.7 and 1.9 cm corresponding to 95, 100 and 300 U, respectively (Fig. 5). As shown in Table 1, the specific activity of *Natto-19* (1187.5 ± 134 U/mg) was slightly higher than *Natto* but was 3-fold that of the fermentation product. Previously, Weng et al., 2009 [5] expressed a wild type subtilisin NAT and two of its mutants T220S and M222A possessing specific fibrinolytic activities of 1760 ± 154, 1230 ± 90 and 933 ± 97 U/mg, respectively, at pH 7.75.

CONCLUSION

The fibrinolytic activities of the cloned Nattokinases, *Natto-1.1kb* and *Natto-1.3kb*, were more or less the same although 19 amino acid residues were missing at the N-terminal of *Natto-1.1kb*. The lyophilized fermentation product of *Bacillus subtilis* (natto) showed quite a good activity compared with the lyophilized pure expressed Nattokinases. Production of such a fermentation product on a larger scale and its incorporation into pharmaceutical preparations is our next goal.

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