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MEDIA OPTIMIZATION FOR THE BACTERIOCIN PRODUCTION OF LACTOBACILLUS PLANTARUM BLN39 AGAINST MYCOBACTERIUM FORTUITUM

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ABSTRACT

Lactic Acid Bacteria (LAB) is a diverse group of gram positive bacteria which grabs the researchers attention in recent times due to due to their Generally Recognized As Safe (GRAS) status. Bacteriocins of LAB found numerous applications in food and dairy industries as they are pH and heat stable, easy to produce and retains their activity after storage. Bacteriocin production by LAB highly depends on the culture condition, physicochemical properties, media composition, etc., In media composition, carbon and nitrogen sources, tween concentration are essential for the bacteriocin production. Most commonly, de Man Rogosa Sharpe (MRS) broth is the medium of choice for the LAB ferementation process. However, this medium is quite expensive and provides hindrance to the large scale economical production of bacteriocin from LAB. There is always a necessity to find an alternative and cheapest source of carbon and nitrogen source and optimization of media composition for the effective bacteriocin production. Mycobacterium fortuitum is one of the rapidly growing non tuberculous mycobacteria which causes diseases in humans includes pulmonary diseases, skin, joints and bone infections and disseminated diseases, etc., The treatment for M. fortuitum disease involves multiple antibiotics, long course treatment and drug related toxicities. This necessitates the development of new drug candidates against M. fortuitum. In this study, eight different media were prepared by supplementing and replacing the existing carbon and nitrogen source with molasses and saw dust and with 2X composition of existing carbon source and tween 80. Lactobacillus plantarum BLN39 was subjected to grown in this different optimized media and their arbitrary unit (AU/ml) was calculated by screening L. plantarum BLN39 against M. fortuitum. Among eight different media optimized, MRS broth supplemented with molasses showed 1600 AU/ml which is double the AU/ml obtained for neat MRS broth (800 AU/ml). MRS broth supplemented with saw dust and MRS broth with 2X tween 80 showed the same AU/ml as of neat MRS broth. Hence, molasses proved as an effective and cheapest supplement for the bacteriocin production of L. plantarum BLN39 against M. fortuitum.

Keywords: Lactobacillus plantarum, Mycobacterium fortuitum, Non tuberculous mycobacteria, media optimization, molasses.

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INTRODUCTION:

Lactic Acid Bacteria is a diverse group of microorganisms which is gram positive, catalase negative bacteria which exists in either cocci or bacilli in shape. LAB grabs a significant attention towards researchers and in biopreservation techniques due to their Generally Recognized As Safe (GRAS) status (Carr et al., 2002). LAB could inhibit the growth of other virulent microorganisms by production of bacteriocins. Bacteriocins is an antimicrobial peptide, ribosomally synthesized, can be produced by many other bacteria (Sahar et al., 2017).

Bacteriocins of LAB have numerous biotechnology applications as they are easy to produce, stable at low pH, non toxic to humans, sensitive to proteases, heat stable and they are able to retain their activity after long term of storage, etc., (Todorov and Dicks, 2009; Bari et al., 2005). Bacteriocin production can be influenced by incubation atmosphere, physicochemical conditions, etc. and also by various medium composition (Ganzle et al., 1999; Zheng et al., 2012; Zhou et al., 2015; Turgis et al., 2016). The most important parameters in the medium composition on bacteriocin production are the concentration of carbon source, nitrogen source and tween 80 (Keren et al., 2004; Mataragas et al., 2004; Abo-Amer, 2011). Most commonly, de Man Rogosa Sharpe (MRS) medium is the choice of medium for LAB fermentations but it has its own limitations (Arakawa et al., 2008). It is quite expensive and acts as barrier for economical large scale of production. Few studies focused on the cheaper source of carbon and nitrogen for LAB fermentations and their bacteriocin production (Sridevi et al., 2017; Han et al., 2011; Verluyten et al., 2004).

M. fortuitum is a clinically significant rapidly growing mycobacteria which causes mostly pulmonary diseases and also extrapulmonary disease, causing localized skin, soft tissue, wound or bone-infections following traumatic injuries or surgery. The treatment for *M. fortuitum* involved with long course, combination of antibiotics and their drug related toxicities necessitates the need for the development of new candidates against *M. fortuitum* (Brown et al., 2002; Griffith et al., 2007; Johanna et al., 2020).

In this study, eight different media has been prepared for the growth of LAB strain BLN39 which includes neat MRS broth, MRS broth supplemented with cheaper carbon sources like molasses and saw dust, MRS broth in which dextrose replaced by molasses and saw dust, MRS broth with 2X carbon and nitrogen source and Tween 80. BLN39 was subjected to grow in this media and screened their bacteriocin production by screening against *M. fortuitum*.

MATERIALS AND METHODS:

Chemicals and Cultures:

All the chemicals and reagents used in this study were purchased from Himedia (Mumbai). The indicator organism used in this research work, *Mycobacterium fortuitum* (MTCC1902) was obtained from Microbial Type Culture Collection, Chandigarh, India. The isolation and identification detail of a LAB isolate BLN39 were published in Revathy et al., 2020.

Media Preparation:

Eight different media for the growth and bacteriocin production of BLN39 was prepared as mentioned in Table 1. The pH of all the prepared media was adjusted to pH 6.5 and autoclaved at 121°C for 15 minutes.

Bacteriocin Production:

An overnight grown culture of BLN39 was inoculated into the prepared eight different media and incubated in shaking condition (100rpm) at 30°C for 18 hours. After incubation, the culture was centrifuged at 5000rpm for 10 mins and the cell free supernatant (CFS) was collected to

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assess the bacteriocin production of BLN39. Then the collected CFS from each prepared media was serially diluted in two fold dilution to measure the arbitrary units per ml (AU/ml) of each optimized media.

Anti *M. fortuitum* activity:

Anti *M. fortuitum* activity of BLN39 with different optimized media was assessed using agar well diffusion method. Briefly, the suspension of an indicator organism, *M. fortuitum* (MTCC1902) was prepared by inoculating a loopful of colonies into 0.3ml of middlebrook 7H9 broth in bijou bottle containing sterile glass beads. The colonies were homogenized by vortex for 30 seconds and kept undisturbed to allow the clumps to settle down. Then the volume of the suspension was made to 5ml using middlebrook 7H9 broth. 200µl of prepared suspension was added to 5ml of molten agar and poured onto middlebrook 7H9 agar plate. Subsequently, the wells were made on the surface of the agar plate. 100µl of the CFS collected and serially diluted was added into the well. Then the plates were incubated at 37°C for 24 hours and the zone of inhibition was measured to calculate AU/ml.

Taxonomy of the strain BLN39:

The genomic DNA of the LAB strain BLN39 was isolated using solute ready genomic DNA kit. DNA was analyzed by gel electrophoresis and quantified using spectrophotometer (NanoDrop ND-1000, Thermo Scientific, Gloucester, UK). The 16S rRNA gene sequence of the strain was amplified using the primers: 27F 5′AGAGTTTGATCMTGGCTCAG3′ (forward) and 1492R 5′TACGGYTACCTTGTTACGACTT3′ (reverse) (Kumar Gothwal et al., 2007). The PCR amplified product of the strain was sequenced and analyzed at National Collection of Industrial Microorganisms (NCIM), National Chemical Laboratory (CSIR-NCL), Pune, India. The partial 16S rRNA nucleotide sequence of BLN39 has been deposited in GenBank database.

RESULTS AND DISCUSSION:

Anti *M. fortuitum* activity:

BLN39 exhibited different level of anti *M. fortuitum* activity in the different optimized media (Figure 1 & 2). MRS broth supplemented with molasses showed 1600 AU/ml which is double the AU/ml obtained for neat MRS broth (800 AU/ml). Molasses was used as an efficient and cheap carbon source for large scale production of various microbial strains (Barbosa et al., 2016; Okafor and Okeke, 2017). This study supported the result obtained by Sridevi et al., 2017 in which molasses were proved as effective and cheaper source of carbon for the bacteriocin production by *Lactobacillus plantarum* sp., A study by Mulyani et al (2019) also showed that molasses can act as low cost component for effective bacteriocin production by Pediococcus pentosaceus. MRS broth supplemented with saw dust and MRS broth with 2X tween 80 showed the same AU/ml as of neat MRS broth. MRS broth in which saw dust replaced for dextrose showed the least inhibitory activity i.e, 100 AU/ml. A study by Sathiyanarayanan et al., 2013 have showed that saw dust can also act as an effective carbon source for the biomass production of Streptomyces sp., A study by Hoda et al., 2013 have showed that MRS broth supplemented with Tween 80 along with other components resulted in high bacteriocin production by Lactobacillus acidophilus. A study by Todorov and Dicks (2009) have proved that inclusion of tween 80 increased bacteriocin production by 50% in Enterococcus mundtii. Elvina et al., showed that tween supply is essential for the bacteriocin activity of L.plantarum. Therefore, molasses act as good carbon supplementary source for the growth and bacteriocin production of BLN39.

Taxonomy of the strain BLN39:

Amplification of 16S rRNA gene from the strain BLN39 resulted in 1455bp sequences. BLAST analysis showed 99.79% sequence similarity with *Lactobacillus plantarum* strain DSM 16365. Hence the strain BLN39 was identified as *Lactobacillus plantarum*.

CONCLUSION:

Lactobacillus plantarum BLN39 showed better bacteriocin production against *M. fortuitum* MTCC1902 in the MRS broth medium supplemented with molasses. Molasses proved to be an effective and cheapest carbon source supplement for the bacteriocin production of *L. plantarum*.

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Table 1: Media Preparation for bacteriocin production

Media	Components	w/v
Media 1	Proteose Peptone	10g/l
	Beef extract	10g/l
	Yeast extract	5g/l
	Dextrose	20g/l

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	Ammonium citrate	2g/l
	Sodium acetate	5g/l
	Magnesium sulphate	0.1g/l
	Manganese sulphate	0.05g/l
	Dipotassium phosphate	2g/l
	Tween 80	8g/l
Media 2	Proteose Peptone	20g/l
	Beef extract	20g/l
	Yeast extract	10g/l
	Dextrose	40g/l
	Ammonium citrate	2g/l
	Sodium acetate	5g/l
	Magnesium sulphate	0.1g/l
	Manganese sulphate	0.05g/l
	Dipotassium phosphate	2g/l
	Tween 80	8g/l
Media 3	Proteose Peptone	10g/l
	Beef extract	10g/l
	Yeast extract	5g/l
	Dextrose	20g/l
	Ammonium citrate	2g/l
	Sodium acetate	5g/l
	Magnesium sulphate	0.1g/l
	Manganese sulphate	0.05g/l
	Dipotassium phosphate	2g/l
	Tween 80	8g/l
	Molasses	20g/l
Media 4	Proteose Peptone	10g/l
	Beef extract	10g/l
	Yeast extract	5g/l
	Ammonium citrate	2g/l
	Sodium acetate	5g/l
	Magnesium sulphate	0.1g/l
	Manganese sulphate	0.05g/l
	Dipotassium phosphate	2g/l
	Tween 80	8g/l
	Molasses	20g/l
Media 5	Proteose Peptone	10g/l
	Beef extract	10g/l
	Yeast extract	5g/l
	Dextrose	20g/l
	Ammonium citrate	2g/l
	Sodium acetate	5g/l
	Magnesium sulphate	0.1g/l

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	Manganese sulphate	0.05g/l
	Dipotassium phosphate	2g/l
	Tween 80	8g/l
	Saw dust	20g/l
Media 6	Proteose Peptone	10g/l
	Beef extract	10g/l
	Yeast extract	5g/l
	Ammonium citrate	2g/l
	Sodium acetate	5g/l
	Magnesium sulphate	0.1g/l
	Manganese sulphate	0.05g/l
	Dipotassium phosphate	2g/l
	Tween 80	8g/l
	Saw dust	20g/l
Media 7	Proteose Peptone	10g/l
	Beef extract	10g/l
	Yeast extract	5g/l
	Dextrose	20g/l
	Ammonium citrate	2g/l
	Sodium acetate	5g/l
	Magnesium sulphate	0.1g/l
	Manganese sulphate	0.05g/l
	Dipotassium phosphate	2g/l
Media 8	Proteose Peptone	10g/l
	Beef extract	10g/l
	Yeast extract	5g/l
	Dextrose	20g/l
	Ammonium citrate	2g/l
	Sodium acetate	5g/l
	Magnesium sulphate	0.1g/l
	Manganese sulphate	0.05g/l
	Dipotassium phosphate	2g/l
	Tween 80	16g/l

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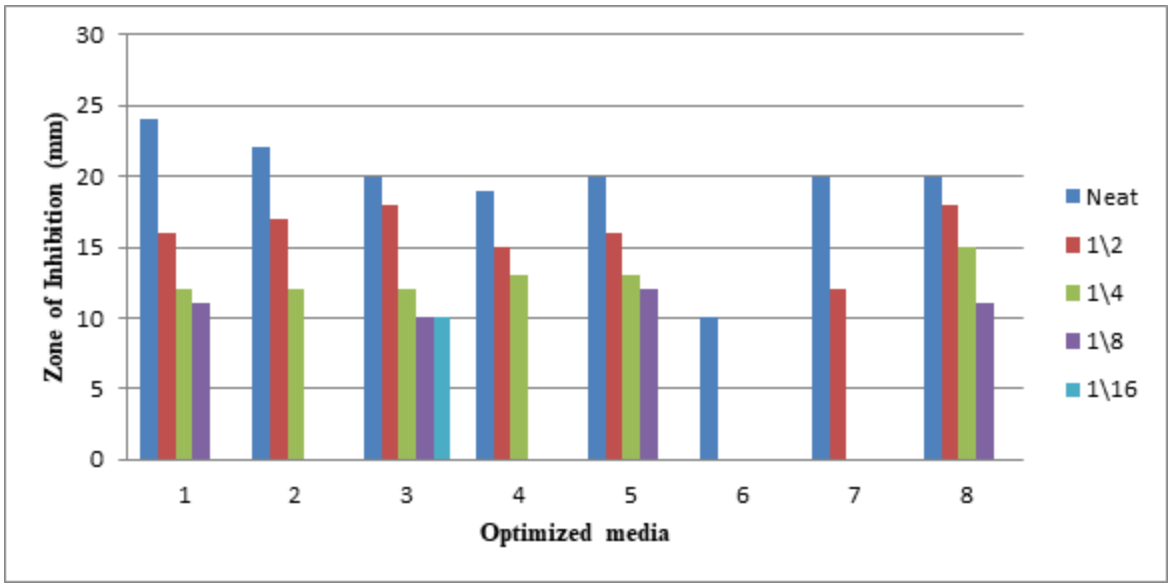

Figure 1: Anti *M. fortuitum* activity of optimized media

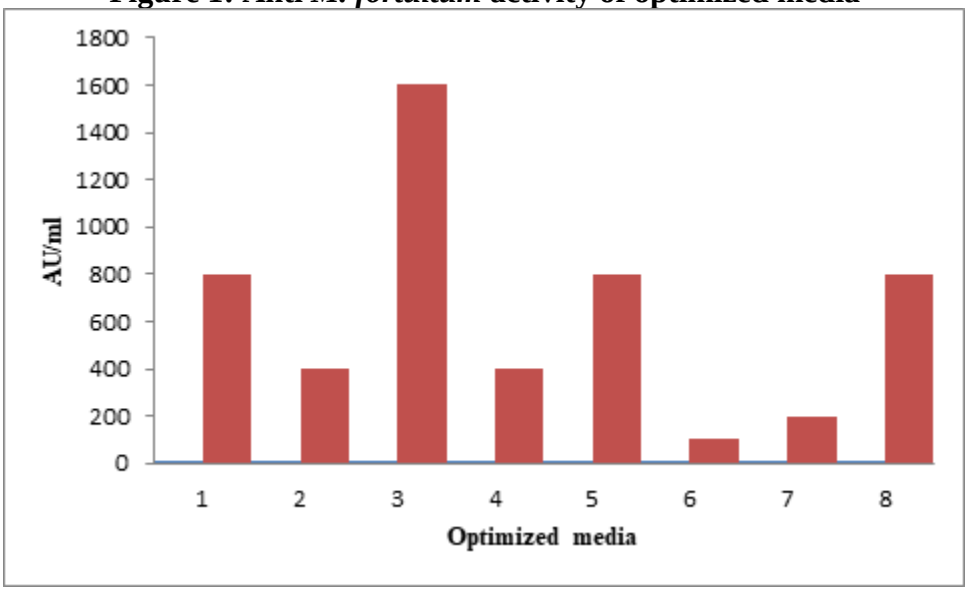

Figure 1: AU/ml evaluation of optimized media