Journal of Applied Biological Sciences 2 (2): 55-61, 2008
ISSN: 1307-1130, www.nobel.gen.tr

Lignin Degradation and Acid Precipitable Polymeric Lignin (APPL) Accumulation by Selected *Streptomyces* Strains in Submerged and Solid State Culture Systems*

Mustafa YAMAC[1*] A. Usame TAMER[2]

[1] Eskisehir Osmangazi University, Science and Arts Faculty, Department of Biology, Eskisehir, Turkey

[2] Celal Bayar University, Science and Arts Faculty, Department of Biology, Manisa, Turkey

* This paper is constitues PhD thesis of the first author

*Corresponding Author
e-mail: myamac@ogu.edu.tr

Received: December 24, 2008
Accepted: February 28, 2008

Abstract

Lignin has connective effect between cell wall components. Therefore, lignin polymer leads to less biological usefulness of substrate. In this study, it has been aimed to decrease lignin proportion in wheat straw by selected actinomycetes strains. For this purpose, the effect of different actinomycetes strains which incubated during six weeks with solid state and submerged culture systems on wheat straw have been investigated. Besides, actinomycetes strains and controls have been compared for lignin loss and production of "Acid Precipitable Polymeric Lignin" (APPL) values. A strong correlation was observed between lignin loss from substrate and APPL production in both culture systems. The maximum lignin loss values for solid state and submerged cultures by *Streptomyces* 07 – 05 strain were determined as 53.22% and 61.03%, respectively. After 3 weeks of growth, the APPL production by this strain reached maximum, 65.52 and 84.12 mg/g substrate for solid state and submerged culture, respectively. It has been determined that, in both of culture systems, amount of APPL which was produced by *Phanerochaete chrysosporium* did not reach any of actinomycetes strains' values.

Key words: Actinomycetes, APPL, Lignin, Wheat Straw

INTRODUCTION

One of the most important natural and renewable sources is lignocelluloses. The amount of lignocelluloses produced by plants is estimated as 10 – 50 x 10^9 ton / year [1, 2]. Lignocelluloses is composed of three polymer; lignin (10-30%, dry weight), cellulose (25-45%, dry weight) and hemicellulose (24-50%, dry weight) [3]. Lignin is a complex aromatic polymer which physically surrounds and protects cellulose and hemicelluloses from enzymatic hydrolysis and is resistant to microbial degradation. In this case, effective uses of lignocellulose depend on delignification of plant material. Only some microorganisms such as white rot fungi and actinobacteria are capable of degrading lignin by an oxidative process.

Actinobacteria are a group of biodegradative bacteria which make an important contribution to degradation of plant biomass and nutrient recycling in soil and composts [4]. Several actinomycetes strains have been shown to be able to degrade the polymeric components of lignocellulosic biomass. A water soluble modified polymeric lignin termed "acid–precipitable polymeric lignin" (APPL) is produced as a major lignin degradation product by *Streptomyces viridosporus* T7A [5] and other actinomycetes such as *Thermomonospora* and other *Streptomyces* spp. [6, 7]. A direct correlation between lignin loss from substrate and APPL production was reported during lignocellulose degradation by *S. viridosporus* T7A [8].

Lignin biodegradation studies almost completely focused on white rot fungi (WRF). Actinomycetes are similar to filamentous fungi because of their mycelial structure. They are adapted to the colonization of solid substrates such as plant biomass due to their mycelial nature and are an excellent source of lignocellulose-degrading strains [9]. It is thought that native lignin degradation by actinomycetes is different from that of fungi and is associated with primary growth rather than during starvation [6]. These microorganisms have many potential biotechnological applications such as delignification in pulp and paper industry, textile dye bleaching, bioremediation, and so forth. Therefore in this report a hundred actinomycetes strains were screened for the lignin degradation and APPL production from wheat straw.

MATERIALS AND METHODS

Microorganisms

Actinomycetes strains were isolated from different wheat field soil samples by plating on Starch Casein Medium and Yeast Extract Malt Extract Medium (ISP2) supplemented with filter sterilized nystatin and cyclohehimide (50 µg/ml). Single colonies were subcultured on ISP 2 medium and the strains were maintained as spore suspensions and hyphal fragments in 15% glycerol (vol/vol) at – 20 ºC until used.

Phanerochaete chrysosporium ME 446 which is used as positive control is obtained from Hacettepe University, Science Faculty, Biology Department.

Wheat straw pretreatment

Wheat straw was dried, ground to pass a 40 mesh screen, and then sequentially extracted with hot water, benzene: ethanol (1:1), ethanol, hot water and then the residue was dried [10]. Wheat straw in 250-ml flasks was autoclaved for 1 h and then

the flasks were plugged with cotton stoppers and autoclaved for an additional 20 min at 1 atm. [11].

Screening of ligninoytic activity

To determine which of the strains has higher ligninolytic activity, amount of produced APPL was measured as turbidometric way. Tween 80 spore suspension (0.1%, vol/vol) of strains was used to inoculate flask containing 100 ml defined fermentation medium (FM): Extracted wheat straw 2.00 g, Yeast extract 6.00 g, Na_2HPO_4 5.30 g, KH_2PO_4 1.98 g, $MgSO_4$. $7H_2O$ 0.20 g, NaCl 0.20 g, $CaCl_2$. $2H_2O$ 0.05 g, Pridham and Gottlieb's trace elements suspension 1.00 ml, distilled water 1000 ml, pH 7.1 – 7.2 [12]. Cultures were incubated at 37ºC, 100 rpm for 7 days [13]. To determinate APPL amount produced by strains, samples were taken and separated by filtration onto filter paper (Whatman No:1) at daily periods [14] Then pH of supernatant was adjusted to 1 - 2 by adding 0.1 ml 12 M HCl per 2 ml culture medium [5]. The amount of APPL was measured by recording the increase in absorbance at 600 nm over a 5-minute period following acidification [10, 15]. Four strains which produced maximum APPL were selected for submerged and solid state culture studies.

Submerged and solid state cultures

Inoculum preparation for both culture types was performed as recommended by Shirling and Gottlieb [16]. Actinomycetes strains were allowed to grow in tripton yeast broth for 48 – 72 hours. After incubation period, cultures were divided into 5 ml portion and transferred into sterile centrifuge tubes equipped with sterile caps. This culture portion was centrifuged at 1600 x g. The precipitated cells were washed with sterile distilled water (or sterile 0.85% NaCl) two times. A sterile 5.0 ml cell suspension was used for inoculation of submerged and solid state cultures.

For submerged culture systems, flasks were inoculated by 5 ml of inoculum and were incubated at 37°C during 4 days in humid incubator. After preincubation on wheat straw, 100 ml fermentation medium was added to cultures and was incubated at 37°C for 6 weeks. Flasks which added 5.0 ml SDW instead of inoculum was used as negative controls. Solid state culture conditions were the same with those of submerged cultures except adding 100 ml fermentation medium.

Phanerochaete chrysosporium ME 446 was used as positive control in both culture systems. *Phanerochaete chrysosporium* fermentation medium (Yeast extract 1.00 g, Na_2HPO_4 5.30 g, KH_2PO_4 1.98 g, $MgSO_4$. $7H_2O$ 0.20 g, $CaCl_2$. $2H_2O$ 0.05 g, Pridham and Gottlieb' s trace elements solution 1.00 ml, Distilled water 1000 ml, pH 4.5) was inoculated with spore suspension (2 x 10^6 spore per 5 ml). Other cultural conditions were the same with those of actinomycetes strains.

Samples were taken and analyzed for lignocellulosic material, lignin, APPL, total carbohydrate, nitrogen and ash contents of samples weekly during incubation period.

Analytical methods

For the harvest of each submerged culture, a flask was steamed at 100°C for 1 hour and the residue was collected by filtration onto preweighed filter paper (Whatman No:54) and then was washed with hot water [5]. Harvest of solid state culture was performed by adding 100 ml SDW to each flask which was applied same treatment with those of submerged cultures. To determine lignocelluloses weight loss, harvested insoluble residues on the filters were dried and reweighed. Samples were homogenized by milling and stored at glass jar until used for other analytical methods.

To determine APPL produced in submerged and solid state cultures, culture filtrate was acidified to pH 1-2 with 12 M HCl. The resulting precipitate was collected by centrifugation (16.000 g, 30 min). After discarding the supernatant, the pellet was washed twice with acidic water, dried at 50°C for 24-48 hr and, was weighted. From these data, produced APPL per gram substrate was calculated for incubation period [10, 17, 18].

The lignin, carbohydrate, and organic nitrogen content of wheat straw were determined by the modified Klason lignin procedure, modified Somogyi-Nelson carbohydrate assay and the micro-Kjeldahl method, respectively [10]. To lignin assay, 50 mg of lignocellulose residue was treated one milliliter concentrated H_2SO_4 for 1 hour with occasional mixing. Then, 28 ml of distilled water was added and the suspension was autoclaved for 1 hour. After cooling, acid insoluble material was collected by preweighed filter paper and after several washes with distilled water, it was dried at 70ºC. , The lignin containing filter paper was reweighed to determine the amount of acid-insoluble Klason lignin in residue.

The filtrate of Klason lignin analysis was used for the carbohydrate assay using a modified Somogyi-Nelson procedure. Firstly, 18 ml 2 N NaOH is added to each filtrate. The volume of each solution was brought up to 50 ml and assay solution was added to the filtrate. Then the filtrate was placed in a boiling water bath for 1 hour and cooled to 4ºC. After the Nelson reagent was added to the filtrate, the entire solution was mixed by vortexing and 10 ml distilled water was added to the solution. Its optical density was determined at 500 nm and then the total reducing sugar amount in Klason lignin filtrate was determined from a standard curve. The amount of amino nitrogen in residue was determined using micro-Kjeldahl assay by Eskisehir Control Laboratory of Agriculture Ministry. Ash content for each sample was determined by combustion of about 100 mg samples at 500°C [5].

The lignin and reducing sugar contents of residues were calculated as a percentage of the initial weight of the sample assayed. Values of lignin and reducing sugar loss were calculated by using the general formula;

Lignin or reducing sugar loss (%) = 100 - (a/b x 100)

where a: lignin or reducing sugar content of residue (mg); b: initial lignin or reducing sugar content of sample (mg). All analysis was performed as two parallels and the results were calculated by average of two parallels.

Taxonomic grouping of strains

International Streptomyces Project (ISP) media and conditions were used for physiological and morphological characterization of the strains [16]. Selected actinomycetes strains were tested for spore chain morphology, color of aerial and submerged mycelia, production of diagnostic soluble pigment and melanin, growth of different culture media, antimicrobial activity against test microorganisms, biochemical

tests, resistance to antibiotics, growth at different conditions, growth in the presence of different chemicals, growth on sole carbon and nitrogen sources. Pigment colors of the strains were assessed in natural daylight and were compared with Inter-Society Color Council National Bureau of Standards (ISCC-NBS) Color Name Charts (United States Department of Commerce, Gaithersberg, Maryland, U.S.A.).

For the chemotaxonomic analysis of the cell wall, the strains were grown in triptone yeast extract broth for a week and harvested bacterial mycelia was washed twice with sterile distilled water (SDW) and dried. Diaminopimelic acid isomer analysis of whole-cell hydrolysates followed previously described procedures [19].

To assess statistical significance, all results were subjected to a one-way analysis of variance (ANOVA) and Tukey post hoc test comparison of the means, $P< 0.05$, using SPSS.

RESULTS

An extended screening for lignin degradation of wheat straw was performed among a hundred newly isolated actinomycetes strains. Four of the screened actinomycetes strains (*Streptomyces* 07-03, 07-05, 09-04 and 10-04) were selected because of their ability to produce APPL from wheat straw in culture media. The overall lignocellulose-decomposing ability of each strain was determined in long-term (6 weeks) growth experiments. The recoveries of APPL production and lignin loss from substrate for solid state and submerged culture systems are summarized in Table 1 and Table 2, respectively.

The time course for production of APPL and loss of lignin during the growth of *Streptomyces* 07-05 and *P. chrysosporium* in submerged and solid state cultures is shown in Figure 1. For *Streptomyces* 07 – 05 strain, the maximum lignin loss values for solid state and submerged culture were obtained after 3 and 6 weeks of growth, respectively. After 3 weeks of incubation, the APPL production reached maximum, 65.52 and 84.12 mg/g substrate for solid state and submerged culture, respectively.

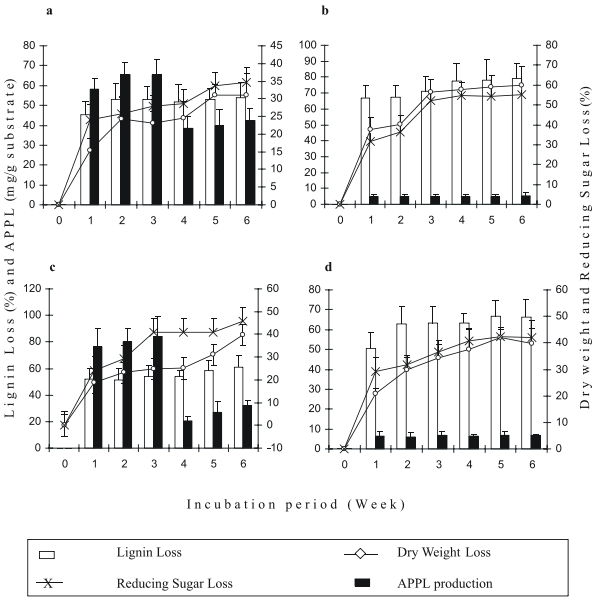

Figure 1. Lignin, dry weight and reducing sugar loss and APPLproduction during incubation period. a. Solid state culture of Streptomyces 07-05, b. Solid state culture of Phanerochaete chrysosporium, c. Submerged culture of Streptomyces 07-05, d. Submerged culture of Phanerochaete chrysosporium.

Table 1. Lignin loss (%) and APPL Production (mg/g subsrate) activities of actinomycetes strains and controls for solid state fermentations during incubation period

Groups	Incubation period (Week)											
	1		2		3		4		5		6	
	LL[a]	APPL	LL	APPL	LL	APPL	LL	APPL	LL	APPL	LL	APPL
Streptomyces 07 - 03	28.31 ± 2.4a	39.32 ± 5.3a	30.99 ± 4.3a	45.34 ± 6.1a	33.08 ± 4.5a	48.30 ± 7.4a	35.43 ± 5.9a	50.28 ± 6.7a	37.13 ± 5.4a	52.96 ± 8.7a	38.83 ± 8.1a	56.00 ± 5.1a
Streptomyces 07 - 05	45.30 ± 6.7b	58.00 ± 5.2b	53.17 ± 7.8b	65.50 ± 6.1b	53.22 ± 6.4b	65.52 ± 7.8b	51.65 ± 8.8b	38.50 ± 6.1b	53.02 ± 5.6b	40.00 ± 8.0b	53.80 ± 7.6b	42.30 ± 6.2b
Streptomyces 09 - 04	15.49 ± 3.2c	18.8 ± 2.4c	15.68 ± 3.2ce	19.52 ± 3.3c	16.40 ± 2.1c	20.66 ± 3.1c	16.74 ± 5.7c	20.56 ± 3.9c	17.00 ± 5.1c	22.08 ± 6.2c	16.74 ± 4.2c	23.94 ± 5.2c
Streptomyces 10 - 04	6.77 ± 2.0c	13.82 ± 3.0d	7.39 ± 1.6c	14.68 ± 2.1c	8.45 ± 2.4c	15.70 ± 3.5c	9.67 ± 3.2c	16.92 ± 4.1c	9.93 ± 2.4c	17.92 ± 5.1c	10.21 ± 2.3c	18.30 ± 3.2c
P. chrysosporium	66.85 ± 8.1d	4.80 ± 1.4e	67.14 ± 7.6d	4.80 ± 0.9d	70.95 ± 9.0d	4.95 ± 1.1d	77.62 ± 11.2d	4.70 ± 1.1d	77.95 ± 13.2d	5.00 ± 1.6d	78.99 ± 9.8d	5.20 ± 2.1d
Negative Control	5.18 ± 1.9e	3.86 ± 1.0f	5.31 ± 1.4e	4.00 ± 0.6e	5.55 ± 1.2c	3.55 ± 1.0d	4.67 ± 1.1c	3.77 ± 0.8c	6.43 ± 1.6c	3.84 ± 1.1c	6.26 ± 2.1c	3.80 ± 2.0d

[a] Mean ± S.D., one way ANOVA. Values in each column followed by the same letter do not differ significantly. LL: Lignin loss

Table 2. Lignin loss (%) and APPL Production (mg/g subsrate) activities of actinomycetes strains and controls for submerged fermentations during incubation period

Groups	Incubation period (Week)											
	1		2		3		4		5		6	
	LL[a]	APPL	LL	APPL	LL	APPL	LL	APPL	LL	APPL	LL	APPL
Streptomyces 07 - 03	39.31 ± 5.5a	58.82 ± 12.7a	38.72 ± 8.7a	64.16 ± 5.6a	38.82 ± 8.2a	66.20 ± 7.5 a	41.17 ± 2.7a	70.00 ± 5.3a	41.76 ± 7.8a	70.28 ± 10.9a	42.80 ± 6.3a	73.36 ± 9.7a
Streptomyces 07 - 05	52.27 ± 8.1a	76.28 ± 14.3b	51.49 ± 8.6b	80.50 ± 9.7b	54.15 ± 8.4b	84.12 ± 14.7b	54.00 ± 4.5b	20.68 ± 3.1b	58.14 ± 8.1b	27.18 ± 8.2b	61.03 ± 8.7b	32.02 ± 3.6b
Streptomyces 09 - 04	18.91 ± 4.3b	24.52 ± 4.2c	19.09 ± 7.2c	26.82 ± 9.6c	18.33 ± 3.2c	27.60 ± 9.3c	18.43 ± 6.1c	25.86 ± 1.9	18.32 ± 4.5c	25.52 ± 6.8b	18.25 ± 5.2c	26.08 ± 8.4c
Streptomyces 10 - 04	8.42 ± 3.1bc	15.92 ± 4.3d	9.95 ± 2.7ce	17.24 ± 5.1c	10.61 ± 3.1c	19.88 ± 7.2c	11.97 ± 2.6c	23.38 ± 4.2bc	12.38 ± 2.3cd	24.18 ± 5.3b	12.83 ± 3.2cs	25.80 ± 7.2c
P. chrysosporium	50.62 ± 9.8a	6.56 ± 2.5e	63.00 ± 6.5d	6.18 ± 1.8d	63.12 ± 6.5b	6.65 ± 2.3d	63.37 ± 3.6d	6.48 ± 1.0d	66.54 ± 9.4b	6.64 ± 2.0c	66.47 ± 9.0b	6.80 ± 0.6d
Negative Control	5.27 ± 0.9c	5.08 ± 2.2 e	5.18 ± 1.8e	5.10 ± 1.2d	6.04 ± 1.7c	5.20 ± 0.7d	6.59 ± 2.3 c	5.30 ± 0.9d	6.78 ± 1.2d	5.26 ± 1.8c	7.32 ± 1.1d	5.30 ± 1.2d

[a] Mean ± S.D., one way ANOVA. Values in each column followed by the same letter do not differ significantly.LL: Lignin loss

Table 3. Physiological, biochemical and morphological properties of the strains

Character	(w/v)	Strains			
		07 - 03	07 - 05	09 - 04	10 - 04
DEGRADATION and BIOCHEMICAL TESTS					
Starch	1	+	+	+	+
Tyrosine	0.5	-	-	-	-
Gelatin	0.4	-	-	-	+
Nitrate reduction	0.2	+	+	+	-
DNase	0.2	+	+	+	-
Urea hyrolysis	2	-	-	-	-
Hydrogen sulphite production		+	+	+	+
PHYSIOLOGICAL TESTS					
Growth at:					
4 °C		-	-	-	-
37 °C		+	+	+	+
pH : 4		+	+	+	+
Growth in the presence of:					
Crystal violet	0.001	-	-	-	-
Phenol	0.1	-	-	-	-
Potassium tellurite	0.001	+	+	+	+
Potassium tellurite	0.01	-	+	-	+
Sodium azide	0.01	+	+	+	-
Sodium azide	0.02	+	+	+	-
Sodium chloride	4	+	+	+	+
Sodium chloride	7	-	-	-	-
Sodium chloride	10	-	-	-	-
Sodium chloride	13	-	-	-	-
Resistance to antibiotics: (µg/ml)					
Lincomycin	100	-	+	+	+
Streptomycin sulphate	100	-	-	-	-
Rifampicin	50	+	+	+	-
Tobramycin	50	-	-	-	-
Vancomycin	50	-	-	-	-
Penicillin G	10 i.u	+	+	+	+
Antimicrobial activity against:					
Bacillus subtilis NRRL B-744		-	-	-	-
Escherichia coli NRRL B-3704		-	-	-	-
Micrococcus luteus NRRL B-4376		-	-	-	-
Pseudomonas fluorescens NRRL B-10		-	-	-	-
Streptomyces murinus ISP 5091		-	-	-	-
Candida albicans CBS 562		-	-	-	-
Saccharomyces cerevisiae CBS 1171		-	-	-	+
NUTRITIONAL TESTS					
Growth on sole carbon sources:					
Adonitol	1	+	+	-	+
D - Fructose	1	+	+	+	+
D - Galactose	1	+	+	+	+
D - Xylose	1	+	+	+	+
D - Lactose	1	+	+	+	+
D - Mannose	1	+	+	+	+
Inulin	1	+	+	+	+
L - Arabinose	1	+	+	+	+
L - Ramnose	1	+	+	+	+
D - Mannitol	1	+	+	+	+
meso - Inositol	1	+	+	+	-
Raffinose	1	+	+	+	+
Salicin	1	+	+	+	+
Sucrose	1	+	+	+	+
Sodium acetate	0.1	+	+	+	+
Sodium citrate	0.1	-	-	+	-
Growth on sole nitrogen sources:					
L - Arginine	0.1	+	+	-	-
L - Phenylalanine	0.1	+	+	+	+
L - Hystidine	0.1	+	+	+	+
L - Serin	0.1	+	+	+	+
L - Valine	0.1	+	+	+	+
Potassium nitrate	0.1	+	+	+	+

All of the studied strains showed L, L-diaminopimelic acid as diagnostic diamino acid in their peptidoglycan. This amino acid distinguishes *Streptomyces* species from all other actinomycetes [20, 21]. Chemotaxonomic and morphological examinations placed the studied four strains in the *Streptomyces* genus. These strains were further characterized for their several properties proposed by Williams et al. [22] (Table 3).

DISCUSSION

Lignin is known as polymer which gives plants rigidity and binds plant cells together in such a manner as to impart resistance towards impact, bending, and compression [23]. Besides, lignin has been reported to protect other cell wall constituents against microbial degradation [24]. Therefore, biological degradation of lignin is accepted as the most important research parameter. In the present study, a hundred actinomycetes strains were screened for their lignin degradation and APPL production capabilities from wheat straw. The ability of strains to degrade lignin was assessed by monitoring lignin losses and APPL production from substrate during growth on wheat straw. Lignin loss percents of actinomycetes strains for solid state and submerged culture systems have changed according to strain types.

It was pointed out that *Streptomyces* species can be used for lignin degradation under solid state conditions. For example, *S. cyaneus* was used to improve the qualities of pulp paper after 2

weeks of incubation [25]. In the present study, it was determined that 45.30% of initial lignin was degraded by *Streptomyces* 07 - 05 strain at the first growth stage under solid state system. At the same stage, lignin contents of substrates have leaned towards reduction. *Streptomyces* 07 - 03 and 07 - 05 strains depleted lignin more extensively than other actinomycetes ($P< 0.05$). On the other hand 78.99% of initial lignin was degraded with solid state culture system by *Phanerochaete chrysosporium* which was used as positive control. None of the actinomycetes strains has reached this value. It was determined that the most nearest value belonged to 07 - 05 strain with 53.80% degradation ratio. As a hopeful result, this strain caused higher depletion of lignin from previously reported *S. griseus* strain (10.5% for softwood and 23.4% for hardwood) under solid state culture system [26].

Streptomyces 07 - 03 and 07 - 05 strains were more successful than others in submerged culture system also. At the end of the 6-week incubation period *Streptomyces* 07 - 03 and 07 - 05 strains, *Phanerochaete chrysosporium* and negative controls have caused 42.80%, 61.03%, 66.47% and 7.32% lignin loss from substrate, respectively. When lignin and carbohydrate losses data compared (Fig. 1), *Streptomyces* 07 - 05 strain exhibited a preference for the lignin component over the carbohydrate component of the wheat straw lignocellulose.

While *Phanerochaete chrysosporium* gave higher lignin loss percents at solid state culture system, actinomycetes strains were more successful at submerged culture system. A lot of studies have reported lignin degradation ability of actinomycetes strains since 1978 [27]. In other studies used Graminae as substrate [14, 17, 28-30], lignin degradation ratios were determined between 3.5% and 52.0%. Therefore, *Streptomyces* strains used in this study could be accepted as significantly better lignin decomposers as compared with the previously studied actinomycetes strains.

The APPL production amounts of actinomycetes strains for solid state fermentations during 6-week incubation period were determined between 18.30 – 56.00 mg/g substrate. Negative and positive controls produced 3.80 and 5.20 mg APPL/g substrate, respectively. *Streptomyces* 07 - 03 and 07 - 05 strains which have caused the highest lignin loss from substrate have also proved more successful than other actinomycetes strains for APPL accumulation. Amount of APPL produced by *Phanerochaete chrysosporium* has not reached any of the actinomycetes strains' values. Whereas we determined that *Phanerochaete chrysosporium* could degrade lignin more than all of the actinomycetes strains ($P< 0.05$). These data have confirmed that actinomycetes have different lignin degradation mechanisms from fungi [4, 6, 29, 30].

The APPL production amounts of actinomycete strains for submerged culture system during 6-week incubation period were determined between 25.80 – 73.36 mg/g substrate. These values are higher than those of solid state fermentations. APPL production difference between culture systems could be accepted as reflection of difference between lignin degradation ratios. In the first two weeks of the growth, a dramatic increase in both lignin loss and APPL accumulation was observed in both culture systems. At the end of the first incubation week 52.27% lignin loss has been synchronized with 76.28 mg/g substrate APPL production by 07 - 05 for submerged culture system. It was reported that amount of APPL produced by eight different actinomycetes strains was determined between 12.00 – 55.00 mg/g substrate [31]. Therefore it can be declared that our actinomycetes strains have been partially successful for this property.

Although some reports have suggested that APPL production may not correlate with lignin degradation [4, 30], our data have supported studies that report APPL as a product of lignin degradation [5, 8, 12, 13, 15]. Increased activities of xylanases [32] and cellulase [33] were also reported in APPL-overproducing strains. It was determined that several actinomycetes strains produce APPL like product from lignin [6, 34, 35] and they have similar degradation mechanisms [7, 8, 36]. All of the lignin degradable strains produce APPL like product [12, 31] and APPL is not produced when lignocellulose is absent [5]. Besides, a correlation was also determined between APPL production and lignin loss [8]. According to these data, APPL production may be accepted as an evidence for lignin degradation activity of actinomycetes [37].

Formation of water soluble polymeric lignin fragments (APPL) during lignin degradation by *Streptomyces viridosporus* findings [5] is one of the milestones of lignin degradation studies. Difference of lignin degradation mechanisms of actinomycetes has acquired them some advantages. APPL is formed by actinomycetes as main product of lignin degradation which is a major difference from white rot fungi [8, 36]. It was reported that if APPL can be produce at large scale and economically, it has great economic importance [35]. It can be used as surfactant [5, 37, 38], polyurethane [5] and for adhesive and resin production [5, 39]. Besides, it is known for its antioxidant [37, 40] and immunoadjuvant [38] properties. There are some studies which confirm that lignin degradation and/or APPL production potentials can be enhanced via genetic manipulation [6, 28, 33]. Because of decrease in incubation time and place, submerged culture system has been proposed for economical APPL production [40]. Our actinomycetes strains' higher APPL production at submerged culture system confirms these suggestions. Crawford et al. (1983) reported higher than 30% lignin content of substrate converted to APPL [5]. In the present study these amounts were 48.20% and 42.04% for 07 - 05 and 07 - 03 strains, respectively. *P. chrysosporium* gives relatively lower lignin degradation ratio and negligible APPL production at submerged culture system. These properties of *P. chrysosporium* seem to have also increased the attractiveness of studies about actinomycetes strains at submerged culture system. In this context, we think that our 07 - 03 and 07 - 05 strains are successful for APPL production in both culture systems and they could be used for this purpose.

Berrocal et al. (2000) suggested that laccase was involved in the solubilization and mineralization of lignin from wheat straw by *Streptomyces* strains [41]. This enzyme has a great potential for biotechnological applications in areas such as biobleaching, increasing the strength of cellulose fibers, textile dye bleaching and bioremediation [42]. Laccase activity has been demonstrated in different species of *Streptomyces* such as *S. cyaneus* [41], *S. coelicolor* [43], *S. lavendulae* [44] and *S. psammoticus* [45].

The degradation of lignin in lignocelluloses by WRF has received much attention from several research groups. Actinomycetes have the same filamentous habit as fungi and provide an alternative system for lignin degradation that may have biotechnological advantages; in particular, a water soluble product (APPL) is formed, the degradation occurs during active growth rather than during secondary metabolism and they do not need nitrogen and sulphur starvation and high oxygen concentration for lignin degradation. Therefore *Streptomyces* 07-05 strain seems to have some major advantages for biotechnological applications. Future works will focus on production of the lignin degrading enzymes and biotransformation of lignin related aromatic compounds to valuable chemicals by this strain.

Acknowledgements

This research was supported in part by Scientific Research and Application Centre (FBAM) of Eskisehir Osmangazi University.

REFERENCES

[1] Kuhad RC, Singh A. 1993. Lignocellulose biotechnology: Current and future prospect, Critical Reviews in Biotechnology. 13: 151-172.

[2] Pedlar SL, Betts WB, Dart RK. 1993. Extracellular protein production by *Thermomonospora mesophila* grown on lignin-related compounds, Microbios. 74: 89-94.

[3] Betts WB, Dart RK, Ball AS, Pedlar SL. 1992. Biosynthesis and structure of lignocellulose. In: Biodegradation: Natural and Synthetic materials, (ed. Betts WB), pp. 139-155. Springer Verlag, London.

[4] Mc Carthy AJ, Williams ST. 1992. Actinomycetes as agents of biodegradation in the environment- a review. Gene. 115: 189-192.

[5] Crawford DL, Pometto III AL, Crawford RL. 1983. Lignin degradation by *Streptomyces viridosporus*: Isolation and characterization of a new polymeric lignin degradation intermediate. Applied and Environmental Microbiology. 45: 898-904.

[6] Mc Carthy AJ, Broda P. 1984. Screening for lignin-degrading Actinomycetes and characterization of their activity against [^{14}C] lignin labelled wheat lignocellulose. Journal of General Microbiology. 130: 2905-2913.

[7] Mc Carthy AJ, Paterson A, Broda PMA. 1986. Lignin solubilization by *Thermomonospora mesophila*. Applied Microbiology and Biotechnology. 24: 347-352.

[8] Borgmeyer JR, Crawford DL. 1985. Production and characterization of polymeric lignin degradation intermediates from two different *Streptomyces* spp. Applied and Environmental Microbiology. 49: 273-278.

[9] McCarthy AJ. 1987. Lignocellulose-degrading actinomycetes. FEMS Microbiology Letters. 46: 145–163.

[10] Crawford DL, Pometto III AL. 1988. Acid-precipitable polymeric lignin: Production and analysis. Methods in Enzymology. 161: 35-47.

[11] Rodriguez J, Hernandez-Coronado MJ, Hernandez M, Bocchini P, Galletti GC, Arias ME. 1997. Chemical characterization by pyrolysis/gas chromatography/mass spectrometry of acid-precipitable polymeric lignin (APPL) from wheat straw transformed by selected *Streptornyces* strains. Analytica Chimica Acta. 345: 121-129.

[12] Pasti MB, Pometto III AL, Nuti MP and Crawford DL. 1990. Lignin-solubilizing ability of Actinomycetes isolated from termite (Termitidae) gut. Applied and Environmental Microbiology. 56: 2213-2218.

[13] Crawford DL, Pometto III AL. 1988. Lignin depolymerizing activity of *Streptomyces*. Methods in Enzymology. 161: 249-258.

[14] Zimmermann W, Broda P. 1989. Utilization of lignocellulose from barley straw by Actinomycetes. Applied Microbiology and Biotechnology. 30: 103-109.

[15] Trigo T, Ball AS. 1994. Is the solubilized product from the degradation of lignocellulose by Actinomycetes a precursor of humic substances? Microbiology. 140: 3145-3152.

[16] Shirling EB, Gottlieb D. 1966. Methods for characterization of *Streptomyces* species. International Journal of Systematic Bacteriology. 16: 313-340.

[17] Antai SP, Crawford DL. 1981. Degradation of softwood, hardwood and grass lignocelluloses by two *Streptomyces* strains. Applied and Environmental Microbiology. 42: 378-380.

[18] Pometto III AL, Crawford DL. 1986. Effects of pH on lignin and cellulose degradation by *Streptomyces viridosporus.* Applied and Environmental Microbiology. 52: 246-250.

[19] Lechevalier MP, Lechevalier H. 1970. Chemical composition as a criterion in the classification of aerobic actinomycetes. International Journal of Systematic Bacteriology. 20: 435-443.

[20] Goodfellow M. 1989. Suprageneric classification of Actinomycetes. In: Bergey's Manual of Systematic Bacteriology, (eds. Williams ST, Sharpe ME, Holt JG), vol. 4. pp. 2333–2339, Williams and Wilkins, Baltimore.

[21] Anderson S, Wellington EMH. 2001. The taxonomy of *Streptomyces* and related genera. International Journal of Systematic and Evolutionary Microbiology. 51: 797–814.

[22] Williams ST, Goodfellow M, Alderson G, Wellington EMH, Sneath PHA, Sackin MJ. 1983. Numerical classification of *Streptomyces* and related genera. Journal of General Microbiology. 129: 1743-1813.

[23] Crawford DL, Crawford RL. 1976. Microbial degaradtion of lignocellulose: The lignin component. Applied and Environmental Microbiology. 31: 714-717.

[24] Topal Ş, Kırat E. 1993. Samanın mikrobiyolojik delignifikasyonunda farklı inokulum teknikleri ve test organizmalarının etkileri. Doğa Türk Biyoloji Dergisi. 17: 133-147.

[25] Berrocal M, Ball AS, Huerta S, Barrasa JM, Hernandez M, Perez-Leblic MI, Arias ME. 2000. Biological upgrading of wheat straw through solid-state fermentation with *Streptomyces cyaneus*. Applied Microbiology and Biotechnology. 54:764–771.

[26] Arora A, Nain L, Gupta JK. 2005. Solid-state fermentation of wood residues by *Streptomyces griseus* B1, a soil isolate,and solubilization of lignins, World Journal of Microbiology and Biotechnology. 21: 303–308.

[27] Crawford DL, 1978. Lignocellulose decomposition by selected *Streptomyces* strains. Applied and Environmental Microbiology. 35: 1041-1045.

[28] Crawford DL. 1981. Microbial conversions of lignin to useful chemicals using a lignin-degrading *Streptomyces*. Biotechnology and Bioengineering Symposium. 11: 275-291.

[29] Barder MJ, Crawford DL. 1981. Effects of carbon and nitrogen supplementation on lignin and cellulose decomposition by a *Streptomyces.* Canadian Journal of Microbiology. 27: 859-863.

[30] Crawford DL, 1988. Biodegradation of agricultural and urban wastes, In: Actinomycetes in Biotechnology (eds. Goodfellow M, Williams ST, Mordarski M), pp. 433-459. Academic Press, London.

[31] Ball AS, Godden B, Helvenstein P, Penninckx MJ, Mc Carthy A.J. 1990. Lignocarbohydrate solubilization from straw by actinomycetes. Applied and Environmental Microbiology. 56: 3017-3022.

[32] Adhi TP, Korus RA, Crawford DL. 1989. Production of major extracellular enzymes during lignocellulose degradation by two Streptomycetes in agitated submerged culture. Applied and Environmental Microbiology. 55: 1165-1168.

[33] Deobald LA, Crawford DL. 1987. Activities of cellulaseand other extracellular enzymes during solubilization by *Streptomyces viridosporus*. Applied Microbiology and Biotechnology. 26: 158-163.

[34] Mc Carthy AJ, Mac Donald MJ, Paterson A, Broda PMA. 1984. Degradation of against [^{14}C] lignin labelled wheat lignocellulose by white-rot fungi. Journal of General Microbiology. 130: 1023-1030.

[35] Gonzalez B, Merino A, Almedia M, Vicuna R. 1986. Comparative growth of natural bacterial isolates on various lignin-related compounds. Applied and Environmental Microbiology. 52: 1428-1432.

[36] Vicuna R. 1988. Bacterial degradation of lignin. Enzyme and Microbial Technology. 10: 646-655.

[37] Mason JC, Birch OM, Broda P. 1990. Preparation of ^{14}C-radiolabelled lignocelluloses from spring barley of differing maturities and their solubilization by *Phanerochaete chrysosporium* and *Streptomyces cyaneus*. Journal of General Microbiology. 136: 227-232.

[38] Crawford DL, Kurdna DA, Pometto III AL. 1986. Lignocellulose bioconversion by *Streptomyces*: Immunoadjuvant properties of a microbially modified lignin polymer. Biotechnology and Bioengineering Symposium. 17: 253-259.

[39] Crawford DL, Pettey TM, Thede BM, Deobald LA. 1984. Genetic manipulations of ligninolytic *Streptomyces* and generation of improved lignin-to-chemical bioconversions strains. Biotechnology and Bioengineering Symposium. 14: 241-256.

[40] Pometto III AL, Crawford DL. 1986. Effects of pH on lignin and cellulose degradation by *Streptomyces viridosporus*. Applied and Environmental Microbiology. 52: 246-250.

[41] Berrocal M, Rodriguez J, Ball AS,Perez-Leblic MI, Arias ME. 1997. Solubilisation and mineralisation of [14C]lignocellulose from wheat straw by *Streptomyces cyaneus* CECT 3335 during growth in solid-state fermentation. Applied Microbiology and Biotechnology. 48: 379–384.

[42] Arias ME, Arenas M, Rodriguez J, Soliveri J, Ball AS, Hernandez M. 2003. Kraft pulp biobleaching and mediated oxidation of a nonphenolic substrate by laccase from *Streptomyces cyaneus* CECT 3335. Applied and Environmental Microbiology. 69: 1953–1958.

[43] Machczynski MC, Vijgenboom E, Samyn B, Canters GW. 2004. Characterization of SLAC: a small laccase from *Streptomyces coelicolor* with unprecedented activity. Protein Science. 13: 2388–2397.

[44] Suzuki T, Endo K, Ito M, Tsujibo H, Miyamoto K, Inamori YA. 2003. Thermostable laccase from *Streptomyces lavendulae* REN-7: purification, characterization, nucleotide sequence and expression. Bioscience Biotechnology and Biochemistry. 67: 2167–2175.

[45] Niladevi KN, Sukumaran RK, Prema P. 2007. Utilization of rice straw for laccase production by *Streptomyces psammoticus* in solid-state fermentation. Journal of Industrial Microbiology and Biotechnology. 34: 665–674.