MedCrave
Step into the World of Research

Journal of Microbiology & Experimentation

Research Article

Open Access

CrossMark

Screening of lectins for specific detection of *listeria monocytogenes*

Abstract

In the present study, a specific binding activity between lectin and *Listeria monocytogenes* was studied by agglutination assay. The ten lectins were screened for their agglutination activity (HA) against *L. monocytogenes* ATCC 15313, 19111, 19115, 19118, 13932 & BAA 751 along with lab isolates of RM –17 and RM 26. Wherein 84.4±1.76% binding was observed with wheat germ agglutinin (WGA) this was followed by 70% with Helix pometia agglutinin(HPA), 51.25% with Arachis hypogea lectin (AHL), 39.375% with Ulex europeas lectin (UEL), 30.625% with Lens culinaris lectin (LCL), and 18.75% with Sambucus nigra agglutinin (SNA), 26.25% with Phytolaccka Americana(PAL), 31.25% Maackia amurensis lectin (MAL) and 16.25% concavalin A. But, <10% HA was observed with Pisium Sativum (PSA). Additionally, these lectins were also screened for binding activity with Gram positive and Gram negative bacteria other than *L. monocytogenes*, but, no agglutination was observed except Salmonella (10–20%), Klebsiella (10–20%), and Citrobacter (45%). The HA of WGA with *L. monocytogenes* was observed under scanning electron microscopy having cell aggregation with gapped and patchier cells, but, in some cases cell rupture was also seen. It was clear from the sugar inhibition assay that the HA of WGA with *L. monocytogenes* was reliably inhibited in presence of N–acetylglucosamine (NAGA) at a rate of 0, 35, 50, 80 and 100%, respectively, at 0, 0.1, 0.25, 0.5, 0.75 and 1.0 M concentration, respectively. Whereas in the case of glucose, the % inhibition was less compared to WGA followed by galactose, in the case of mannose, a least inhibition of 25% was observed at 1 M concentration. The complete inhibition of agglutination in the presence of NAGA indicates that the binding of *L. monocytogenes* cells to WGA lectin was NAGA specific. These results will help in the use of WGA in the development of rapid test for detection of *L. monocytogenes* in milk.

Keywords: lectin, listeria monocytogenes, agglutination

Volume 5 Issue 3 - 2017

Raghu HV, Naresh Kumar, Komal Arya, Pradeep Kumar Sharma
National Dairy Research Institute, Deemed University, India

Correspondence: Raghu HV, Dairy Microbiology Division and National Referral centre, ICAR–National Dairy Research Institute, Deemed University, Karnal–132001, India, Tel +91–184–2259534, +91–9466963599; Email raghu@ndri.res.in , 4rvsy.dmndri@gmail.com

Received: May 09, 2017 | **Published:** July 19, 2017

Abbreviations

WGA: Wheat Germ Agglutinin; PSA: Pisium Sativum; HPA: Helix Pometia Agglutinin; SNA: Sambucus Nigra Agglutinin; PAL: Phytolaccka Americana; LCL: Lens culinaris Lectin; UEL: Ulex Europeas Lectin; MAL: Maackia Amurensis Lectin; FSSAI: Food Safety Standard Authority of India; ATCC: American Type Culture Collection; EMCCD: Electron Multiplying Charged Coupled Device

Introduction

Listeria monocytogenes is the causative agent of listeriosis, a disease that can be serious and is often fatal in high risk groups such as pregnant women, neonates, and immunocompromised adults with a rate which may reach 75%.[1] Different food types including raw vegetables, raw milk, soft cheeses, fish, poultry, processed chicken, and beef have been found to be contaminated with *L. monocytogenes*.[2] In particular, the ability of the organism to grow at refrigeration temperatures.[3] and on dry surfaces.[4] and its ability to tolerate acidic conditions make it well adapted to food environments which normally restrict bacterial growth. The primary sources of *L. monocytogenes* in milk and dairy products include the feed, bedding, vegetation, soil, animal faeces, contaminated water, diseased and unclean udders and teats, human hands and handling equipment.[5] Both facts, i.e. occurrence of *L. monocytogenes* in food.[6] and the risk of contracting listeriosis have been very well established. The linkage of dairy products to both invasive and noninvasive listeriosis outbreaks is well known.[7] Various outbreaks have been associated with raw milk, pasteurized milk.[8] soft cheeses.[9] Crave Brothers Farmstead cheeses.[10] hard cheese.[11] semisoft cheese.[12] and ice cream.[13] (CDC, 2015). The food safety regulations of some countries (e.g. USA) require zero tolerance of *L. monocytogenes* in ready–to–eat (RTE) foods.[14] For others, therefore, *L. monocytogenes* has also become an issue of global concern because of its increased presence in milk and other food products.[15] Recently, Food Safety standard Authority of India (FSSAI) has established a microbiological criteria for dairy products that *L. monocytogenes* should be absent in per 25g of every dairy product in India.

Conventional methods for the detection of food pathogens, although typically sensitive, often require multiple time–consuming steps such as extraction, isolation, enrichment, counting, etc., prior to measurement, resulting in testing times which can be in days.[16] Rapid detection of foodborne pathogens is a key step in the control of food related diseases. There is an urgent necessity to develop rapid and sensitive detection methods. The advent of new technologies, namely biosensors, has brought in new and promising approaches comparable to or better than the conventional analytical systems in terms of performance (e.g. reliability, sensitivity, selectivity, specificity and robustness), that can identify the contaminants much faster, more efficiently and can give effective real–time monitoring.

A range of molecules with bio–recognition powers are available naturally such as antibodies, enzymes, and nucleic acids, which are used in the development of rapid techniques for detection. Lectins are carbohydrate–binding proteins other than enzymes or antibodies that exist in most living organisms, ranging from viruses and bacteria to plants and animals.[17] In particular, their role in cell recognition, as well as their employment as invaluable tools for the study of complex carbohydrates in solution and on cell surfaces, is

contributing markedly to advances in glycobiology. Lectins interact with carbohydrates, non–covalently in a manner that is usually reversible and highly specific.[18] Specially, plant–isolated lectins that are well characterized and used as new bioreceptors play a pivotal role in many biosensing devices due to their exquisite specificity for their cognate carbohydrates on the bacterial cell surfaces.[19] Furthermore, the molecular size of lectins is much smaller than antibodies; thus, they allow higher densities of carbohydrate–sensing elements leading to higher sensitivity and lower nonspecific adsorption.[20] Therefore, in our present investigation, we screened different plant lectins for the rapid detection *L. monocytogenes* in dairy products.

Materials and methods

Procurement and maintenance of culture

Different strains of Listeria monocytogenes 15313 (serotype 4b), 13932 (serotype 4b), 19111 (serotype 1/2a), 19115 (serotype 4b), 19118 (4e) and BAA 751.American type culture collection (ATCC), Manassas, Virginia), *L. ivanovii* 19119 (ATCC), *Listeria innocua* 33090 (ATCC), *Enterococcus faecalis* 14506 (ATCC), *Staphylococcus aureus* (ATCC), *Bacillus cereus* 14569 (ATCC), *Lactobacillus* GG.National collection of Dairy cultures (NCDC), *E. coli* 25992 (ATCC), *Salmonella choleraesuis* 10708 (ATCC), *Enterobacter aerogenes* 13048 (ATCC), *Klebsiella pneumonia* 138 (NCDC), *Citrobacter freudenii* ATCC 43864, *Yersinia enterocolitica* ATCC 23715 and *Serratia marcescens* 13880 (ATCC) used in this study were procured from their respective mentioned sources and checked for purity. Cultures were activated by incubating at 37oC for overnight followed by streaking on respectively; selective agars reported in Table 1, which were procured from Himedia Chemicals, Mumbai. Incubations were done overnight at 37OC in a shaker incubator (New Brunswick™ Innova® 42/42R, Eppendorf, Germany). A single pure colony from a respective selective medium after microscopic examination was done to check the purity of the culture was picked up and maintained on its respective maintenance media by routine sub–culturing after every 15 days. Pure colonies from a selective medium of each test organism was transferred to 5.0 ml of respective maintenance broth (Table 1) and incubated for 24 hrs at 37°C. The culture broths were then centrifuged at 8994×g (5810R, Eppendorf, Germany) for 5 min at 10°C. The cell pellet was washed once with 10mM phosphate buffer (PB), pH 6.8 in 2.0mL eppendorf tubes to eliminate broth components. Final suspension for each test organism was prepared in 10mM PB, pH 6.8 and the concentration of each organism was set to an OD of 1.0 using Tecan M pro 200 multimode plate reader (Bioscreen Instruments, Chennai, India) for further agglutination assays.[21,22]

Table 1 Selective and maintenance medium used for *L. monocytogenes* and other contaminants [need to spell out the acronyms used for maintenance broth, selective medium, and maintenance media]

Cultures	Strains	Procured from	Maintenance Broth	Selective Media	Maintenance Media
Listeria	*L. monocytogenes* 15313, 13932, 19111, 19115, 19118, BAA 751; *L. ivanovii* 1911, *L. innocua* 33090	ATCC	Brain Heart Infusion	Polymyxin-Acriflavin-Lithium chloride-Ceftazidime-Aesculin-Mannitol PALCAM) agar	Brain Heart Infusion agar (BHI) slants
Enterococci	*E. faecalis* 14506	ATCC	deMann Rogosa Sharpe (MRS)	Citrate azide agar	BHI slants
Lactobacilli	*Lactobacillus* GG 347	NCDC		MRS agar	MRS agar slants
Bacillus	*Bacillus cereus* 11778	ATCC	Nutrient broth	Bacillus cereus Agar	Nutrient agar (NA) slants
Staphylococcus	*S. aureus* 9144	ATCC	Brain Heart Infusion	Baird Parker Agar	BHI agar slants
Escherichia	*E. coli* 25922	ATCC		Violet red bile agar (VRBA)	NA slants
Salmonella	*S. choleraesuis* 10708	ATCC		Xylose lysine deoxycholate agar	NA slants
Enterobacter	*E. aerogenes* 13048	ATCC		VRBA	NA slants
Klebsiella	*K. pneumoniae* 138	NCDC	Nutrient broth	VRBA	NA slants
Citrobacter	*Citrobacter freundii*	ATCC		VRBA	NA slants
Yersinia	*Y. enterocolitica* 23715	ATCC		VRBA	NA slants
Serretia	*S. marcescens* 14756	ATCC		VRBA	NA slants

Screening of lectin for the specific binding with L. monocytogenes

Ten lyophilized lectins (1mg/mL) such as *Canavalia ensiformis* (Con A), *wheat germ agglutinin* (WGA), *Helix pomatia agglutinin* (HPA), *Pisium sativum* (PSL), *Arachis hypogaea* (PNA), *Ulex europaeus* (UEL), *Lens culinaris* (LCL), *Sambucus nigra* (SNL), *Maackia amurensis* (MAL) and Phytolaccka Americana (PAL) were procured from Sigma Aldrich Chemicals Pvt Ltd., (Bengaluru, India) and dissolved individually in PB (10mM, pH 6.8) followed by dilution to an optimum concentration of 200µg/ mL. About 50µL of each lectin mixed with 50µL of L. monocytogenes cells at a concentration of 107 CFU per ml.(15313 (4b), 13932 (4b), 19111 (1/2a), 19115 (4b), 19118 (4e) & BAA 751, RM–17 and RM–26) in suspension were added together in U bottom microliter plate procured from Cole–Parmer (Mumbai, India). For a control, *L. monocytogenes* suspension was mixed with 50µl of PB in separate wells of U Bottom microliter plate. All the experiment related to screening of lectin was done in triplicate (n–=3). The microtiter plate was incubated at 37oC for 10min in a shaker incubator (New Brunswick™ Innova® 42/42R, Eppendorf, Germany). After incubation, the microtitre plate was observed for agglutination visually.[21] with the appearance of ground glass in transmitted light was taken as a negative reaction. Further, agglutinated cells were stained with crystal violet dye (Himedia, Mumbai, India) and observed under iXon Electron Multiplying charged coupled device (EMCCD) using light microscopy (ANDOR, Bengaluru). Images of agglutination were captured using a camera Sony Lightshot (Sony New Delhi, India). The microscopic observation taken during the agglutination assay was interpreted as per Naughton et al.[22] who described 80–90% agglutination as +4, 60–70% agglutination as +3, 40–50% as +2, 10–20% as +1 and < 10% as negative.

Citation: Raghu HV, Naresh K, Komal A, et al. Screening of lectins for specific detection of listeria monocytogenes. *J Microbiol Exp.* 2017;5(3):1–6. DOI: 10.15406/jmen.2017.05.00150

Screening of lectin with gram positive and gram negative bacteria

Further, all ten lectins were screened individually with different Gram positive bacteria (GPB) other than *L. monocytogenes* (*Listeria ivanovii*, *Listeria innocua*, *Bacillus cereus*, *Staphylococcus aureus*, *Lactococcus lactis*, *Lactobacillus fermentum*, *Leuconostoc mesenteroides*) and Gram negative bacteria (GNB).*Escherichia coli* 25992 (ATCC), *Salmonella choleraesuis* 10708 (ATCC), *Enterobacter aerogenes* 13048 (NCDC), *Klebsiella pneumonia* 138 (NCDC), *Citrobacter freudenii* ATCC 43864, *Yersinia enterocolitica* ATCC 23715 and *Serratia marcescens* 13880 (ATCC) in respective growth medium for agglutination activity.[21,22]

Scanning electron microscopic study of agglutination

The selected WGA lectin was further studied for agglutination with *L. monocytogenes* using scanning electron microscopy (SEM)) using the method described by Facinelli et al.[21] After the agglutination reaction, aliquots of samples (5–10µl) were air dried on glass cover slips and fixed in a solution of 2.5% glutaraldehyde in 75 mM phosphate buffer, (pH 7.4) for 45–60min. The fixed specimen was rinsed three times each for 15 min, in 75 mM phosphate buffer (pH 7.4). After the rinsing, samples were serially dehydrated in ethanol concentrations of 30, 50, 70, 80, 90 and 100% each for 15 min, respectively. After this step, the sample was air dried, and the specimen sample was mounted on a stub followed by gold coating. In gold coating, samples were encrusted with gold at approximately 100–200 Å thickness using an ion coater (Hitachi IB–3, Tokyo, Japan). The ion current was kept at 6–8mA with a fine vacuum of 0.05–0.07 torr for 2–4 minutes. The coated samples were then visualized using a SEM at 7000–30,000 X magnifications (EVO® 18, Carl ZEISS Special Edition, Cambridge,–UK).

Effect of sugars on lectin banding activity of L. monocytogenes

Different sugars such as D–glucose (Glc), D–galactose (Gal), D–mannose (Man) and N–acetylglucosamine (NAGA) were screened for sugar specificity of selected lectins with L. monocytogenes, using an inhibition assay.[21,22] Different concentrations of sugar (0.1 to 1 M, 50µl) were mixed with 50µl of each selected lectin followed by incubation for 1 hr at 37oC. After incubation, the mixture was studied for agglutination using *L. monocytogenes*.[21,22] Further, the percentage inhibition of agglutination activity was calculated by the ratio of agglutination activity lectin in the presence of the sugar to the agglutination observed with *L. monocytogenes* without addition of sugar.

Results and Discussion

Screening of lectin for the specific binding with L. monocytogenes

Among ten lectins screened for agglutination activity with different strains of *L. monocytogenes* cited above, HPA and WGA showed 80–90% activity with *L. monocytogenes* cells, having 8.38 log counts per mL, followed by 60–70% activity with AHL, 40–50% activity with UEL, LCL and SNA; 10–20% activity with PAL and MAL, respectively as shown in Figure 1. However, <10% agglutination was observed with PSA. Figure 2 depicts the microscopic observation of agglutination of *L. monocytogenes* with different lectins by simple staining technique and analysis through EMCCD. Maximum agglutination with all strains of *L. monocytogenes* was observed with WGA followed by HPA, AHA, UEL, MAA, LCL, PAL, SNA, CON A and PSA with an HA of 84.4%, 70%, 51.25%, 39.375%, 31.25%, 30.625%, 26.25%, 18.75%, 16.25% and 9.375%, respectively. The maximum HA was observed with WGA in the presence of different strains of *L. monocytogenes*; therefore, WGA was selected for further screening study with Gram positive and Gram negative bacteria. *L. monocytogenes* could interact with HPA and WGA whereas, *Arachis hypogaea* (PNA), *Glycine max* (SBA), and *Vicia villosa* (VVA) lectins failed to agglutinate *L. monocytogenes* 15313 or any of the other test strains under the experimental conditions.[21] Another study conducted by Facinelli et al.[23] observed that 26 strains of *L. monocytogenes* 15313 were agglutinated by WGA, 17 by Griffonia simplicifolia–1 (GS–1) by RCA–I (ricinus communis agglutinin), and nine by HPA. Therefore, WGA from TVA was selected for further study.

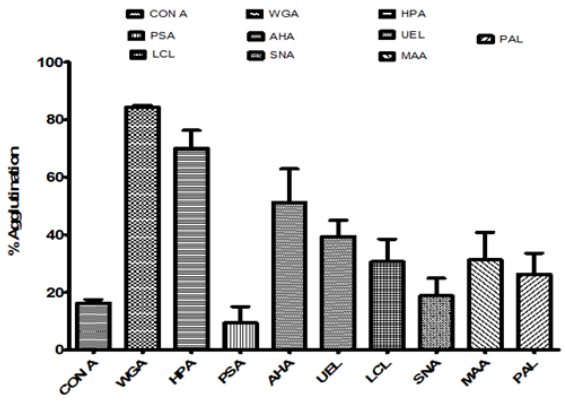

Figure 1 Agglutination activity of different strains of *L. monocytogenes* with various lectins.

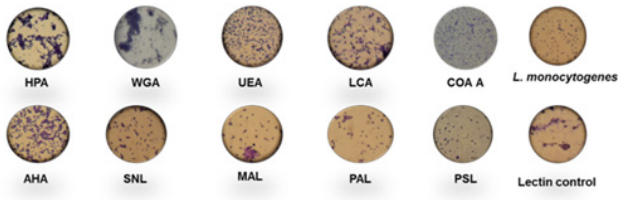

Figure 2 Light microscopic observation of agglutination of *L. monocytogenes* 15313 with different lectins by crystal violet staining. Lectin control contains 200µg/ml lectin dissolved in 10mM phosphate buffer, pH 6.8.

Screening of gram positive and gram negative bacteria for Lectin agglutination

All ten lectins including WGA were also further checked for agglutination activity with GPB (other strains of *Listeria spp.* such as *Listeria innocua* 33090, *Listeria ivanovii* 19119, *Enterococcus faecalis*, *Bacillus cereus and Staphylococcus aureus*)and GNB (*E. coli* O157:H7 *Escherichia coli* 25992 (ATCC), *Salmonella choleraesuis* 10708 (ATCC), *Enterobacter aerogenes* 13048 (NCDC), *Klebsiella pneumonia* 138 (NCDC), *Citrobacter freudenii* ATCC 43864, *Yersinia enterocolitica* ATCC 23715 and *Serratia marcescens* 13880). In our study, no or <10% HA activity was observed with WGA among GPB as shown in Figure 3. Figure 4 depicts the WGA agglutination activity in GNB wherein, zero agglutination was observed among E. coli, *E. coli* O157:H7, Enterobacter, and Serretia whereas, in the case of *Salmonella* and *Klebsiella* HA was 10–20% followed by 45% activity in Citrobacter. Jebor et al.[24] found HA of PSA immobilized with glutaraldehyde in presence *lus*, *Salmonella*, *Staphylococcus*, *Streptococcus* and *Pseudomonas* as compared to *E. coli*, *Enterobacter* on the basis of increase in optical density at 660nm and through microscopic examination using Gran staining technique. In an another study by Payne et al.[25] wherein

Citation: Raghu HV, Naresh K, Komal A, et al. Screening of lectins for specific detection of listeria monocytogenes. *J Microbiol Exp.* 2017;5(3):1–6.
DOI: 10.15406/jmen.2017.05.00150

they immobilized Helix pometia, Canavalia ensiformis, Agaricus bisporus and Triticum vulgaris i.e., WGA on magnetic microspheres for the evaluation of binding activity with *L. monocytogenes*, *S. aureus*, *Salmonella* and *E. coli* and observed 87–100% agglutination activity with *L. monocytogenes* in the presence of WGA, >92% binding with *S. aureus* in the presence of HPA, but poor binding with GNB in the presence of WGA and HPA. The membrane of GNB has not shown increased reactivity to WGA when exposed to increased salinity of the reaction mixture due to loosening of the rigid structure and it has improved the staining with the lectin.[26] Based on these results, it was confirmed that the WGA was highly selective for *L. monocytogenes*. Therefore, WGA was selected for further study.

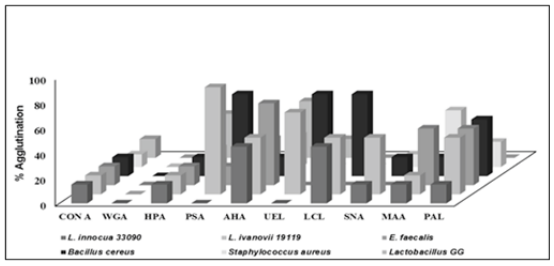

Figure 3 Lectin agglutination activity of different gram positive bacteria.

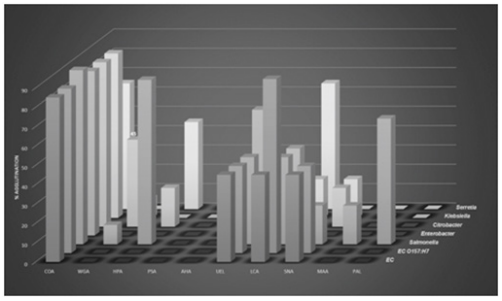

Figure 4 Lectin agglutination activity of different gram negative bacteria.

Scanning electron microscopic study of agglutination

Figure 5a & b shows the arrangements of the *L. monocytogenes* ATCC 15313 in the presence (Figure 5a) or absence of (Figure 5b) 200µg/ml of WGA lectin. Figure 5c shows the pure WGA lectin without *L. monocytogenes* ATCC 15313 cells. The electron microscopic observations depict that the control cells of *L. monocytogenes* ATCC 15313 cells were not induced to aggregate but they arranged in singles and pairs. However, the *L. monocytogenes* ATCC 15313 cell aggregation in the presence of lectin appeared to be gapped and patchier, but, cell rupture was only observed in some cases (as shown in Figure 5).

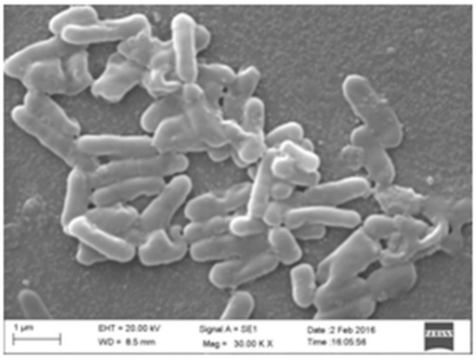

Figure 5a SEM image of lectin agglutination with *L. monocytogenes* ATCC 15313.

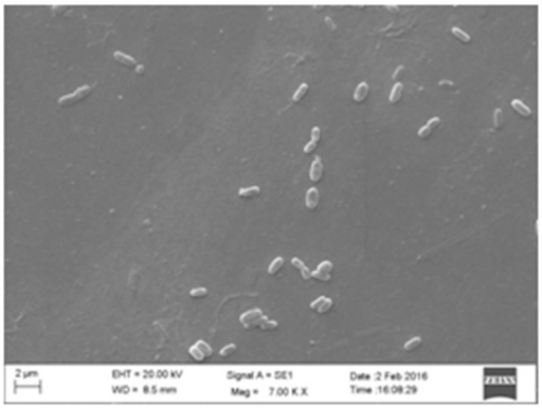

Figure 5b SEM image of *L. monocytogenes* ATCC 15313 in the absence of lectin.

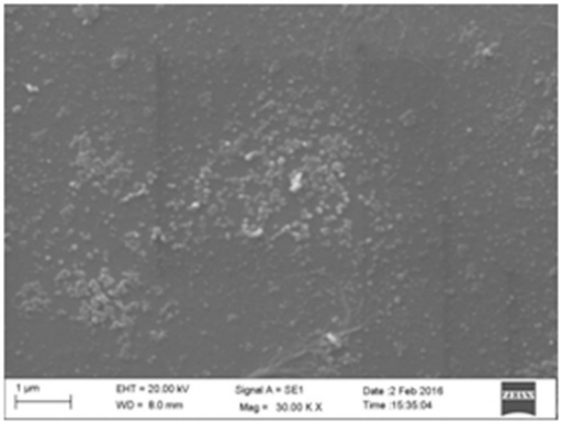

Figure 5c SEM image of WGA lectin.

Inhibition of lectin binding activity of L. monocytogenes by different sugars

Carbohydrate inhibition studies were performed with L. monocytogenes 15313 using Glc, Gal, Man and NAGA. The WGA was consistently inhibited in presence of NAGA with an inhibition % of 0, 35, 50, 80 and 100% inhibition of WGA binding activity at 0.1, 0.25, 0.5, 0.75 and 1M concentration, respectively. In case of glucose, the % inhibition was 0, 25, 35, 55 and 100 and was observed at 0.1, 0.25, 0.5, 0.75 and 1M concentration, respectively. Whereas, in the case of galactose, the inhibition was only observed at 0.75 and 1M concentration with 75 and 90% inhibition respectively, the least inhibition was observed with mannose where only 25% inhibition was found at 1M concentration (Figure 6). The maximum or complete inhibition was observed in presence of NAGA, and indicates that the binding of L. monocytogenes cells to WGA lectin was highly specific for NAGA followed by glucose, galactose and mannose. The binding of *L. monocytogenes* cells to WGA was partially inhibited by glu and gal, suggesting that glu and gal moieties are also present on the cell surface structure of *L. monocytogenes*. The mannose inhibition of WGA agglutination was supported by Neu et al.[27] that wide range of carbohydrates such as glu, gal, man, arabinose, rhamnose, etc. can inhibit the WGA binding in biofilm and about 31% inhibition in WGA binding of biofilm were seen under florescent microscopes using FITC–labelled WGA staining of biofilm. This work is supporting our claim of mannose inhibition of WGA binding with L. monocytogenes. Volf et al.[28] & McGreal et al.[29] reported that carbohydrate binding specificity is an important factor in the recognition of carbohydrates

on pathogen cell surfaces. Further, Loessner et al.[30] has shown that WGA lectin requires terminal NAGA residues present on the teichoic acid of the bacterial wall (WTAs) for attachment. Nir Paz et al.[31] described the binding of fluorescently labeled WGA, a lectin that specifically binds terminal NAGA residues in cell wall polymers using Alexa Fluor 594®. They found that WGA was able to bind wild strains of L. monocytogenes such as 10403S and DP–L5415.

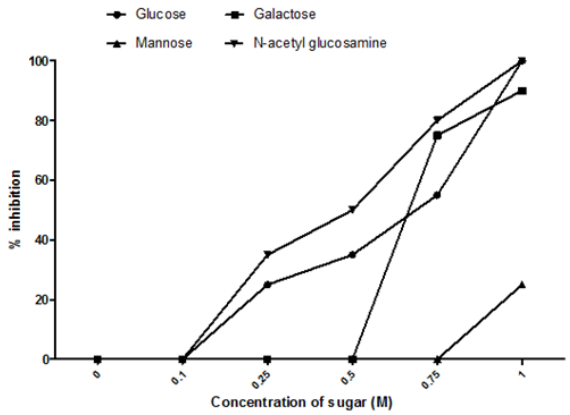

Figure 6 Inhibition of agglutination of *L. monocytogenes* by different sugars.

Conclusion

In our present study, *L. monocytogenes* has shown strong agglutination activity (HA) with WGA followed by HPA, AHA, UEL, MAA, LCL, PAL, SNA, CON A and PSA. The GPB have also shown HA with all lectins screened except WGA wherein HA was <10%. The GNB, especially Enterobacteriaceae groups, haven't shown HA in presence of WGA except Citrobacter (45%), *Klebsiella* and *Salmonella* (10–20%) and maximum HA was observed in presence of ConA. The WGA activity with *L. monocytogenes* was also well established with SEM and by sugar inhibition assays. Based on the inhibition test, it was clear that WGA was specifically bound with NAGA followed by Glc, Gal was present on the cell wall of L. monocytogenes and whereas in case of man it was only 25% inhibition. From the above results, it is clear that WGA may be used as a marker protein for the detection of *L. monocytogenes* in milk and milk products.

Acknowledgments

This work was supported by the National Agriculture Innovation Project (NAIP), Niche area of Excellence (NAE), Outreach Programme (ICAR). The authors express their appreciation to personnel to Dr. S. K. Tomar, Principal Scientist & Incharge, SEM lab and Dr. Vaibao Lule Patil, PhD scholar, Dairy Microbiology Division for SEM study of lectin agglutination with *L. monocytogenes*. Director and Vice Chancellor, ICAR–National Dairy Research Institute (NDRI), Deemed University, Karnal for providing necessary research facility for conducting research work on Lectin binding of *L. monocytogenes*.

Conflicts of interest

None.

References

1. Jalali M, Abedi D. Prevalence of *Listeria* Species in Food Products in Isfahan, Iran. *Int J Food Microbiol*. 2008;122(3):336–340.
2. Schlech WF, David A. Foodborne Listeriosis. *Clin Infect Dis*. 2000;31(3):770–775.
3. Walker SJ, Archer P, Banks JG. Growth of *L. monocytogenes* at refrigeration temperatures. *J Appl Bacteriol*. 1990;68(2):157–162.
4. Wong ACL. Biofilms in food processing environments. *J Dairy Sci*. 1998;81:2765–2770.
5. Ragimbeau DG, Malle P. Use of PFGE typing for tracing contamination with *L. monocytogenes* in three cold smoked salmon processing plants. *Int J Food Microbiol*. 2001;64(1–2):51–61.
6. Ryser ET, Marth EH. Listeria, Listeriosis and Food Safety. 2nd edn, Marcel Dekker Inc New, USA; 1999.
7. Bula CJ, Bille J, Glauser MP. An epidemic of food–borne listeriosis in western Switzerland: description of 57 cases involving adults. *Clin Infect Dis*. 1995;20:66–72.
8. Gaya P, Sanchez J, Medina M, et al. Incidence of *L. monocytogenes* and other *Listeria* species in raw milk produced in Spain. *Food Microbiol*. 1998;15:551–555.
9. Filiousis G, Anders J, Joachim F, et al. Prevalence, genetic diversity and antimicrobial susceptibility of *L. monocytogenes* isolated from open–air food markets in Greece. *Food control*. 2009;20:314–317.
10. CDC Multistate Outbreak of Listeriosis Linked to Crave Brothers Farmstead Cheeses (Final Update). Centre for diseases control and prevention, USA; 2013.
11. Rudolf M, Scherer S. High incidence of *L. monocytogenes* in European red smear cheese. *Int J Food Microbiol*. 2001;63(1–2):91–99.
12. Colak H, Hamparsun H, Enver BB, et al. Prevalence of *L. monocytogenes* and *Salmonella spp.* in Tulum cheese. *Food control*. 2007;18(5):576–579.
13. CDC Multistate Outbreak of Listeriosis Linked to Raw Milk Produced by Miller's Organic Farm in Pennsylvania. Centre for diseases control and prevention, USA; 2015.
14. Meloni D, Pietro G, Anna M, et al. *Listeria Monocytogenes* in RTE Foods Marketed in Italy: Prevalence and Automated Ecori Ribotyping of the Isolates. *Int J Food Microbiol*. 2009;129(2):166–173.
15. WHO Emerging Food–borne Diseases. World Health Organization, Switzerland, 2002;124:1–3.
16. Paul C, Li Y, Cui T, et al. Nanoparticles based sensors for rapid detection of foodborne pathogens. *Int J Agri Biolog Eng*. 2013;6(1)–28–35.
17. Goldstein IJ, Hayes CE. The lectins: carbohydrate–binding proteins of plants and animals. *Adv Carbohydr Chem Biochem*. 1978;35:127–340.
18. Ambrosi M, Cameron NR, Davis BG. Lectins: tools for the molecular understanding of the glycocode. *Org Biomol Chem*. 2005;3(9):1593–1608.
19. Rahaie M, Kazemi SS. Lectin–Based Biosensors: As Powerful Tools in Bioanalytical Applications. *Biotechnol*. 2010;9(4):428–443.
20. Wang Y, Zunzhong Y, Yibin Y. New trends in impedimetric biosensors for the detection of foodborne pathogenic bacteria. *Sensors*. 2012;12(3):3449–3471.
21. Facinelli B, Eleonora G, Chiara C, et al. Interactions with lectins and agglutination profiles of clinical, food, and environmental isolates of Listeria. *J Clin Microbiol*. 1994;32(12):2929–2935.
22. Naughton PJ, Grant G, Bardocz S, et al. Modulation of *Salmonella* infection by the *lectins* of Canavalia ensiformis (Con A) and Galanthus nivalis (GNA) in a rat model *in vivo*. *J Appl Microbiol*. 2000;88(4):720–727.
23. Facinelli B, Giovanetti E, Magi G, et al. Lectin reactivity and virulence among strains of *L. monocytogenes* determined in vitro using the enterocyte–like cell line Caco–2. *Microbiology*. 1998;144(1):109–118.
24. Jebor MA, Yasser HJ. Interaction of Some Pathogenic Bacteria with Phaseolus Vulgaris *L. Seeds Lectin* (as Biosensor). *Int J Biosci Bioche Bioinforma*. 2013;3(3):239–241.

25. Payne MJ, Shona C, Patchett RA, et al. The Use of Immobilized *Lectins* in the Separation of *S. aureus*, *E. coli*, *Listeria* and *Salmonella Spp.* From Pure Cultures and Foods. *J Appl Bacteriol*. 1992;73(1):41–52.

26. Jespersen L, Holm C. Method for Differential Analysis of Bacteria in a Sample. Google Patents WO2002057482 A2; 2002.

27. Neu TR, Swerhone GD, Lawrence JR. Assessment of lectin–binding analysis for in situ detection of glycoconjugates in biofilm systems. *Microbiol*. 2001;147(2):299–313.

28. Volf P, Skarupova S, Man P. Characterization of the lectin from females of Phlebotomus duboscqi sand flies. *Eur J Biochem*. 2002;269(24):6294–6301.

29. McGreal EP, Martinez Pomares L, Gordon S. Divergent roles for C–type lectins expressed by cells of the innate immune system. *Mol Immunol*. 2004;41(11):1109–1121.

30. Loessner MJ, Kramer K, Ebel F, et al. C–terminal domains of *L. monocytogenes* bacteriophage murein hydrolases determine specific recognition and high–affinity binding to bacterial cell wall carbohydrates. *Mol Microbiol.* 2002;44(2):335–349.

31. Nir Paz R, Eugster MR, Zeiman E, et al. *L. monocytogenes* tyrosine phosphatases affect wall teichoic acid composition and phage resistance. *FEMS Microbiol Let*. 2012;326(2):151–160.

Citation: Raghu HV, Naresh K, Komal A, et al. Screening of lectins for specific detection of listeria monocytogenes. *J Microbiol Exp.* 2017;5(3):1–6. DOI: 10.15406/jmen.2017.05.00150