Mysore J. Agric. Sci., 59 (1) : 132-138 (2025)

Optimization of Growth Parameters and Ethanol Tolerance Levels for Growth of Lignocellulolytic Microorganisms for Enzymatic Hydrolysis

M. Keerthi Patel, R. Muttu Raju, Sathwik M. N. Raj, M. K. Murali and Ashwini
Department of Agricultural Microbiology, College of Agriculture, UAS, GKVK, Bengaluru - 560 065
e-Mail : keerthipatelm@gmail.com

Authors Contribution

M. Keerthi Patel :
Conceptualization, carried out experiment, data analysis and draft prepration

R. Muttu Raju :
Supervision and draft correction

Sathwik M. N. Raj;
M. K. Murali & Ashwini :
Data analysis, manuscript prepration and correction

Corresponding Author :
M. Keerthi Patel

Received : September 2024
Accepted : October 2024

Abstract

Bacillus inaquosorum UASBLCB_03, *Phanerochaete chrysosporium* UASBLCF_01 and *Streptomyces viridosporus* UASBLCA_04 are three efficient lignocellulolytic microorganisms used in the hydrolysis of lignocellulosic biomass. To maximize their hydrolytic efficiency, the optimization of growth parameters is essential. Efficient microbial degradation of lignocellulose is critical for biofuel production, particularly ethanol, as these microorganisms produce key enzymes like cellulases and hemicellulases. With this view, study was conducted to optimize temperature, pH and NaCl concentration for microbial growth. The study also evaluates ethanol tolerance and carbon utilization to assess the suitability of these strains for biomass degradation. Results revealed that all the three microorganisms exhibited optimal growth at 30°C and pH of 7.0. *B. inaquosorum* and *P. chrysosporium* exhibited increased growth at NaCl concentrations up to 5 per cent while, *S. viridosporus* preferred lower concentrations. In terms of ethanol tolerance, *B. inaquosorum* tolerated up to 6 per cent, whereas, *P. chrysosporium* and *S. viridosporus* could tolerate up to 8 per cent. Carbon utilization tests indicated that glucose and galactose were the preferred carbon sources for all the three microorganisms with trehalose and ribose being not utilized. These findings provide insights into the optimization of lignocellulolytic microorganisms for improved biomass degradation and highlights the importance of fine-tuning growth parameters, understanding ethanol tolerance and carbon preferences to enhance the efficiency of microbial systems in lignocellulose bioconversion processes.

Keywords : Lignocellulosic biomass, cellulose, hemicellulose, lignin, Bioconversion

Lignocellulosic biomass, a renewable and abundant resource, holds significant potential for producing biofuels and other valuable products through microbial bioconversion (Maki *et al.,* 2011). Globally, an approximate of 181.5 billion tons of lignocellulosic biomass is generated annually (Singh *et al.,* 2022). Composed primarily of cellulose, hemicellulose and lignin, lignocellulosic biomass forms the structural framework of plant cell walls, making it a robust yet difficult material to degrade. The efficient breakdown of these complex materials into fermentable sugars, such as glucose, is crucial for producing biofuels like ethanol (Held *et al.,* 2012). This conversion relies heavily on microorganisms capable of secreting lignocellulolytic enzymes, which hydrolyze the cellulose and hemicellulose into simpler sugars (Sanghi *et al.,* 2010 and Bajaj & Abbass, 2010). Therefore, the optimization of microbial systems for lignocellulose degradation is a key area of research in the development of sustainable biofuels (Sukumaran and Pandey, 2009).

Microorganisms such as bacteria, actinomycetes and fungi play a pivotal role in lignocellulose hydrolysis

(Bollok and Reczey, 2005). Among them, fungi are particularly valuable as they secrete large quantities of extracellular enzymes, enhancing the efficiency of biomass degradation. Fungi such as *Phanerochaete chrysosporium*, known for its lignin-degrading capabilities and *Streptomyces viridosporus*, which excels at cellulose and hemicellulose hydrolysis are widely studied for their potential in this field. Additionally, bacteria such as *Bacillus inaquosorum* contribute to the synergistic breakdown of lignocellulosic materials when used in co-cultures with fungi, forming highly efficient consortia for biomass hydrolysis (Jagadeesh and Muthuraju, 2022).

Enhancing the growth conditions of lignocellulolytic microorganisms is crucial for maximizing enzymatic activity and improving biomass degradation efficiency. Environmental factors such as temperature, pH, salinity and nutrient composition play vital roles in influencing microbial growth and enzyme production (Sharma & Bajaj, 2005 and Bocchini *et al.,* 2008). Inadequate conditions can lead to a significant reduction in enzymatic activity, limiting the capacity of microorganisms to hydrolyze biomass effectively. To increase the efficiency of lignocellulose breakdown in large-scale applications, it is important to adjust these parameters. Temperature and pH in particular, have a major impact on growth and enzyme function with specific ranges required for each microorganism. While some thrive in neutral or slightly acidic conditions, others may have different preferences for optimal enzyme secretion (Subramaniam *et al.*, 2023).

Salinity also affects microbial growth, as some strains are sensitive to high NaCl concentrations, inhibiting growth and enzyme production (Gao *et al.,* 2018). Ethanol tolerance is crucial for biofuel production, as higher ethanol levels can inhibit microbial growth and their enzyme activity (Vinayavekhin *et al.,* 2020 and Annu & Suvarna, 2015). Additionally, understanding the carbon source preferences of microorganisms helps to optimize lignocellulose hydrolysis and enhancing the yield of fermentable products (Shivsharan & Kadam, 2019). Thus, finding a balance in growth conditions that supports all the organisms of the co-culture is crucial in maximizing the degradation efficiency.

Hence, this study aims to optimize the growth conditions of three lignocellulolytic microorganisms such as *Bacillus inaquosorum* UASBLCB_03, *Phanerochaete chrysosporium* UASBLCF_01 and *Streptomyces viridosporus* UASBLCA_04 by determining their optimal temperature, pH and NaCl concentrations for enhanced growth. Additionally, it will also assess their ethanol tolerance to evaluate their suitability in bioethanol production. A carbon utilization test will identify preferred carbon sources, offering insights into their efficiency in degrading lignocellulosic biomass. This research aims to optimize the growth parameters of lignocellulolytic microorganisms.

Material and Methods

Collection of Lignocellulolytic Microorganism

Lignocellulolytic microorganisms were isolated from degraded wood, paddy straw piles, cow dung, forest soil, termite midguts, paddy field soil, tree bark, compost and vermicompost. A total of 173 isolates were obtained and screened for their qualitative cellulolytic and ligninolytic activities by Jagadeesh and Muthuraju (2022). Among these, the efficient lignocellulolytic microorganisms, *Phanerochaete chrysosporium*, *Bacillus inaquosorum* and *Streptomyces viridosporus*, were obtained from the Department of Agricultural Microbiology, GKVK, Bangalore - 560 065 (Plate 1).

Optimization of the Growth Parameters for Multiplication of Efficient Lignocellulolytic Microorganisms for Enzymatic Hydrolysis

The growth of *Bacillus inaquosorum*, *Phanerochaete chrysosporium* and *Streptomyces viridosporus* was evaluated under varying conditions to optimize their growth. All microorganisms were assessed for temperature, pH, NaCl and ethanol tolerance by inoculating active culture to nutrient broth, PDB and starch casein agar while, growth was measured *via.,* optical density (600 nm), biomass (g) and visual assessment respectively. These organisms were tested

Plate 1 : Lignocellulolytic microorganisms; A- *Phanerochaete chrysosporium* UASBLCF_01; B-*Bacillus inaquosorum* UASBLCF_01; C- *Streptomyces viridosporus* UASBLCA_04

at temperatures ranging from 18°C to 30°C, pH levels from 4 to 8, NaCl concentrations of 2.5-7.5 g L^{-1} and ethanol concentrations of 4-14 per cent. Carbon source utilization was evaluated using fermentation medium, indicated by a color shift from red to yellow.

Statistical Analysis

The WASP 2.0 was used for data analysis, significant different groups were calculated with one-way analysis of variance (ANOVA). The Duncan Multiple Range Test (DMRT) was used for multiple comparisons and the level of significance was set at ≤0.05.

Results and Discussion

Optimization of Temperature for Lignocellulolytic Microorganisms for Hydrolysis

Optimization of temperature is crucial for lignocellulolytic microorganisms in hydrolysis because temperature directly influences enzyme activity, microbial growth rates and substrate degradation efficiency. Each microorganism has an optimal temperature range where its lignocellulolytic enzymes are most effective, ensuring maximum biomass breakdown. Inappropriate temperatures can reduce enzyme activity, slow microbial metabolism and impair hydrolysis performance.

All lignocellulolytic microorganisms exhibited the highest growth at 30°C, with the lowest growth observed at 18°C. Specifically, *B. inaquosorum* UASBLCB_03 and *P. chrysosporium* UASBLCF_01 achieved a maximum growth (OD_{600}= 0.33 and 0.53 g) at 30°C and *S. viridosporus* UASBLCA_04 showed the most significant growth on agar media at 30°C based on visual assessment (Table 1 and 6). The optimal temperature for the growth of all the three lignocellulolytic microorganisms was found to be 30°C, where maximum growth was observed. Temperature plays a crucial role in microbial metabolism and enzyme activity and deviations from this optimal level significantly inhibits the growth. These findings are consistent with those of Alwakeel (2008), who identified 30°C as the ideal temperature for enhancing lignocellulolytic microbial activity. Similarly, Malviya *et al.* (2012) reported 28°C as the optimal temperature for *Streptomyces* species. The slight difference in optimal temperature for *Streptomyces* in their study suggests temperature tolerance variation within microbial groups, but overall, 30 °C remains ideal for the consortium studied here.

Optimization of pH for Lignocellulolytic Microorganisms for Hydrolysis

Optimization of pH is vital for lignocellulolytic microorganisms as pH affects enzyme stability, activity and microbial metabolism during hydrolysis. The growth of all the three lignocellulolytic microorganisms was assessed across different pH levels. *B. inaquosorum* showed optimal growth at pH 7.0 (OD_{600} = 0.50), while *P. chrysosporium*

TABLE 1

Optimization of temperature for growth of *Bacillus inaquosorum* (UASBLCB_03) and *Phanerochaete chrysosporium* (UASBLCF_01) for enzymatic hydrolysis

Temperature (°C)	Growth of *Bacillus inaquosorum* (Absorbance at OD_{600})	Biomass of *Phanerochaete chrysosporium* (g)
18	0.07 ^{e}	0.11 ^{e}
20	0.09 ^{d}	0.19 ^{d}
25	0.14 ^{c}	0.25 ^{c}
28	0.25 ^{b}	0.40 ^{b}
30	0.33 ^{a}	0.53 ^{a}
35	0.26 ^{b}	0.42 ^{b}

Note : Numerical values are expressed as mean of four replicates. Treatment with different superscripts in the same column represent a significant difference as determined by DMRT ($p \leq 0.05$).

exhibited the highest biomass at pH 6.0 (0.54 g) with on par growth at pH 7.0 (0.49g), indicating adaptability from slightly acidic to neutral pH. *S. viridosporus* showed better growth at pH 8.0 but also demonstrated better growth at pH 7.0 (Table 2 and 6). The optimal growth of consortia of all the three organisms in pH 7.0, where all strains showed substantial growth, likely due to macromolecular stability at neutral pH. The present findings align with Ali *et al.* (2017) and Malviya *et al.* (2012), evaluated adaptability of microorganisms to neutral and alkaline pH conditions, which is crucial for optimizing microbial processes in various industrial applications.

TABLE 2

Optimization of pH for growth of *Bacillus inaquosorum* (UASBLCB_03) and - *Phanerochaete chrysosporium* (UASBLCF_01) for enzymatic hydrolysis

pH	Growth of *Bacillus inaquosorum* (Absorbance at OD_{600})	Biomass of *Phanerochaete chrysosporium* (g)
4.0	0.003 ^{d}	0.08 ^{c}
5.0	0.004 ^{d}	0.08 ^{c}
6.0	0.129 ^{b}	0.54 ^{a}
7.0	0.493 ^{a}	0.49 ^{a}
8.0	0.116 ^{c}	0.30 ^{b}

Note : Numerical values are expressed as mean of four replicates. Treatment with different superscripts in the same column represent a significant difference as determined by DMRT ($p \leq 0.05$).

Optimization of NaCl for Lignocellulolytic Microorganisms for Hydrolysis

Optimization of NaCl concentration is important for lignocellulolytic microorganisms as salt levels can impact cell growth, enzyme stability and overall hydrolysis efficiency. While some microorganisms tolerate or even thrive in moderate salinity, high NaCl concentrations can inhibit enzyme activity and microbial metabolism, reducing biomass degradation. Proper NaCl optimization ensures balanced growth and effective hydrolysis.

The growth of all three organisms was evaluated at different NaCl concentrations. *B. inaquosorum* and *P. chrysosporium* exhibited optimal growth at 5 per cent NaCl, with OD_{600} of 0.46 and biomass of 2.61 g, respectively. In contrast, *S. viridosporus* showed the most significant growth at 2.5 per cent NaCl based on visual assessment. Higher NaCl concentrations (7.5%) inhibited the growth of all strains likely due to osmotic stress and plasmolysis, which disrupt cellular function. These findings are consistent with Ibrahim *et al*. (2015), who reported that NaCl concentrations up to 5 per cent supported optimal activity in lignocellulolytic microorganisms. The data suggests that *B. inaquosorum* and *P. chrysosporium* tolerate higher NaCl levels whereas, *S. viridosporus* prefers lower concentrations (Table 3 and 6).

TABLE 3

Optimization of sodium chloride concentration for growth of *Bacillus inaquosorum* (UASBLCB_03) and *Phanerochaete chrysosporium* (UASBLCF_01) for enzymatic hydrolysis

NaCl (%)	Growth of *Bacillus inaquosorum* (Absorbance at OD600)	Biomass of *Phanerochaete chrysosporium* (g)
2.50	0.40 b	1.75 a
5.00	0.46 a	2.61 b
7.50	0.11 c	0.94 c

Note : Numerical values are expressed as mean of four replicates. Treatment with different superscripts in the same column represent a significant difference as determined by DMRT ($p \leq 0.05$).

Effect of Ethanol Concentrations on the Growth of Lignocellulolytic Microorganism

Ethanol concentration affects the growth of lignocellulolytic microorganisms by influencing cell membrane integrity and enzyme activity. At low concentrations, ethanol can enhance microbial tolerance and enzyme production, but at higher levels, it can inhibit growth, reduce metabolic efficiency and impair the hydrolysis process. The *P. chrysosporium* UASBLCF_01 and *S. viridosporus* UASBLCA_04 tolerated ethanol concentration up to 8 per cent, while *B. inaquosorum* UASBLCB_03 exhibited growth up to 6 per cent ethanol. No growth was observed beyond these levels, indicating inhibitory effects ethanol. This inhibition is likely due to ethanol induced disruption of cell membranes integrity, causing leakage of cellular contents and impaired metabolic functions. These findings align with previous studies such as, Wadhwani *et al.* (2009), which demonstrated that higher ethanol concentrations disrupt membrane stability and decreased microbial viability. The gradual decline in growth as ethanol concentration increased suggests that all three microorganisms are sensitive to ethanol toxicity beyond certain thresholds, limiting their tolerance beyond 6-8 per cent (Table 4 and 6).

Table 4

Optimization of ethanol tolerance on the growth of *Bacillus inaquosorum* (UASBLCB_03) and *Phanerochaete chrysosporium* (UASBLCF_01) for enzymatic hydrolysis

Ethanol (%)	Growth of *Bacillus inaquosorum* (Absorbance at OD600)	Biomass of *Phanerochaete chrysosporium* (g)
4.00	0.01 (0.72^{a})	0.15 (0.81^{a})
6.00	0.01 (0.71^{b})	0.09 (0.77^{b})
8.00	0.00 (0.71^{b})	0.06 (0.75^{c})
10.00	0.00 (0.71^{b})	0.00 (0.71^{d})
12.00	0.00 (0.71^{b})	0.00 (0.71^{d})
14.00	0.00 (0.71^{b})	0.00 (0.71^{d})

Note : Numerical values are transformed values and expressed as mean of four replicates. Treatment with different superscripts in the same column represent a significant difference as determined by DMRT ($p \leq 0.05$).

Utilization of different Carbon Source from Lignocellulolytic Microorganism

The utilization of different carbon sources by lignocellulolytic microorganisms in bioethanol production is essential because the efficiency of biomass breakdown and fermentation depends on the ability of microorganisms to metabolize various sugars. Different carbon sources such as, glucose, xylose and galactose can impact microbial growth. All the three lignocellulolytic microorganisms effectively utilized glucose, maltose, mannitol, lactose, sucrose, starch, arabinose, cellobiose and galactose as carbon sources. However, they were unable to utilize trehalose while, *B. inaquosorum* and *S. viridosporus* could not utilize ribose (Table 5; Plate 2 and 3). Our results align with Hamad *et al.* (2014), showing preference of *Aspergillus niger* for fructose and sucrose over glucose and maltose with starch being the least favored carbon source (Table 5).

Table 5

Utilization of carbon sources by different lignocellulolytic microorganisms

Carbon source	*Bacillus inaquosorum* UASBLCB_03	*Phanerochaete chrysosporium* UASBLCF_01	*Streptomyces viridosporus* UASBLCA_04
Glucose	+++	+++	+++
Maltose	++	++	++
Mannitol	+	+	+
Lactose	++	++	++
Sucrose	++	++	++
Starch	+	+	+
Trehalose	-	-	-
Cellobiose	+	+	+
Arabinose	++	++	++
Galactose	+++	+++	+++
Ribose	-	+	-

Note : (+++)- vigorous growth; (++)- moderate growth; (+)- light growth; (-)- no growth

TABLE 6

Effect of growth parameters and ethanol tolerance on growth of *Streptomyces viridosporus* UASBLCA_04 hydrolysis

Lignocellulolytic microorganism	Temperature (°C)						pH				NaCl (%)				Ethanol (%)				
	18	20	25	28	30	35	4.0	5.0	6.0	7.0	8.0	2.5	5.0	7.5	4.0	6.0	8.0	10.0	12.0	14.0
Streptomyces viridosporus UASBLCA_04	+	+	+	++	+++	++	+	+	+	++	++	+++	++	+	+	+	+	-	-	-

Note : '+'- Good, '++'- Very good, '+++'- Excellent growth, '-'- No growth

In this study, the physiological and biochemical growth parameters of *B. inaquosorum* UASBLCB-03, *P. chrysosporium* UASBLCF-01 and *S. viridosporus* UASBLCA-04 were optimized to enhance their lignocellulolytic activity. Optimal growth for all the three microorganisms occurred at 30°C and pH 7.0. Both *B. inaquosorum* and *P. chrysosporium* showed increased growth in the presence of NaCl up to 5 per cent, while *S. viridosporus* exhibited reduced growth with increasing NaCl concentrations. Ethanol tolerance varied with *B. inaquosorum* tolerating up to 6 per cent ethanol while, *P. chrysosporium* and *S. viridosporus* showed tolerance up to 8 per cent. Glucose and galactose were identified as the preferred carbon sources for all three microorganisms, enhancing their growth and enzymatic activity, crucial for effective lignocellulosic biomass hydrolysis.

REFERENCES

AGUSTINI, L., EFIYANTI, L., FAULINA, S. A. AND SANTOSO, E., 2012, Isolation and characterization of cellulase-and xylanase-producing microbes isolated from tropical forests in Java and Sumatra. *Inter. J. Environ. Bioenergy,* **3** : 154 - 167.

ALI, S. R., FRADI, A. J. AND AL-AARAJI, A. M., 2017, Effect of some physical factors on growth of five fungal species. *Eur. Acad. Res.*, **2** (2) : 1069 - 1078.

ALWAKEEL, S. S., 2008, Indoor fungal and bacterial contaminations on household environment in riyadh, Saudi Arabia. *Saudi J. Biol. Sci.*, **15** (1) : 113 - 119.

ANONYMOUS, 2023, Ethanol production capacity in the country is 1380 crore litres of which 875 crore litres is molasses based and 505 crore litres is grain based. Ministry of Consumer Affairs, Food and Public Distribution, Government of India.

ANNU, T. AND SUVARNA, V. C., 2015, Ethanol tolerance of yeasts isolated from fruits. *Mysore J. Agric. Sci.*, **50** (2) : 263 - 265.

BAJAJ, B. K. AND ABBASS, M., 2010, Studies on an alkali-thermostable xylanase from *Aspergillus fumigatus* MA28.3. *Biotech.*, **1** : 161 - 171.

BOCCHINI, D. A., GOMES, E. AND DASILVA, R., 2008, Xylanase production using Bacillus circulans D1 using maltose as carbon source. *Appl. Biochem. Biotechnol.,* **146** : 29 - 37.

BOLLOK, M. AND RECZEY, K., 2005, Cellulase enzyme production by various fungal strains on different carbon sources. *Acta Aliment*., **29** : 155 - 168.

GAO, X., YIN, Y. AND ZHOU, C., 2018, Purification, characterisation and salt-tolerance molecular mechanisms of aspartyl aminopeptidase from *Aspergillus oryzae 3*.042. *Food Chem.*, **240** : 377 - 385.

HAMAD, H., ALMA, M., ISMAEL, H. AND GOCERI, A., 2014, The effect of some sugars on the growth of *Aspergillus niger*. *KSU Doda Bilimleri Dergisi*, **17** (4) : 7 - 11.

HELD, P., 2012, Enzymatic digestion of *polysaccharides*. Part II : Optimization of polymer digestion and glucose production in microplates. Application note of biofuel research. BioTek Instruments. *Inc., Vermont,* USA.

IBRAHIM, A. S., AL-SALAMAH, A. A., ELBADAWI, Y. B., EL-TAYEB, M. A. AND IBRAHIM, S. S. S., 2015, Production of extra cellular alkaline protease by new halotolerant alkaliphilic *Bacillus* sp. NPST-AK15 isolated from hyper saline soda lakes. *Electron. J. Biotechnol. Electron.*, **18** (3) : 236 - 243.

JAGADEESH, U. AND MUTHURAJU, R., 2022, Isolation and screening of potential lignocellulolytic micro organisms from different ecosystems. *Mysore J. Agric. Sci.*, **56** (4) : 202 - 208.

MAKI, M. L., BROERE, M., LEUNG, K. T. AND QIN, W., 2011, Characterization of some efficient cellulase producing bacteria isolated from paper mill sludges and organic fertilizers. *Int. J. Biochem. Mol. Biol*., **2** : 146 - 154.

MALVIYA, M. K., PANDEY, A., SHARMA, A. AND TIWARI, S. C., 2012, Characterization and identification of actinomycetes isolated from 'fired plots' under shifting cultivation in northeast Himalaya, India. *Ann. Microbiol.*, **63** : 561 - 569.

SANGHI, A., GARG, N., GUPTA. V. K., MITTAL, A. AND KUHAD, R. C., 2010, One step purification and characterization of a cellulose free xylanase produced by alkalophilic *Bacillus subtilis* ASH. *Braz. J. Microbiol.,* **41** : 467 - 476.

SHARMA, P. AND BAJAJ, B. K., 2005, Production and partial characterization of alkali-tolerant xylanase from an alkaliphilic *Streptomyces* sp. CD3. *J. Sci. Ind. Res.,* **64** : 688 - 698.

SHIVSHARAN, R. AND KADAM, D., 2019, Improvement of Local *Saccharomyces cerevisiae* strain by mutagenesis for enhanced production of Bioethanol. *Int. J. Pharm. Biol. Sci.,* **9** (2) : 159 - 167.

SINGH, N., SINGHANIA, R. R., NIGAM, P. S., DONG, C. D., PATEL, A. K. AND PURI, M., 2022, Global status of lignocellulosic biorefinery: Challenges and perspectives. *Bioresour. Technol*., **344** : 126 - 415.

SRIVASTAVA, S. AND DAFALE, N. A., 2024, Tailored microbial consortium producing hydrolytic enzyme cocktail for maximum saccharification of wheat straw. *Bioresour. Technol.*, **399** : 130 - 560.

SUKUMARAN, R. K. AND PANDEY, A., 2009, Ethanol from biomass. In: bioprocess and bioproducts - emerging trends. (Eds.) S. Biswas, P. R. Basak and N. Kaushik. TIFAC, New Delhi, pp. : 13 - 36.

SUBRAMANIAM, S., VELU, G. AND JACKSON, K. M., 2023, Exploring and optimizing the termite hill bacterial diversity for cellulase production. *Eco. Env. Cons.*, **29** : 89 - 93.

VINAYAVEKHIN, N., KONGCHAI, W., PIAPUKIEW, J. AND CHAVASIRI, W., 2020, *Aspergillus niger* upregulated glycerolipid metabolism and ethanol utilization pathway under ethanol stress. *Microbiology Open*, **9** (1) : 100.

WADHWANI, T., DESAI, K., PATEL, D., LAWANI, D., BAHALEY, P., JOSHI, P. AND KOTHARI, V., 2009, Effect of various solvents on bacterial growth in context of determining MIC of various antimicrobials. *Internet J. Microbiol.*, **7** (1) : 1 - 8.