BIOLOGICAL PRE -TREATMENT USE LOCAL WILD STRAIN OF LIGNOLYTIC FILAMENTOUS BACTERIA TO IMPROVE *IN VITRO* DRY MATTER DIGESTABILITY AND REDUCTION LIGNIN CONTENT OF LOW QUALITY ROUGHAGES .

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ABSTRACT

This study being performed at the central laboratory for graduate students Animal Resources Department / College of Agriculture / University of Baghdad/Aljadrea and in laboratory of applied microbiology Department, Food and Biotechnology Center /Directorate of Agriculture Research / Baghdad, Iraq. Wild strain of local ligninolytic bacteria of genus *Streptomyces MS* isolated from Iraq camel degrade feces was used to pre- treated of low quality roughages in *in vitro* fermentation. Roughages wheat straw (WS), corncobs (Cc), alfalfa hay (AH) was pre- treated with wild strain of genus *Streptomyces MS*. The results showed higher significant improvement ($p<0.01$) for *in vitro* dry matter digestibility (IVDMD %),*in vitro* Organic matter digestibility (IVOMD %) for most of roughages (WS Cc, AH). Moreover, greatest ($p<0.01$) improvement in(IVDMD,IVOMD %) was observed with pre-treated AH using *Streptomyces MS* prior of use for 21 days from 45 untreated to 67.07,67.08% treated, the smallest improving was observed ($P<0.05$) with WS from 20.78 to 30.22%. This result indicated that ability of *Streptomyces MS* to improve (IVDMD %) was significantly ($P<0.05$) affected by the roughages sources. Treated roughages with *Streptomyces MS* showed significant reduction in lignin content ($p<0.01$).However, the highest ($p<0.01$) reduction in lignin content was shown in Cc pre-treated with *Streptomyces MS*(from 9.8 to 6.87%), while lowest ($P<0.05$)reduction in lignin content was shown in AH (from8.26 to 7.64%), These results support the possibility application of this wild strain of bacteria to improve low quality roughages in ruminants feed.

Key words:*Streptomyces*, roughages,IVDMD, lignin

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المعاملة البايولوجية المسبقة باستخدام عزلة محلية من البكتريا الخيطية *Streptomyces MS*المحللة للكنين لتحسين معامل الهضم المختبري للمادة الجافة وتقليل محتوى اللكنين للاعلاف الخشنة الرديئة النوعية

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المستخلص

اجريت هذه الدراسة في المختبر المركزي للدراسات العليا\ كلية الزراعة – جامعة بغداد ومختبر الاحياء المجهرية التطبيقية\ دائرة البحوث الزراعية– وزارة التعليم العالي والعلوم والتكنولوجيا المدمجة. لدراسة تاثير المعاملة المسبقة للاعلاف الخشنة مثل تبن الحنطة WS، كوالح الذرة Cc، دريس الجت AH) بالبكتريا المحلية المحللة للكنين التابعة الى جنس *Streptomyces MS* المعزولة من الفضلات المتحللة للجمال العراقية لمدة21 يوم قبل تجربة الهضم المختبري *in vitro*. اظهرت النتائج ان المعاملة ببكتريا *Streptomyces MS* ادت الى تحسين عالي المعنوية ($p<0.01$) في معامل الهضم المختبري للمادة الجافة IVDMD% ومعامل هضم المادة العضوية IVOMD% لكافة المخلفات الزراعية المستخدمة. لكن تفوقت معاملة دريس الجت (AH) بمستوى معنوية ($p<0.01$) في معامل الهضم المختبري للمادة الجافة والعضوية على باقي المعاملات من 45 الى 67.07و67.08% بنسبة تحسين 22.07و 22.08%، ومن ناحية اخرى عند دراسة تاثير المعاملتين على تخفيض محتوى جدار الخلية النباتية من اللكنين. لوحظ تاثير معنوي للعزلة على تقليل محتوى الاعلاف الخشنة من اللكنين، تفوقت معاملة كوالح الذرة بمستوى عالي المعنوية حيث خفضت اللكنين من 9.8 الى 6.87 % بنسبة تخفيض 2.93% واقل تخفيض كان باستخدام دريس الجت بالمقارنة مع الغير معاملة. نستنتج من ذلك ان معاملة الاعلاف الرديئة النوعية بالبكتريا الخيطية ادت الى تحسين معامل الهضم المختبري للمادة الجافة وتقليل محتوى اللكنين للاعلاف الخشنة.

الكلمات المفتاحية :*Streptomyces MS*، الاعلاف الخشنة، IVDMD، للكنين.

INTRODUCTION

Forages represent an important cost effective feed resource in ruminant nutrition. However, the relatively low quality of tropical forages combat against their use as the best feed for actively growing or high-performing ruminants and feed costs account for nearly 75% of the total input costs (24). Several experiments have been made to improve forages quality, including, chemicals or biological treatments. One of the important targets in this regard is to improve the fiber digestibility of the forages (3). the great important contribution of forages to animal feed is fiber. Sufficient fiber supplies is necessary to maintain the rumen mat; slowing the passage rate of feed also increases the amount of nutrients that can be digested and absorbed from the feed. Forages fiber also increases chewing and rumination and therefore increases the production of saliva, which conserve normal ruminal pH and decrease theincidence of ruminal acidosis (16), (22) Lignin very resistant to degradation, since most microorganisms do not possess enzyme systems that are capable of degrading lignin.(8). A wide range of microorganisms including bacteria and fungi is capable of producing cellulases and hemicellulases but only a limited number of these microorganisms are capable of producing lignin degrading enzymes. Lignolytic enzymes generally attack lignin directly (1), (21)(29).Pasti et al., (25) found that the bacteria like *Streptomyces* previously isolated from digestive tract termites (White ants) with great ability to lignining-solubilizing of lignocellulose-decomposing similar result (34). The problems of feeding low quality roughages to farmanimals are in general, low protein content, high crude fiber, low digestibility and the content of some anti-nutrition factors such as tannins and alkaloids (16).Thus,to improve the digestibility of these roughages , it is important to destroy the bonds between cellulose, hemicellulose and lignin (17). The aims of this current study areto: Examine the ability of wild local strain of filamentous bacteria *Streptomyces MS*(isolated from Iraq camel degrade feces)to Degrade various lignin and improve %IVDMD of roughages. The aim of this experiment was to investigate screening and isolation best microorganism capable of degrade lignin.

MATERIALS AND METHODS

Screening and isolation of ligninolytic microorganism

We tried to isolate microorganism species capable of metabolizing and solubilizing of phenolic monomers which may act as catalysts for forage fiber breakdown through increasing microbial access to cell wall polysaccharides.by Two isolation procedures were used. liquid-enrichment technique and a dilution pour plate technique(27)(26)(29).

Samples collection

Samples were collected using sterilized polyethylene bags as sources used for the isolation of lignin degrading microorganisms from domestic and wild rumen Iraqi animal degrade feces (Camel, Buffalo, Cow, Lama camel, Deer, and Horse)., samples were air dried and mixed thoroughly to increase homogeneity and kept at 5°C.

Isolation of lignin degrading bacteria

The medium Using MSM-L contain media

Chemical	Gram
Alkaline lignin	10
K_2HPO_4	4.55
KH_2PO_4	0.53
$MgSO_4$	0.5
NH_4NO_3	5.0
deionized water	1000ml

Were used for isolation .Media were supplemented with anti- fungal agents such as (cychloheximide[0.05mg/ml]andnystatin[200 U/ml]) .Plate was inoculated at 28^0C for 7 days (19). Pure cultures were cultivated on MSM-L slant until sporulation .Physiological characteristics were determined based on several biochemical tests such as catalase, oxidase, VogesProskaeur , methyl red, hydrolysis casein ,starch and gelatin(3),(7),(14),(28),(30),(32).

Roughages preparation: All roughages were chopped (approximate 1 mm) and divided into two treatments [Untreated roughages, Biological treatments roughages with *Streptomyces MS* for 21day(5).

Rumen collection: Ruminal contents used to prepare the treatment systems were collected from the rumen of slaughtered lamb. Collected ruminal fluids were strained through four layers of cheesecloth into separating tube previously gassed and flush these with CO_2

beforeuse.Thestrainedrumenfluidiskeptat39°C undercarbondioxideandshouldbepreparedjustbe forestartoftheincubation.Astheamountoffeedta kenis500mg,compositionofthemediumisaccordi ngto (21) ,(35).

Preparation artificial saliva: Phosphate-carbonate buffer (artificial salvia) was prepared as described by modified (3). Kept at 39°C. CO_2 was passed through the solution according to (7; 8) The pH of the artificial saliva was adjusted to 7.4 before use (2)(1).

***In vitro* digestibility procedures:** Approximately 1-2 g finely ground (particle size<1mm) samples of each roughages, was prepared as described by modified by (3) .Tubes were incubated at 39°C with periodic shaking for 72 h (11). After 72 h of incubation, fermentation was stopped by attrition of few drops of Hcl to each tube and kept under freezing. The contents of tubes were centrifuged at 2500 rpm for 15 minutes after which the supernatant is poured off. The insoluble residue was filtered off, dried according to standard method of AOAC (6), (12)(19). DM residues were recorded after drying and igniting to be subtracted from the sample content of DM to provide an estimate of lignin, DM digested, as described by (35).

Chemical analysis: Proximate chemical analysis of untreated and treated roughages in triplicate per each determination was carried out for dry matter (9). The *In Vitro* digestibility of DM,IVOMD for untreated and treated roughage was determined using the method of (18)(35).

Statistical Analysis: The statistical analysis system- SAS (31) program was used to effect of treatment (*Streptomyces MS*) on roughage IVDMD,IVOMD% and lignin content bass study parameters according to factorial experiment (3 x 2). Duncan (9) multiple range tests was used to significant compare between means In this study. Statistical model:

Yijk = μ + Ai + Bj + AB (ij) + eijk.

RESULTS AND DISCUSSION: The effects of pre-treated roughages with *Streptomyces MS* on IVDMD, IVOMD%. are shown in table 1. all roughages (WS,Cc,AH) treated with the *Streptomyces MS* shown significantly ($p<0.01$) increased in IVDMD,IVOMD%.However, the greatest improved in IVDMD,IVOMD% was showed high significant ($p<0.01$) with biological treated AH using *Streptomyces MS* from 45 to 67.07%, the average improvement was 22.07,22.08%,The smallest improving was observed ($P<0.01$) with WS from 20.78 to 30.22% average 9.44,9.46%. The current result indicated that the ability of *Streptomyces MS* to improve IVDMD,IVOMD % were affected by source or type of roughages ($p<0.01$) and that also referred to lignin content and lignification (13).This improving in IVDMD,IVOMD % due to capability of *Streptomyces MS* to degrade anti-nutritional factors, Similar results was obtained by Streeter et al., (33) who reported that treated roughages with *P. ostreatus* and *E. carotovora* ($P<0.05$) increased IVDMD from 32.7 to 47.7%. However, (16) found that treated wheat straw with six *white-rot fungi* improved IVDMD from 41.4 (control) to 59.2% .The improve of IVDMD% in decay WS. Was probably related to decrease of lignin content (13).It is known that some species of fungi increase WS IVDMD% such as *P. ostreatus*, whereas others decrease IVDMD% . (10) Reported treated WS with fungi significantly improvement IVDMD ($P<0.05$) from 28.1 to 40.3%, the lately report revealed that IVDMD improved ($P<0.05$) when WS per-treated using *basidiomycetes* white-rot fungi (21)this agrees with the report published by (36). Similar results were obtained by (4)(5). In contrast, Montañez-Valdez et al., (23) report that no differences were observed in DM, OM and CP digestability .When treated maize Stover with *Pleurotus djamor* and suggest that treated maize Stover is not ideal forage for ruminants. Another outcome agreement with some report, *In vitro* digestibility of Lucerne hay was significantly higher from control (1)(20).

Table 1.Effect of *Streptomyces MS* treatments on IVDMD% of roughages

Source of roughages	DM in Fresh%	Treatments				
		Untreated	*Streptomyces MS*	IVDMD% improvement	IVOMD% improvement	Level of sign.
Wheat straw	94.18	20.78	30.22	9.44	9.46	**
Corn cob	94.08	30	45.01	15.01	15.3	**
Alfalfa hay	91.72	45	67.07	22.07	22.08	**

*** (P<0.01).**

***Streptomyces MS* :isolated from Iraq camel degrade feces**

Table2.The effect of treated roughages with *Streptomyces MS* on lignin content

Source of roughages	Lignin content (%)	Untreated	*Streptomyces MS*	Average(Reduction in lignin content)%	Level of sig.
Wheat straw	10.01	9.4	8.12	1.28	*
Corncob	11.5	9.8	6.87	2.93	**
Alfalfa hay	8.9	8.26	7.64	0.62	**

***(P<0.05). **(P<0.01).**

CONCLUSION

It could be concluded that ruminant diet pre-treated with wild strain of ligninolytic bacteria improved IVDMD,IVOMD % and reduction lignin content for all roughages, this would be achieved by digestion of structural carbohydrate by destroy lignin –carbohydrate linkage.in addition, biological treatment are preferable than other treatments such as chemical and physical treatments friendly environmen.

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