Measurement of Growth Rates of Dictyostelium discoideum for search of Social Behavior

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Abstract

Dictyostellium Discoideum is often used as a model organism for differentiation and patterning, because it has unicellular stage and multicellular stage within its relatively short life cycle. When mixed the same number of Dictyostelium amoeba from genetically different wild strains, it is known that these amoebae construct fruiting body together. However the final number of spores on top of fruiting body differs on each combination of strains. Because these genetically different strains are found in same area, this social conflict (cheating) might be compensated by different stage of development. The aim of this study is to examine which part of development among the life cycle compensates the cheating.

19 wild genotypes were used in this study (Figure 1). When these isolates were developed clonally, no big differences were observed among strains except for hatching rate. This hatching rate seems to be controlled by hatching inhibitor. Widespread variations of production of and response to the inhibitor are observed and social cooperation might be started from unicellular stage.

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Introduction

Dictyostelium Discoideum

D. discoideum, belonging to the phylum Mycetozoa, eukaryote is one of the model organism. The sequence of the complete genome of Dictyostelium was reported [1]. The life cycle starts from spores released from fruiting body. Unicellular amoebae hatch from spores and feed on bacteria as dividing by mitosis. This is called as vegetative stage. When they get starved, they enter aggregation stage. The amoebae aggregate and stick together. In migration stage, they form a slug which is capable of movement. Finally, the slug differentiates into fruiting body which has spores raised in the air onto a stalk. This is called culmination stage. Finally, spores are released from the fruiting body [2].
Spores are on top of stalk which is composed of dead cells which is no longer capable of reproduction. Furthermore, social behavior is observed between genetically different strains of Dictyostelium [3-7]. In aggregation stage, amoebae of different genotypes aggregate and form a chimeric fruiting body. This phenomenon results in social outcome in which strains will differentiate into stalk as a sacrifice and which will become spores, with direct reproductive fitness. Spore: stalk allocation in chimera highly varies and depends on pairing of genotypes [3, 8]. Chimaerism seems to occur frequently because these genotypes co-exist in same area [4]. Therefore, disruptive cheating (social conflict) [9-13] might be compensated by social cooperation. Otherwise, some strains are forced to extinct.

To test this idea, each parts of life cycle (growth rates of amoebae, final numbers of amoebae, hatching rates of spores, hatching rates of washed spores, hatching rates of supernatants-crossed spores) are measured.
In part, this idea is supported by idea of pleiotropy. When a knockout mutant which ignore stalk-inducing factor forms a chimeric fruiting body with parental-wild strain, lose out the allocation of spores vice versa [14].

Methods and Material

Strains

Nineteen wild strains of D. discoideum used among these studies are labeled as strain 1 to 20 to simplify the experiments. This is real name corresponds to the label (Figure 1). All study are maintained with *Klebsiella aerogenes* bacteria (KA).

simple No	strain
1	28.1
2	34.1
3	34.2
4	39.1
5	41.2
6	43.1
7	52.3
8	59.2
9	60.1
10	63.2
11	67.2
12	73.1
13	78.2
14	80.1
15	85.2
16	88.2
17	96.1
18	98.1

19	105.1

Figure 1 real strain name corresponds to label

Growth Rate Measuring

Total of 2.5×10^5 spores and 2.0×10^{10} of concentrated KA are used in this study. To harvest spores, hatching out plates are made by spreading 400 µl of KA (LB) with freezed stock of wild strains of Dictiostelium on SM plates, and used from 5 days later. This is because all the 19 wild strains used in this study are ready from 5days later (5 to 12 days old plates are used). Spores are harvested with tips by the side of Bunsen burner into 1ml of KK2 in eppendorf tube, and counted under microscope with hemocytometer. KA plates are made by spreading 400 µl of KA (LB) on SM plate and left for 4days, and harvested with spatula by the side of Bunsen burner into 1 ml KK2 per 1 plate in falcon tube. To check this KA stock, 50 µl of KA is diluted with glass tubes from $\frac{1}{1}$ to $\frac{1}{10^{10}}$. Then, $\frac{1}{10^{10}}, \frac{1}{10^9}, \frac{1}{10^8}, \frac{1}{10^7}, \frac{1}{10^6}$ diluted stocks are spotted on LB plates 3 times 10 µl for each dilution and left in 37 degrees Celcius for 12 to 16 hours, and counted the number of colony under the stereoscope. From this scoring, the concentration of KA is calculated.

The fixed number of spores and concentrated KA are mixed in 200 µl of KK2 for each plate and spread by the side of Bunsen burner on 90-minutes-dried (room temperature) KK2 agar plate (60mm). To make influence of time of spreading less, KA is mixed shortly before spreading.

Every 3 hours between 15 h to 30 h from spreading, 1 plate for each strain is harvested with two times of 500 µl of KK2 in total 1ml. By pipetting up and down with KK2, ameba on the plate are harvested into eppendorf tube. And the number is scored under microscope with hemocytometer.

Final Amoeba Numbers Measuring

The growth plates made by the same way with growth rate measuring is used for this study, and cells are harvested when they formed slugs (usually between 40 h to 45 h, sometimes 46 to 65 h), with 500 µl ×2 of KK2 (20mM EDTA) into eppendorf tubes. Shortly after harvesting into eppendorf tubes, stocks are disaggregated by pipetting up and down with 19 gauge needle for 10 times, and 100 µl of original stocks are diluted with 900 µl of KK2. And these diluted stocks are pipetted up and down again and scored under microscope with hemocytometer.

Hatching Rate Measuring

The plates made by the same way with growth rate measuring, except which were made 24 h before using is used for this study, and scored every hour between 0 h to 4 h. Shortly after spreading, plates are marked with a red marker on their bottom with 3 spots avoiding very wet region, and scored number of unhatched spores in the field with microscope (20× objective lenz). To make sure to count the same field every time, the spot is every time located in the middle of the field.

Hatching Rate of Washed Spores Measuring

The same way used with hatching rate study is

used, except for using washed spores. After harvesting spores, stocks were centrifuged for 90 seconds with 13,000 rpm and supernatants were disposed. One ml of new KK2 were added into every pellet and after well-shaking, it is centrifuged again and supernatants were disposed. At the last, 1ml of KK2-added spores were scored and used for this study as used in other studies.

Hatching out plates were used between 5to 8 days. This is because when older spores are used, spores could not be observed properly in the field. This might be because old spores were damaged by centrifuging.

Cross Inhibition of Spore hatch Measuring

It is clear that supernatants include something inhibit hatching of spores, from hatching rate of washed spores study. In this study, 10 and 20 µl of supernatants are mixed with washed spores of each strain. To make sure density of inhibitors in supernatants will be the same with the other supernatants from different strains, spore density of stocks are made even before centrifuging by adding KK2 to the higher concentrated stocks to become 1.25×10^7 spores / ml. When spores were spread, they were scored again after washing. Only strain 1, 10, 13 are used in this study. Strain 1 as a strain of middle speed hatching, strain 10 as a strain of fast hatching, strain 13 as a strain of slow hatching.

Results

Measurement Error

Standard deviation (STDEV) is used to estimate errors from minimum of 3 replicates among every study. In growth rate study, far-off data from exponential approximate equation is left out in order to make sure R-squared value of the approximate equation becomes more than 0.98.

Growth Rate Measuring

Raw numbers scored on hemocytometre are charted by exponential function graph as a growth rate. Therefore from each exponential approximate equation of the graph, multiplier of the equations are charted to compare slope of the growth rate. (Figure 2) Strain 5 seems to have lowest growth rate, and biggest growth rate for strain 4.

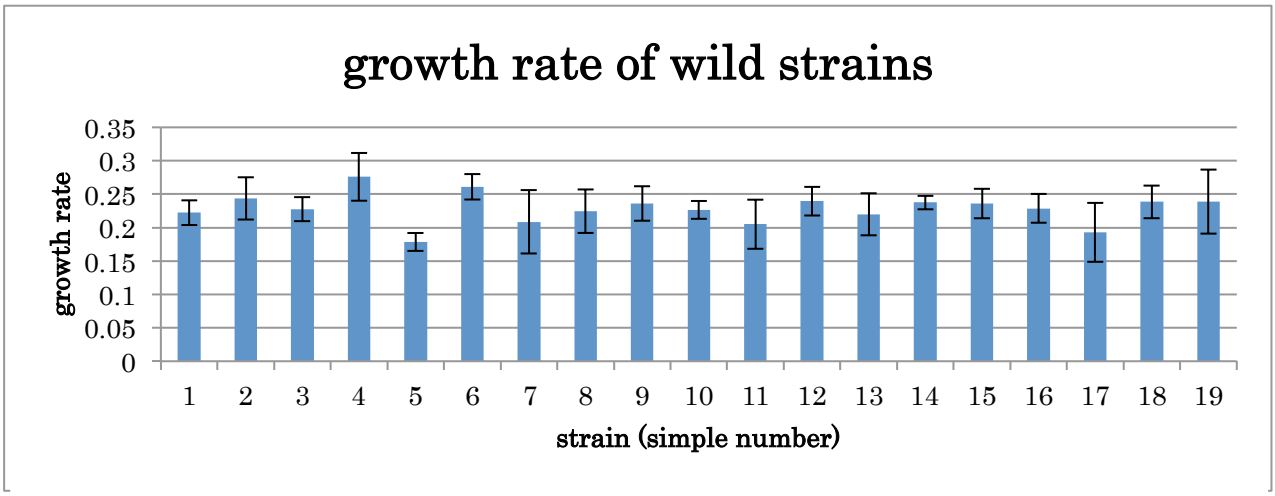


Figure 2 growth rates of wild strains

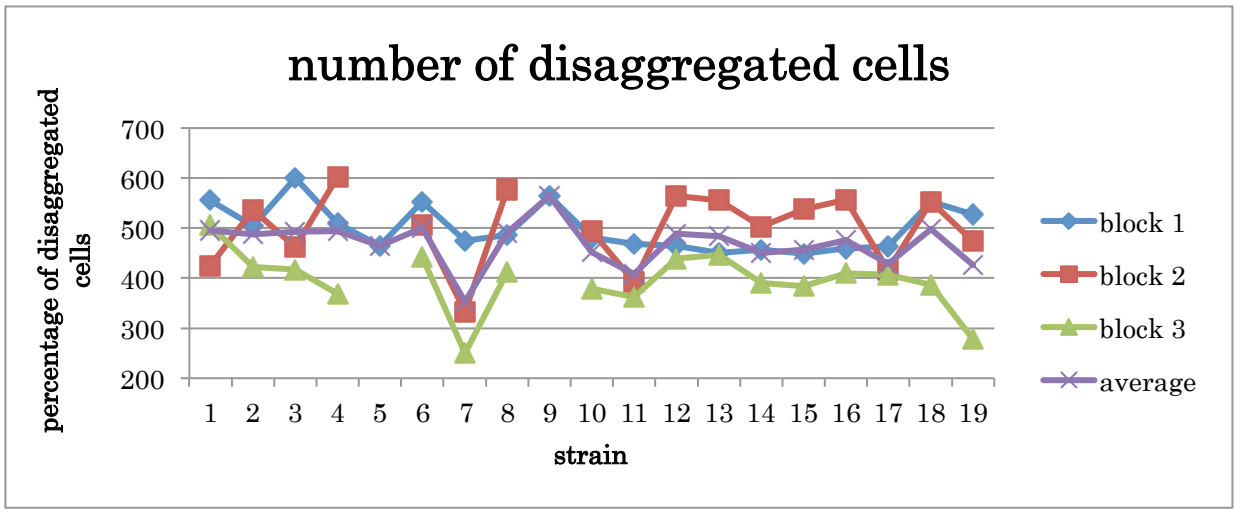


Figure 3 number of dissaggregated cells.

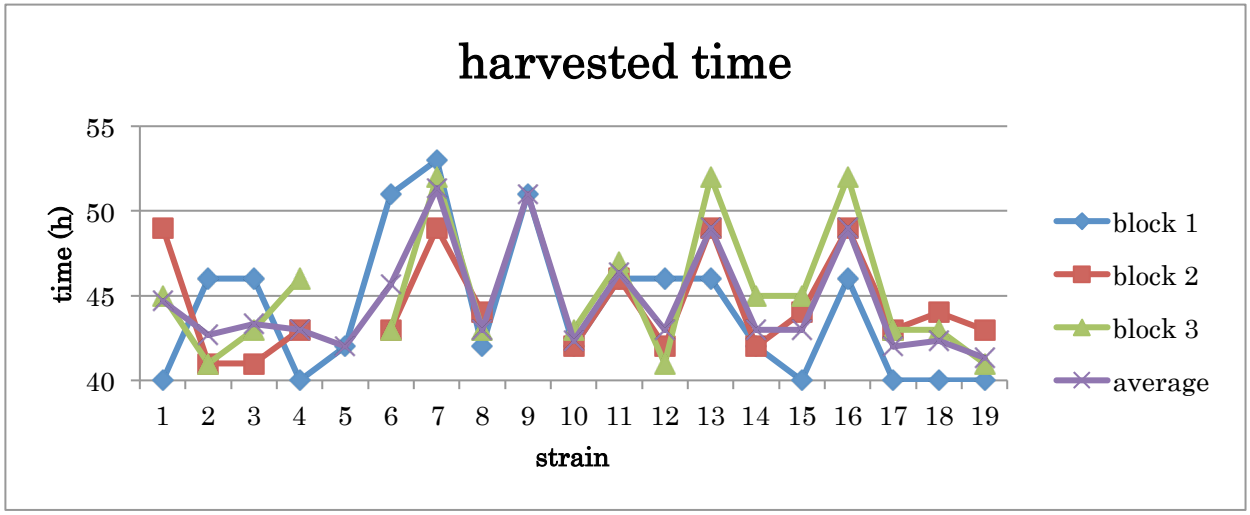


Figure 4 harvested time.

Final Amoeba Numbers Measuring

Raw numbers scored on hemocytometre are charted. (Figure 3) Raw date of harvested time is charted. (Figure 4)

Hatching Rate Measuring

Percentages of unhatched spores after 1 hour from spreading (Figure 5) and after 4 hour from spreading (Figure 6) are charted.

Hatching Rate of Washed Spores Measuring

Percentages of unhatched washed spores after 1 hour (Figure 7) and 4 hour (Figure 8) from spreading are charted.

Cross Inhibition of Spore hatch Measuring

Ten (Figure 10, 11) and twenty µl (Figure 12, 13) of supernatants are used in this study. Simple numbers are used in this study to indicate combinations of supernatants and spores. (Figure 14)

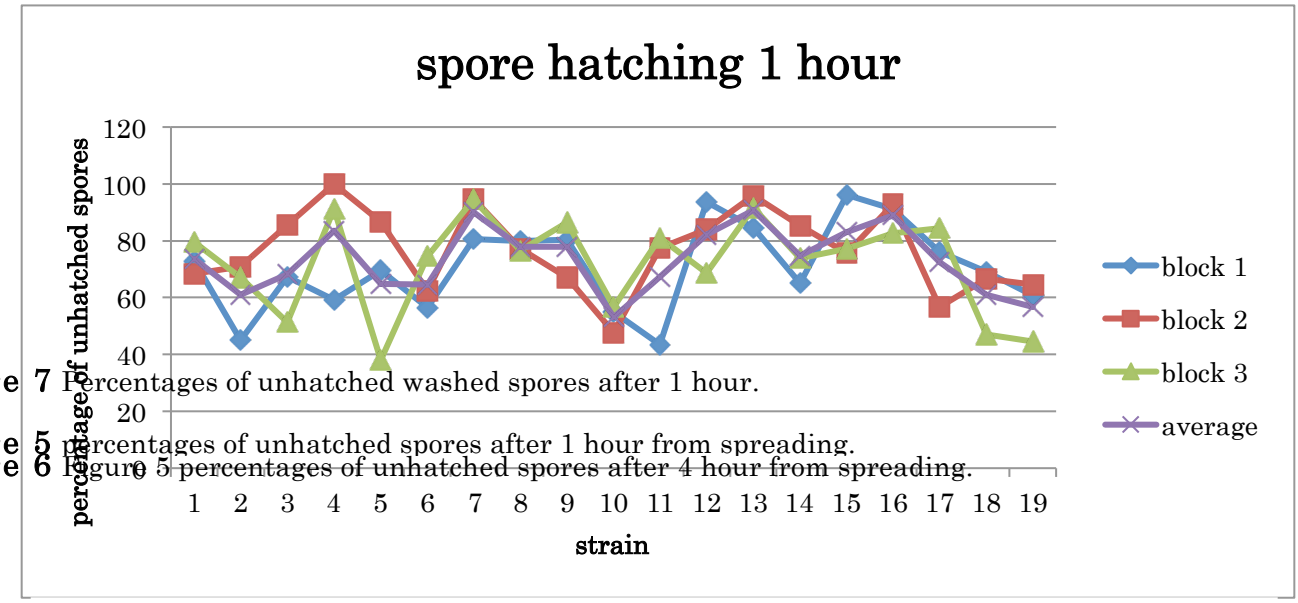
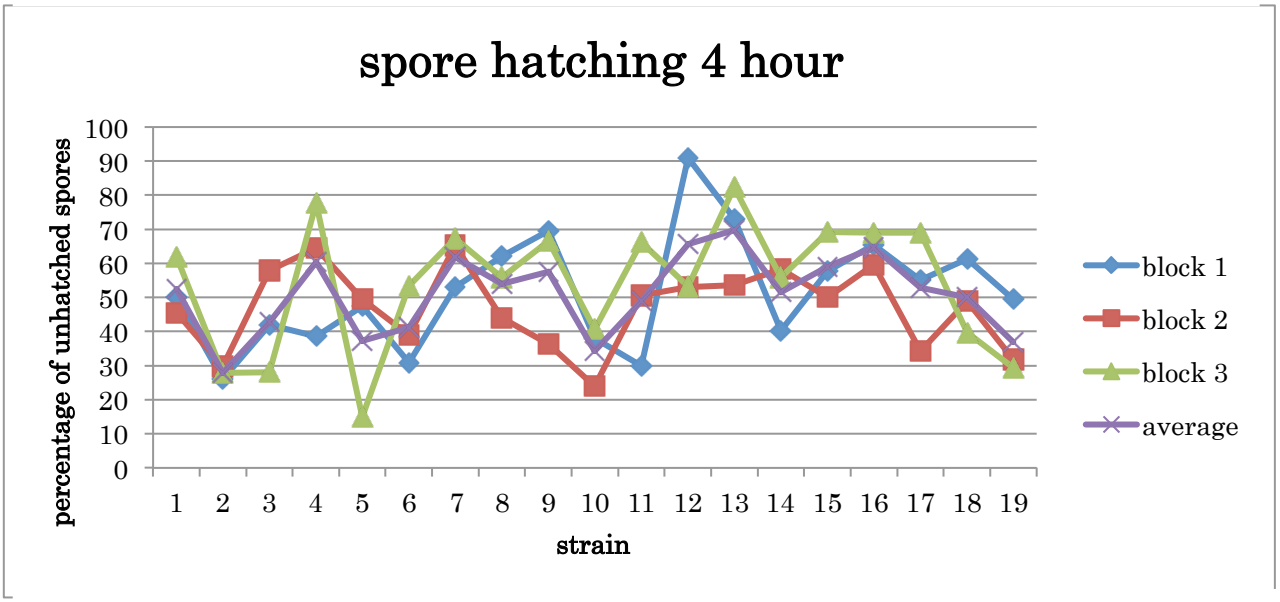
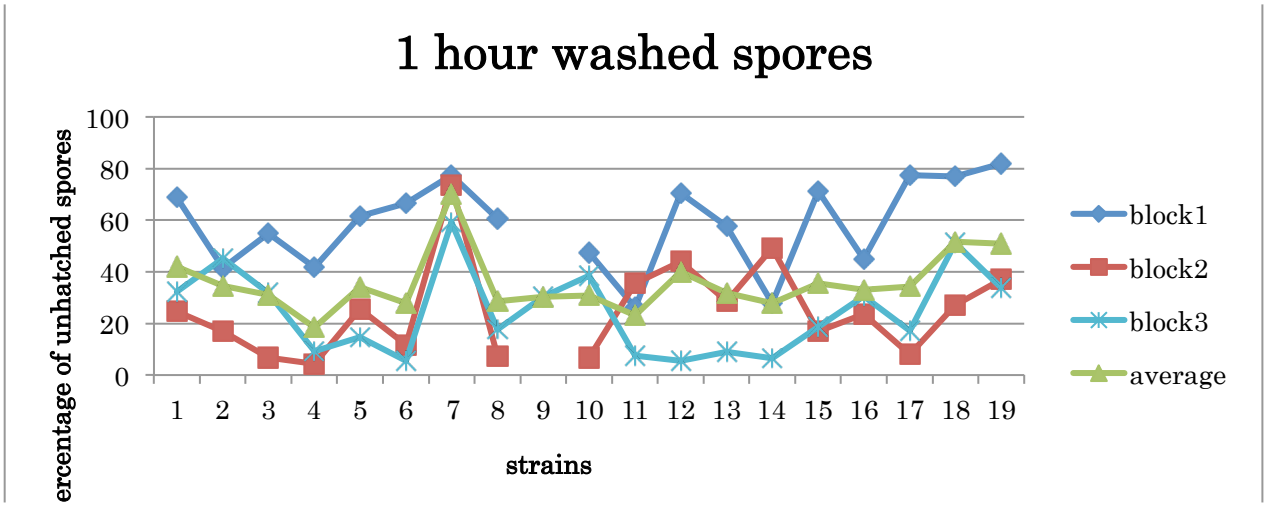


Figure 7 Percentages of unhatched washed spores after 1 hour.

Figure 5 percentages of unhatched spores after 1 hour from spreading.

Figure 6 Figure 5 percentages of unhatched spores after 4 hour from spreading.





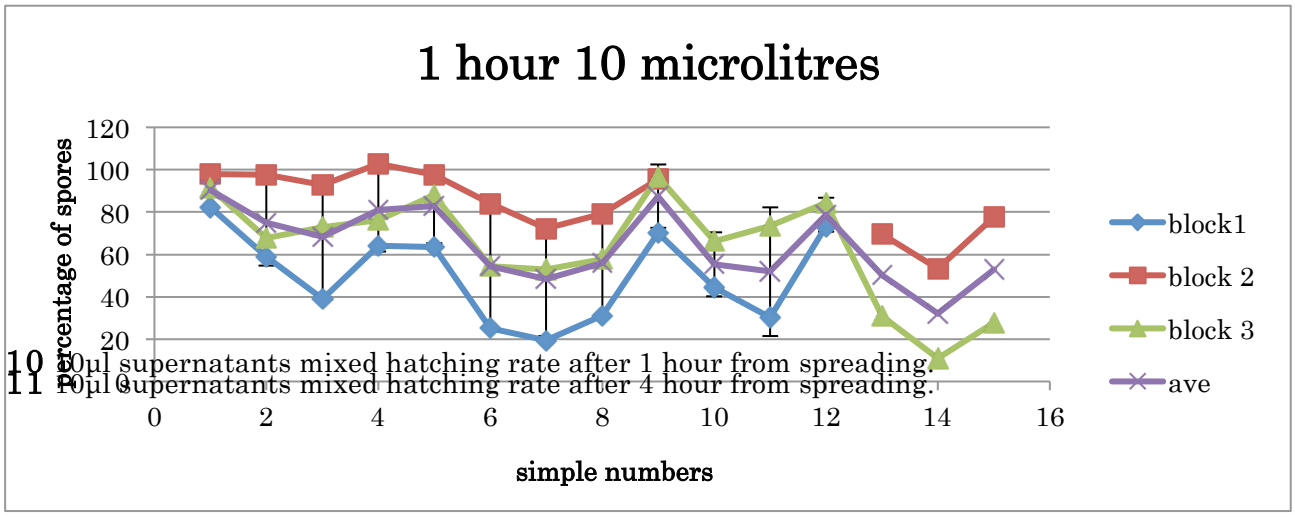


Figure 10 10μl supernatants mixed hatching rate after 1 hour from spreading.

Figure 11 10μl supernatants mixed hatching rate after 4 hour from spreading.

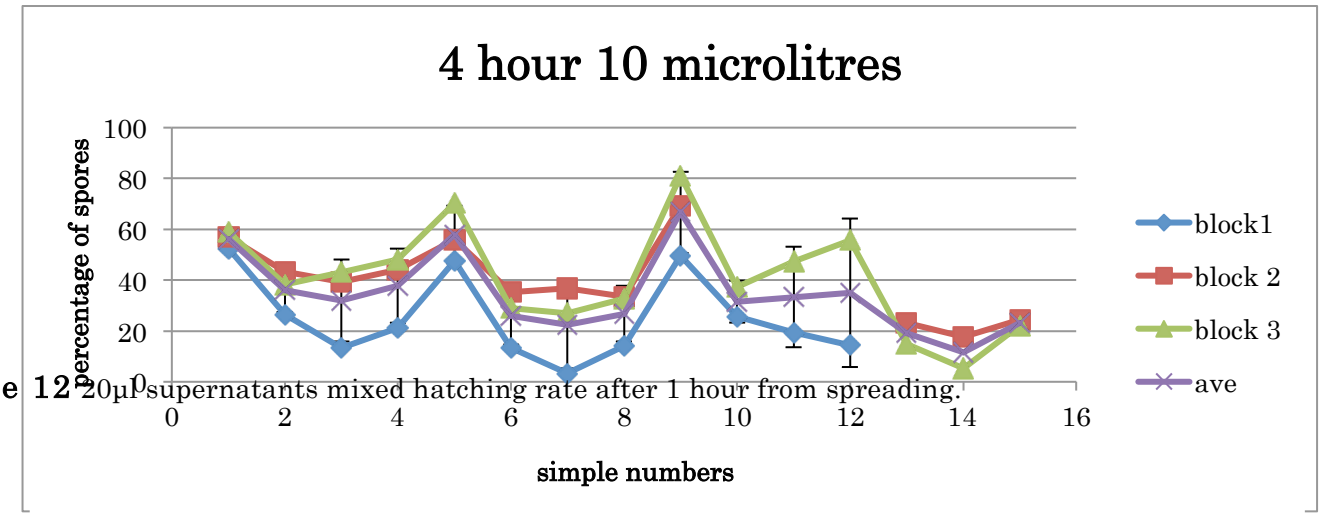
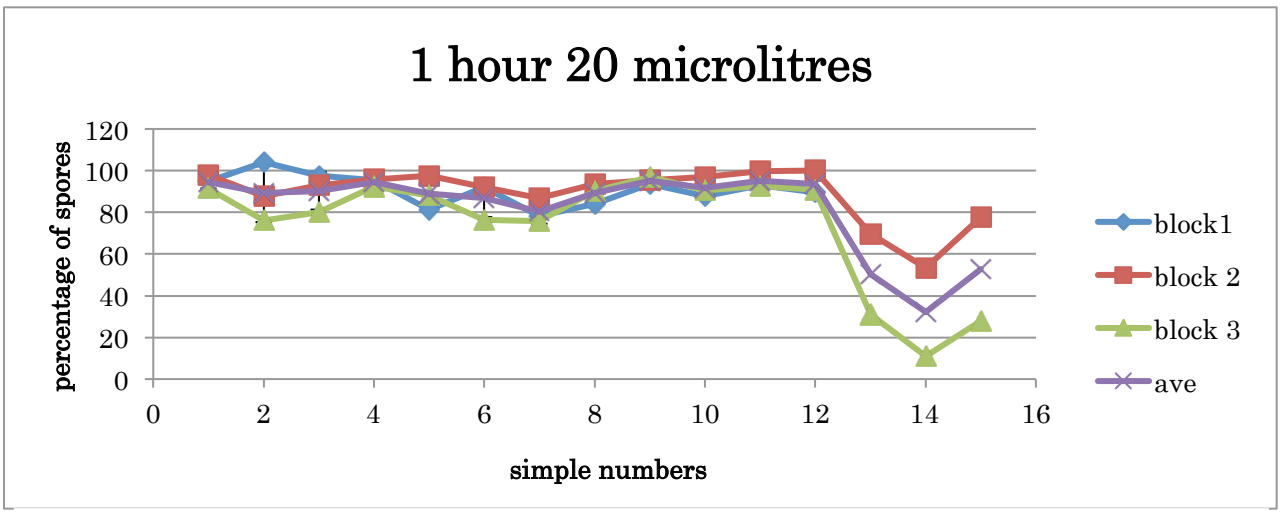


Figure 12 20μl supernatants mixed hatching rate after 1 hour from spreading.



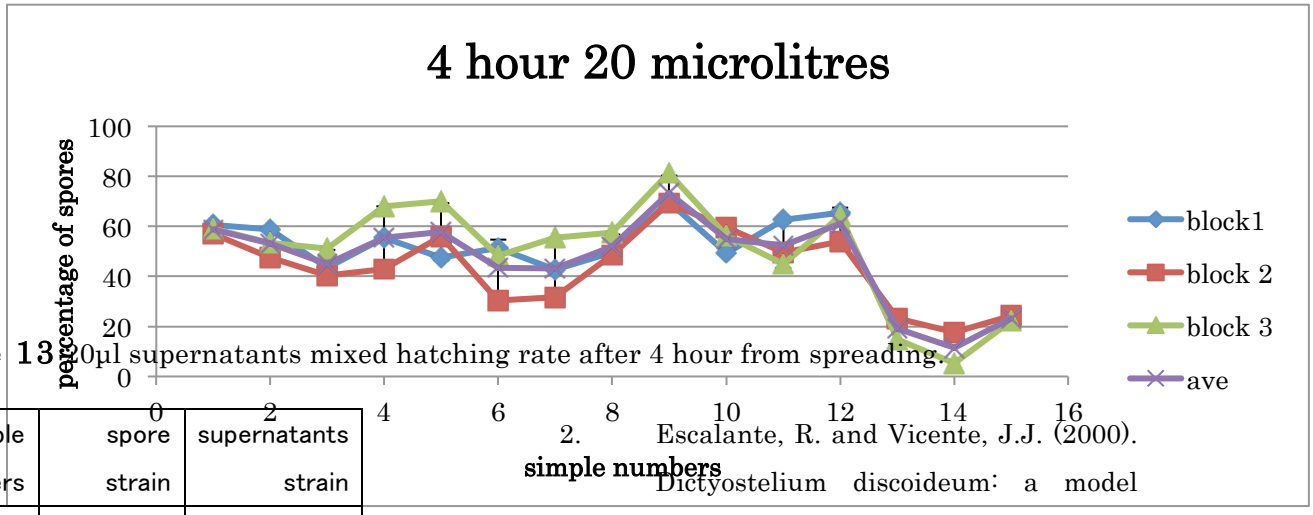


Figure 13 20μl supernatants mixed hatching rate after 4 hour from spreading.

simple numbers	spore strain	supernatants strain
1	1	unwash
2	1	1
3	1	10
4	1	13
5	10	unwash
6	10	1
7	10	10
8	10	13
9	13	unwash
10	13	1
11	13	10
12	13	13
13	1	wash
14	10	wash
15	13	wash

Figure 14 simple numbers of combinations.

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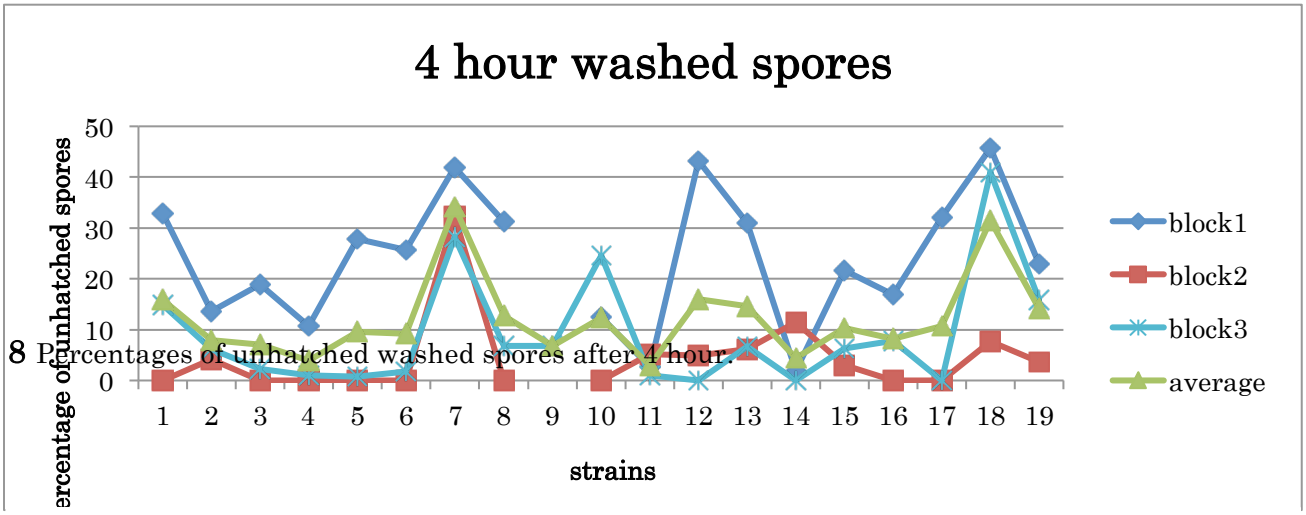
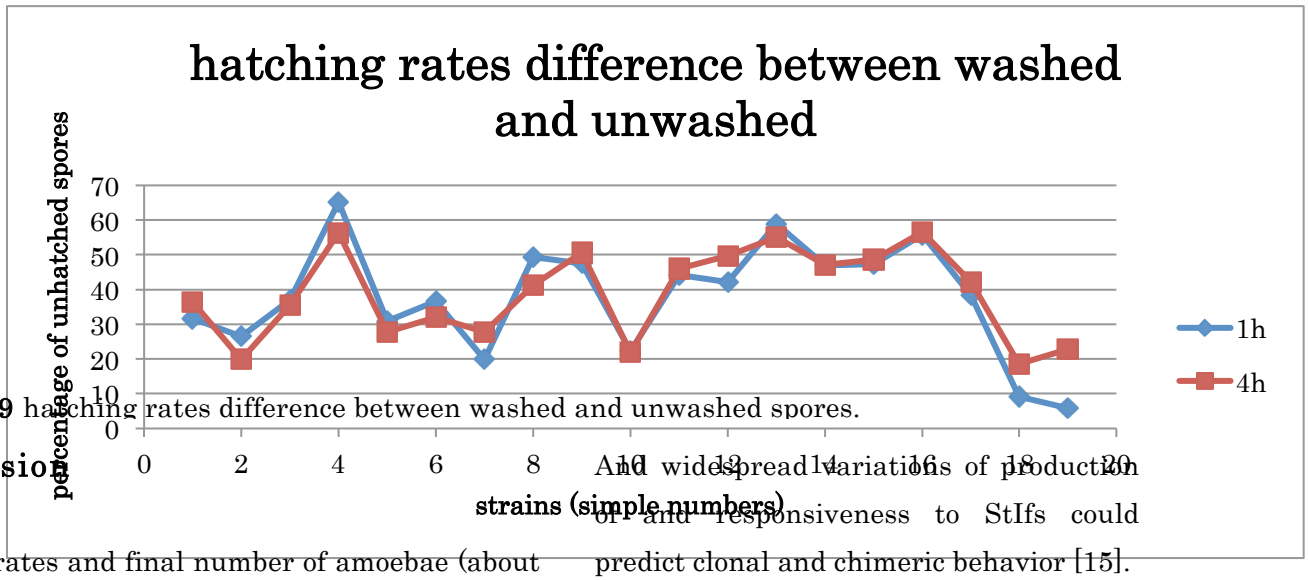


'igure 8 Percentages of unhatched washed spores after 4 hour.



'igure 9 hatching rates difference between washed and unwashed spores.

)iscussion

Growth rates and final number of amoebae (about .5-fold difference between highest and lowest) as no great difference between strains. On the ther hand, differences of hatching rates between ashed and unwashed are big (about 11-fold ifference between highest and lowest). (Figure 9) 'his result indicates that production of and esponsiveness to hatching inhibitors differs ramatically between genotypes.

talk-inducing factors (StIFs) are reasoned to be a ıajor determination of spore : stalk proportion [8]. And widespread variations of production of and responsiveness to StIfs could predict clonal and chimeric behavior [15]. Cross inhibition data indicate that inhibitor is common among genotypes and it might be used as a cooperation factor similarly StIFs. When cross inhibition data are accumulated and compared to chimeric cheating behavior, it might be clear that Dictyostelium has social behavior from its unicellular stage.