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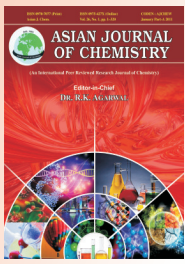

Separation of Cellulose-Decomposing Strain

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In order to find mixed strains which are highly effective in decomposing straw cellulose to decompose straws so as to utilize resources optimally, this research starts from separating strains and takes the soil chronically covered by straws and other plants as the sample. Through repeatable enrichment cultivation, separation and purification, fourteen natural strain stubs which can grow on solid cultivation medium with carboxymethyl cellulose medium-Na (CMC-Na) as the only carbon source are gradually obtained. Then, through the degradation test of filter paper and the identification of congo red cellulose, ten fungi with strong adaptability and excellent effect of decomposing cellulose are screened, from which four combination ways are formed. A combination of mixed strains with the best effect to decompose cellulose is finally gained by comparing the CMC, FPA enzyme activity and the syngenesis property of the four combinations. Through identification, the two strains are, respectively *Mucor sp*. and *Aspergillus sp*.

Keywords: Cellulose, Mixed strains, Separation, *Mucor sp.*, *Aspergillus sp*.

INTRODUCTION

Cellulose is the most abundant reproducible resource in the world. Converting it into effective energy through microbial degradation is the useful approach to utilize it rationally. While, the strains in practical industrial production have the disadvantages of low activity, low energy conversion rate, high cost, *etc*. Therefore, it is quite important to separate and screen natural strains high-yield enzyme, fast growth, strong adaptation and low requirements for culturing and yielding enzymes. This research tries to make up the defects of incomplete elements and low activity of enzyme in natural monoxenie strains and to set an experimental basis for industrial production by utilizing the reciprocal syngenesis effect of strains, culturing different strains mixedly and screening the best mixed strains combinations.

EXPERIMENTAL

Straw powder: The straw powder is gained by crashing the straws which have been dried naturally and sifting out the crushed straw from 1mm sieve and the bran is also crushed and sifted out in 1 mm sieve.

Experimental culture medium

Enrichment media: K_2HPO_4 0.2 %, $(NH_4)_2SO_4$ 0.14 %, $MgS0_4{\cdot}7H_2O$ 0.03 %, $CaCl_2$ 0.03 %, $FeSO_4{\cdot}7H_2O$ 5×10^{-4} %, $MnSO_4$ 1.6×10^{-4} %, $ZnSO_4$ 1.7×10^{-4} %, $CoCl_2$ 2×10^{-4} %, filter strip 2 %, pH 5.5, sterilizing for 0.5 h at 121 ºC.

Separating plating medium-carboxymethyl cellulose medium (CMC medium) 15 g of CMC-Na, 1 g of NH_4NO_3, 1 g of yeast extract, 0.5 g of $MgSO_4{\cdot}7H_2O$, 1 g of KH_2PO_4, 1000 mL H_2O, 2 % agaragar, natural pH value, sterilizing for 0.5 h at 121 ºC.

Strain storage medium-potato glucose slant medium (PDA yeast extract): Peeling off and slicing 200 g of potatoes, boiling the sliced potatoes for 0.5 h first and filtering the boiled potatoes next; adding 20 g of glucose and 20 g of yeast extract into the filtrate, adding water to the amount of 1,000 mL until the glucose and the yeast extract being dissolved into the water; pH value being natural, sterilizing the filtrate for 0.5 h at 121 ºC and then producing slant for refrigeration and reservation.

Filter strip identifying the culture medium: $(NH_4)_2SO_4$ 0.10 %, KH_2PO_4 0.10 %, $MgSO_4{\cdot}7H_2O$ 0.05 %, K_2HPO_4 0.2 %, yeast extract 0.01 %, one filter strip (1 × 7 cm),natural pH value.

Congo red cellulose identifying the medium: $(NH_4)_2SO_4$ 0.2 %, 0.05 % $MgSO_4{\cdot}7H_2O$, KH_2PO_4 0.1 %, NaCl 0.05 %, CMC-Na 2.0 %, congo red 0.02 %, agaragar 2.0 %, natural pH value.

Liquid enzyme-production identifying the medium: 15 g of (CMC-Na), 1 g of NH_4NO_3, 0.5 g of $MgSO_4{\cdot}7H_2O$, 1 g of KH_2PO_4, 1000 mL H_2O, natural pH value, sterilizing for 0.5 h at 121 ºC.

Liquid enzyme-production identifying the medium: 90 % of straw powder, 10 % of bran and nutrient fluid; the proportion being every 5 g of solid being added with 15 mL of nutrient fluid; natural pH value and sterilizing for 1 h at 121 ºC.

Nutrient fluid: $(NH_4)_2SO_4$ 2 %, KH_2PO_4 0.1 %, $MgSO_4 \cdot 7H_2O$ 0.5 %, $CaCl_2$ 0.01 %, NaCl 0.01 %, $FeSO_4 \cdot 7H_2O$ 0.005 %, $MnSO_4 \cdot H_2O$ 0.0016 %, $ZnSO_4 \cdot 7H_2O$ 0.0014 %, $CoCl_2$ 0.002 %.

Major reagents: 1 mg/mL of glucose standard solution; dinitrosalicylic acid reagent (DNS reagent); 0.2 M of acetid acid-sodium acetate buffer solution (pJ4.8); 1 % CMC buffer solution; Xinhua number one filter paper.

Experimental strains: Strains for strains separation are selected from the humic soil samples in rural straw piles.

Experimental methods

Samples collection: The purpose of this experiment is to find high-efficient decomposing cellulose strains. According to the principle of ecological simulation, the samples should be selected from the natural environment abundant in cellulose decomposing strains. The samples of this experiment are the soil covered by straws and plants all the year round. With the moderate temperature and humidity in autumn, the soil covered by the various plants has the largest number of microorganisms and thus autumn is the best time to collect the soil samples. The soil samples are taken from the 5-25 cm depth down the surface[1].

Enrichment cultivation of strains: To obtain cellulose decomposing strain, this experiment utilizes liquid enrichment medium with filter paper as the only carbon source. As the main ingredients of the filter paper are cellulose, the target strain can grow vigorously under the special cultivation condition rich in cellulose and that is good for separation and purification. The concrete methods are as follows: filling 50 mL of sterile water in the sample bottle, adequately oscillating the sample bottle after glass beads are added in to clean the microorganism on the samples and then standing the samples bottle; filtering the samples with trilaminated sterile gauze and standing the samples bottle again; taking out 1 mL of supernatant fluid from the filtrate and inoculating the supernatant fluid in a 250 mL triangular flask which contains enrichment medium. After that placing the conical beaker containing the enrichment medium of sampled strain on an oscillating table and keep culturing the enrichment medium of sampled strain for 72 h under the condition of 3000 rpm and 30 ºC. After the culturing is ended, placing the filter paper into a sterile fresh enrichment medium, repeating that process for 3 times to enrich the strains utilizing cellulose.

Separating strains: After the enrichment cultivation, this experiment selected the agar plate with CMC-Na as its unique carbon source to further separate and purify the target strains. The detailed operations are as follows:

Taking 0.1 Ml of enrichment cultivation solution and placing it on the CMC-Na medium for painting the plate, cultivating it at homothermal 30 ºC; when there are strains growing on the plate, taking the strains and making line-marking separation on the CMC-Na plate; next, inoculating the individual strain colony growing on the plate on the slant medium for storage.

Picking out uncerated filter sheet from the enrichment cultivation solution and inoculating it on the CMC-Na medium plate for homothermal 30 ºC cultivation; observing the forms changes of the filter sheet on the medium and the growing of the strains.

Strains screening

Filter paper disintegration testing: The liquid part of the filter paper medium is, respectively put into several testing tubes, with each test tube loading 2 mL of the liquid. A filter strip (weighed after being dried) of 50 mg (1 cm × 6 cm) is put into the test tube and is sterilized for reservation. Different strains in different forms are, respectively inoculated on the filter strip of the filter strip medium and then are cultivated at homothermal 30 ºC.

The test tubes displaying filter paper decomposing are diluted. Line-marking separation for CMC medium is made on the plate, after 72 h of cultivation at 30 ºC and the growing individual strain colony is inoculated in the filter strip medium for 10 days' cultivation.

Weightlessness measuring: The filter strip which has been inoculated for 10 days is cleaned by water, after being dries and it is weighed. Then an equation is used for calculation:

$$X = \frac{m - m_1}{m} \times 100\ \%$$

wherein X is the weight loss rate (%) of the filter strip; m is the weight (g) of the filter strip before the inoculation and m_1 is weight (g) of the filter strip after the inoculation.

Plate identification of Congo red cellulose: The individual strain colony obtained by separation is inoculated on the congo red cellulose agar plate and then is cultivated for 7 days in the 30 ºC thermotank. The individual strain colonies with fast growing speed, dense red colour, transparent and large-ring shapes are taken out from the plate. Then, line-marking separation is done. Determine the individual strain colonies with pure strains and then these kinds of colonies are inoculated on the medium for storage.

Strain identification: According to the classification and identification methods of fungus, observe the forms of strain stubs, hyphaes, sporophores and spores and observe the consolidation relationship between the spores and the nutrient body. Find out the classification status of the fungus according to relevant data. The specific methods include following several points.

Individual form observation: Water dipping and sliding method: selecting clean glass slide, dipping a drop of sterile water on it, selecting a small amount of fungus with an inoculating loop and putting the fungus into sterile water and covering the glass slide for microscopical examination.

Dipslide culture method: making the sterilized PDA medium into a plate with a thickness of 0.5 mm, cutting it into 1 cm^2 pieces after it is cooled, taking a small piece of the medium and putting it at the two ends of the glass slide in a sterilized flat utensil (putting a piece of filter paper and a glass slide on the flat utensil); next, making inoculation around the small medium, covering the glass slide, filling in sterile water on the filter paper in the flat utensil for cultivation at 28 ºC; observing the growing of the small medium every 6 h and observing the hyphae and the spore with a microscope.

Strain colony form observation: Dibble strains on PDA medium plate and cultivating the strains for 2-3 d at 28 ºC; Observe the growing speed, size, shape, colour and the generation of soluble pigment of the strains.

Stains preservation: Inoculate the strains into the PDA slant, cultivating them for 24, 48, 72, 96 and 120 h, respectively and observing the growing of the strains and the spores.

Spore suspension preparation: Scrape several rings of spores from the activated slant into the sterile water with glass beads; scatter the spores by oscillating; measure the concentration of the spores with a hemocytometer plate and finding the concentration of 10^6-10^7 per mL.

Enzymatic production experiment

Liquid state fermentation: Inoculate 1 mL of spore suspension into a 250 mL conical bottle which contains 100 mL of liquid enzymatic production and identification medium and place the conical bottle, respectively on the oscillating table (150 r/min; cultivated by oscillation for 5 d at 30 ºC) and a biochemical incubator (standing the conical bottle at 30 ºC for 5d); observe the viscosity change of the liquid medium and the strain growing and measure the sugar yields, respectively.

Solid state fermentation: Inoculate 1 mL of spore suspension into a 250 mL conical bottle which contains 5 g of solid enzymatic production and identification medium and next place the conical bottle in a biochemical incubator. Measure the sugar yield after cultivation for 5 d at 30 ºC[2].

Enzyme activity measuring

Indexes selection: The cellulose enzyme is a complex enzyme with multiple ingredients, while currently the measuring method for the activity has not been standardized. This research mainly measures the diastatic power of carboxymethyl cellulose (CMC) and the enzymatic activity of the diastatic power of filter paper.

Enzymatic liquid preparation: The extractions of the crude enzyme solution in solid fermentation is added with distilled water whose dry weight is ten times that of cellulases and then stir the mixture. The mixture is put into 40 ºC water bath for heat preservation for 45 min. Next, absorbent cotton is filtered and then put into a centrifugal tube. Then, a frequency of 4,000 r/min is done for 10 min in the tube. Finally, the supernatant fluid is distilled for reservation.

Measuring method: CMC enzyme activity measuring: Add 1 mL of 1 % CMC buffer solution into test tubes A and B, respectively and then add 0.5 mL partly distilled enzyme liquid (preheated) in the test tube A and mix the materials to a balanced state; put the test tubes A and B into 50 ºC water bath for heat preservation for 0.5 h and then take out the two test tubes and add 3 mL LDNS reagent into them, respectively, add 0.5 mL enzyme liquid again into the test tube B, oscillate the materials in the test tube B to a balanced state and then boil the test tube B into the water bath to be boiled for 5 min and take out the test tube B. Put the test tube B in cold water until it is cooled to room temperature. Then, add distilled water to dilute the materials in the test tube B to 25 mL and mix the materials to a balanced state and measure the light absorbency (the test tube B is used as an empty contrast) at the 540 nm wave length point to measure the OD value. By consulting the standard curve and calculate the glucose content in the solution[3].

Measuring the filter paper activity (FPA) of enzyme: Add 1 mL of pH 4.8 acetic acid-sodium acetate buffer solution into the test tubes A and B and add one filter sheet (10 mm × 60 mm, weighing 50 mg) into each of the two test tubes; add 0.5 mL partly diluted enzymatic liquid (preheated) to the test tube A, preserving the heat for 1 h and then taking the test tube A out to be added with 3 mL LDNS reagent. Add another 0.5 mL enzyme liquid to the test tube B, oscillate the mixture and the materials in test tube B to a balanced state. Then boil the test tube B into water bath to boil for 5 min and take out the test tube B; put the test tube B in cold water until it is cooled to room temperature; next, add distilled water to dilute the materials in the test tube B to 25 mL; mix the materials to a balanced state and compare the colours (the test tube B is used as an empty contrast) at the 540 nm wave length point to measure the OD value and consult the standard curve and calculate the glucose content in the solution[4].

Enzyme activity calculation: The enzyme unit (U) = $G \times n \times 60 \times 10^3 / (0.5 \times t \times M_G)$ [glucose (μmol/(mL h 50 ºC)], wherein G is the glucose weight (mg) measured by the standard curve; N is the enzyme liquid dilution times when the enzyme activity is measured; 0.5 means we take 0.5 mL enzyme liquid of proper concentration to take part in the reaction; T means the homothermal period (min); M_G is the mole mass (g/mol) of the glucose.

RESULTS AND DISCUSSION

Strains separation and initial screening: Through enrichment cultivation for the collected samples and making plate painting and line-marking separation on the formed enrichment cultivation solution, 14 individual strains were gained. The shape features of each strain is shown is the Table-1. Filter paper disintegration experiment and Congo red identification are done on the 14 individual strain colonies which were gained by separation and the testing results are shown in Table-2.

From the data gained from the initial screening identification experiment, it can be seen that the weight loss rates of the filter paper of strains 13# and14# are far lower than those of others and the ratio between the soluble rings and strain colony rings of these two strains are also far lower than others. Therefore, these two strains are excluded. For strains 11# and12#, they have strong cellulose decomposing capacities from their weight loss rates of the filter paper and the diameters ratios between their soluble rings and strain colonies and their efficiencies are high. However, in the process of marking lines, it is found that both the strain 11# and the strain 12# grow in the inside of the medium and are difficult to be picked out and therefore it can be concluded that both the two strains are anaerobic bacteria. The strains screened in this experiment should not only have high activity, but also strong adaptability, low reliance on the growing environment. In other words, aerobic strains or oxygen-compatible strains are needed in this experiment, so 11# and 12# are excluded.

Further, it can be seen from the two groups of tests, the two methods are both effective in initially screening cellulose

TABLE-1
SHAPE FEATURES OF EACH STRAIN

Strain number	Shape features
1#	The strain is round and radial; the colour is white at first but later deepens to hoary; and it is dry.
2#	The strain is round and radial; the colour is white at first and changes to light pink; it is dry.
3#	The strain is white, lvet and covers the whole plate; it's dry. The strain is light pink and lvet; the hyphae covers the hole plate; the strain is dry.
4#	The strain colony is round, radial, white and dry.
5#	The strain takes on black grain shape and radiates outwards and it is dry.
6#	The strain is white, lvet, radiating outwards and dry.
7#	The strain is gray, lvet, radiating outwards and dry.
8#	The strain is green, lvet, radiating outwards and dry.
9#	The strain is white at first and turns to green and gray and it is radiating outwards and dry.
10#	The strain is white at first and turns to green and gray and it is radiating outwards and dry.
11#	The strain is white, distributed in the inside of the medium and wet.
12#	The strain is yellow, is distributed in the inside of the medium and wet.
13#	The strain is purple and distributed on the surface and in the inside of the medium.
14#	The strain is red and distributed on the surface and in the inside of the medium.

TABLE-2
IDENTIFICATION RESULTS FOR INITIAL SCREENING OF ENZYME PRODUCTION

Strain number	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Weight loss rate of the filter paper (%)	15	16	18	18	19	32	37	39	28	25	17	18	5	4
Soluble ring/strain	0.75	0.77	0.81	0.79	0.82	0.97	1.14	1.01	0.90	0.87	0.78	0.80	0.44	0.37

decomposing strains. According to the weight loss rate of the filter paper, the size of the transparent ring and the time sequences, the enzymatic productions of each strain can be estimated and that greatly reduces the work of screening. However, using above mentioned standard to judge the quantitative indexes of cellulose enzyme activity of strains is not reliable, for the enzyme activity is also influenced by temperature, humidity, time and other factors. Therefore, a second screening is indispensible.

Second screening of strains

Enzyme production of each strain: The 10 screened strains are, respectively inoculated in a 250 mL conical flask and are fermented in solid forms under the same condition. The enzyme activity of CMC within a week and the variation trend of the enzyme activity of the filter paper are measured, respectively. The results are shown in Table-3.

From the test data, it can be found that the peaks of 1#-5# among the 10 strains are lower than the peaks of others, wherein 1# and 3# show evident increase trend and the two strains have shorter time, about 5 d for producing enzyme. The enzyme activity peaks of 6#-10# are high, wherein the enzyme activity peaks of 7# and 8# are, respectively 154.42 and 144.65 U and these two peaks are obviously higher than the peaks of other strains. Further, the best enzyme production periods of 7# and 8# are short, about 4d. Thus, taking the peak of enzyme activity and the best enzyme production time into comprehensive consideration, this experiment selects out 1# and 3 # with low enzyme activity and 7# and 8# with high enzyme activity as the strains to be studies in the research of mixed strains in the next step.

As the single ingredient and unbalanced mixture ratio of the enzyme natural individual strain, therefore different strains need to be cultured mixedly. In this way, the strains can complement the disadvantages of each other and that is good for improving the ingredients of cellulose enzyme, the enzyme activity, shortening the enzyme production period and improving the decomposing efficiency of cellulose. For that reason, this experiment would further on to screen the best mixture strain combinations with the best decomposing effect.

After referring to several data, it is foundthat the combination of two strains is better than the combination of multiple strains. The main reason lies in that the growing conditions suitable for different strains are different and multiple strains

TABLE-3
ENZYME PRODUCTION OF EACH STRAIN

Strain number	CMC		FPA	
	Best time for enzyme production (d)	Largest enzyme activity [μmol/(mL h)]	Best time for enzyme production (d)	Largest enzyme activity [μmol/(mL h)]
1	4	43.37	5	24.33
2	5	44.32	5	25.56
3	5	46.09	5	23.79
4	7	46.50	6	25.42
5	5	46.50	5	25.62
6	6	127.27	4	35.40
7	4	154.42	4	52.78
8	4	144.65	4	49.52
9	4	84.24	4	28.54
10	4	66.72	4	27.74

are difficult to coordinate and coexist. Reciprocal inhibition always happens among multiple strains and that's why we choose the four mentioned strains to make up pair combinations. In the mixing process, combination methods like the combination of two inferior strains, the combination of superior and inferior strains, the combination of two superior strains, are adopted. Through these methods, the four mixed strains combinations are, respectively 1#-3#, 3#-8#, 1#-7# and 7#-8#. The enzyme production of these four combinations is measured to screen the best strains combination.

Enzyme production of each mixed strain: The four preserved strains are inoculated into the slant for activation cultivation and are made into spore suspension after four days. According to said combination method, the four strains are, respectively inoculated into solid medium according to a proportion of 1:1 and an inoculation amount of 10 % and under the same condition, the four strains are tested in solid fermentation. The CMC enzyme activity of the four combinations and the changing trend of the enzyme activity of the filter paper are measured and later a broken line graph is drawn to describe the results. The results are shown in Table-4.

Table-4 showed that the enzyme activity of the first combination kept stable increasing in the first 9 days and reached 113.28 U on the ninth day. The second combination took on comparatively high CMC enzyme activity from the 6th day to the 9th day, wherein its enzyme peak appeared on the 7th day and the peak value was 163.25U. The third combination kept relatively high CMC enzyme activity from the 4th day to the 8th day, with its peak appearing on the 7th day and the peak value was 182.94U. The fourth combination showed vast turning on the 3rd day in the first 9 days and it is estimated that certain antagonism happened in the initial stage of the fermentation. While later it kept stable increase and reached its peak on the 8th day, with the peak value as 167.05 U. Compared with the enzyme activities of the four individual strains screened at the last step, the largest enzyme activities of the four combinations are increased more or less, which indicates that the four combinations have different degrees of symbiotic relationship and form excellent biodegradation systems. However, the best enzyme production time was delayed for about 3 days, which is due to two reasons. On one hand, to keep the two strains completely adapting to each other by artificially mixing the two strains for cultivation needs time and domestication. On the other hand, to gain higher cellulose decomposing efficiency needs to find out the best cultivation conditions and the best fermentation conditions, such as temperature, humidity, inoculation proportion, *etc*. Through the continuous optimization of experimental condition, the enzyme production effect and the decomposing efficiency can reach the best states. Therefore, taking all the factors into consideration, this experiment selects the third combination (1#-7#) as the combination for subsequent saccharity fermentation technique research.

Identification for 1# strain

Shape features: The shape features include obvious and substantial membranes, nonporous ascocarps, tenuous and separated hyphaes, straight mitogenetic sporophores, smooth diameters of sporophores, subsphaeroidal vesicles, multiple cores, dense and irregular branches, spherical cleistothecium, having handles, unbalanced distribution on the surfaces of the strain colonies, irregular stocking of ascus in the softgel shells and no transudate.

Properties in cultivation: The strain stubs are cultivated on the medium plate at 28 ºC. The surfaces of the strain colonies are firstly in the shape of white tenuous and planar grains, while the grains gradually become bigger and denser as the passing of time. After 4 days, the colour is gradually deepening and darkening and no taste exists there.

Identification result: According to the shape features and the properties in cultivation, together with referring to the book fungal identification manual, 1# strain is initially identified as *Aspergillus* [*Aspergillus* sp.].

Identification of 7# strain

Shape features: The shape features include no rhizoids on the hyphae, no bunches formed on the sporangiophore, single growing, dense layers, sporangiophores on the top, large and spherical sporangiophores, many spores, smooth and easy melting epigonium, white columella and no apophysis.

Properties in cultivation: The strain stubs are cultivated on the PDA medium plate at 28 ºC. At first, they are in the shape of white lvet, but after two or three days, they can be strew the whole plate. As the passing of time, their colours are gradually deepening and darkening and finally changing to charcoal grey lvet shapes. No soluble pigment nor taste is generated.

Identification result: According to the shape features and the properties in cultivation, together with referring to the book fungal identification manual, 7# strain is initially identified as *Mucor* [*Mucor* sp.].

TABLE-4
ENZYME PRODUCTION OF DIFFERENT COMBINATIONS [μmol/(mL h)]

Time	Combination 1		Combination 2		Combination 3		Combination 4	
	CMC	FPA	CMC	FPA	CMC	FPA	CMC	FPA
First day	39.30	22.43	28.76	25.90	88.17	41.37	79.76	32.14
Second day	47.71	30.10	107.59	40.69	120.35	47.89	109.89	47.82
Third day	64.01	33.36	118.04	46.80	92.79	50.18	88.31	44.09
Fourth day	69.30	22.71	122.25	31.53	167.05	47.68	121.16	46.80
Fifth day	79.49	18.90	116.68	32.89	169.87	43.54	130.94	45.58
Sixth day	81.66	33.77	159.99	44.36	174.61	35.74	153.88	47.00
Seventh day	85.59	31.53	163.25	47.35	182.94	41.44	165.69	45.51
Eighth day	102.16	29.90	159.17	48.30	176.82	33.43	167.05	50.74
Ninth day	113.28	28.76	157.36	41.91	146.28	25.22	166.71	37.03

Conclusion

This research is put forward under dual backgrounds *i.e.*, worsening energy crisis and serious waste of straw cellulose, with the aim to find mixed strains which are highly effective in decomposing straw cellulose to decompose straws so as to utilize resources optimally. Based on present aim, this research starts from separating strains and takes the soil chronically covered by straws and other plants as the sample. Through repeatable enrichment cultivation, separation and purification, fourteen natural strain stubs which can grow on solid cultivation medium with carboxymethyl cellulose medium-Na (CMC-Na) as the only carbon source are gradually obtained. Then, through the degradation test of filter paper and the identification of Congo red cellulose, ten fungi with strong adaptability and excellent effect of decomposing cellulose are screened, from which four combination ways are formed. A combination of mixed strains with the best effect to decompose cellulose is finally gained by comparing the CMC, FPA enzyme activity and the syngenesis property of the four combinations. Through identification, the two strains are, respectively *Mucor* [*Mucor* sp.] and *Aspergillus* [*Aspergillus* sp.].

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